IBRO-APRC Associate School 2018

School on Basic Techniques in Neuroscience — The 1st Ulaanbaatar School

Ulaanbaatar, Mongolia
17-22 SEPTEMBER, 2018

Core Laboratory, Science and Technology Center, MNUMS

Welcome Address —
President of Mongolian National University of Medical Sciences

WE CORDIALLY WELCOME YOU TO IBRO ASSOCIATE SCHOOL ULAANBAATAR

Ladies and gentlemen,

It is my greatest pleasure to congratulate Mongolian Neuroscience Society for organizing the first ever IBRO-APRC Associate School on behalf of the Mongolian National University of Medical Sciences. In recent years Mongolian Neuroscience Society has grown tremendously under the support of International Brain Research Organization and others as well. Mongolian Neuroscience Society is full of talented researchers who are committed to develop the neuroscience in Mongolia and there is no doubt that the society will nurture the next generation of scientists onto the next stage.

Brain mysteries are now being unlocked and knowledge of it has already begun to become innovation alleviating the suffered ones from neurodegenerative incurable diseases. The future of neuroscience is infinite and advances of modern technology are enabling us to witness the beginning of thy future including artificial intelligence. Therefore I would like to mention that the Mongolian National University of Medical Sciences will be the hub of brain research bridging the world level scientist and our people in order to collaborate towards the bright intelligent future of Mongolia.

This year's annual meeting is extraordinarily fruitful because we are proudly hosting the very first Neuroscience School in Mongolia. It is as important as water and sun for the crop to enhance young scientists' skills to conduct basic laboratory studies. It is also my greatest pleasure to host the Neuroscience school at our institution that we wish to host many other neuroscience schools in Mongolia.

One occasion is making their annual meeting a historic remark that is the official visit of Nobel laureate in Physiology or Medicine ever to visit in our beautiful country. Professor Moser has been honored 2014 Nobel Prize for discoveries of brain's navigational function and conscience of one's location. We hope that many of you will witness the honored public lecture.

I wish you the most fruitful training and pleasant journey in Mongolia. The Mongolian National University of Medical Sciences will support scientists in neuroscience for every aspect and wishes you to flourish magnificently.

Best regards,

Tsolmon Jadamba, PhD

President, Mongolian National University of Medical Sciences

Welcome Address – Message from Host Organizer Laboratory

First of all, I would like to express my appreciation to distinguished scientists, doctors, and invited speakers for involving in the IBRO-APRC Associate School on Basic Techniques in Neuroscience – the 1st Ulaanbaatar school 2018. In the great support of IBRO, the first IBRO-APRC Associate School at Mongolia is giving us a great chance and unique opportunity for educating and improving our capacity, knowledge, ability and experience, and taking a motivation on the future scientific activities.

I currently hold the position a Specialist of Core Laboratory, Science and Technology Center, Mongolian National University of Medical Sciences (MNUMS). The Core Laboratory is the biggest RD lab in the MNUMS. It has established on 2015 and consists of 6 kinds of laboratories, such as Cell culture, Molecule biology, Protein and chemical analysis laboratory, Pathology, Live cell imaging laboratory, and Experimental animal laboratory. We have a big interest to conduct world level scientific project using the Core Laboratory. In addition, we are very happy to be a host organizer laboratory of this IBRO-APRC Associate School 2018.

Finally, I hope that the first IBRO-APRC Associate School will provide participants be stronger on RD basic techniques such as Cell Culture preparation, Nucleic Acid isolation/ quantification, and Nitric Oxide determination protocols. Based on this training, I believe that we have plenty of new ideas for our participants and build our tight relationships.

Thanking you most sincerely for your time and consideration.

Sincerely yours,

Ts. Bilegtsaikhan

Bilegtsaikhan Tsolmon, MD, PhD

Head of Professional Board for Bio-product and Reagents, Ministry of Health, Mongolia

Specialist of Core Laboratory, Science and Technology Center, MNUMS

Board Member of Mongolian Neuroscience Society

Contents

IBRO-APRC ASSOCIATE SCHOOL 2018

Detailed program, Sep 17-22, 2018 (day by day activities)

The IBRO-APRC Associate School 2018 (Ulaanbaatar, Mongolia) will provide a 6-day program including comprehensive lectures, group discussions, hands-on techniques, and entrepreneur tour. All lectures will be conducted in the Core Laboratory, Mongolian National University of Medical Sciences (MNUMS).

Day 1 : Monday, September 17th	
9:00-9:30	Registration
9:30-10:00	a) Opening ceremony b) Interactions between school faculties and students c) School Memorial Photography
10:00-11:00	Lecture 1: Cell signaling in the brain
11:00-11:15	Tea break
11:15-12:00	Technical lecture 1: Cell culture using ENDD cell line
12:00-13:00	Lunch
13:00-14:00	Lecture 2: Molecular cloning and applications of CRISPR-CAS
14:00-14:15	Tea break
14:15-15:00	Technical lecture 2: DNA extraction protocols
15:00-17:00	Lab training course 1: Preparation for cell culture
17:00-19:00	Welcome dinner & back to hotel
Day 2 : Tuesday, September 18th	
9:00-10:00	Lecture 3: Neuroendocrinology
10:00-10:15	Tea break
10:15-11:15	Lecture 4: Neuroinflammation
11:15-12:00	Technical lecture 3: Essentials in RT-qPCR
12:00-13:00	Lunch
13:00-14:00	Lecture 5: Gene sequencing in neurodegenerative disorders
14:00-14:15	Break
14:15-15:00	Technical lecture 4: Genotyping methods
15:00-17:00	Lab training course 2: Culturing cell lines
17:00-18:00	Dinner & back to hotel
Day 3 : Wednesday, September 19th	
9:00-10:00	Invited lecture 3: Language Organization in The Human Brain
10:00-10:15	Tea break
10:15-11:00	Technical lecture 5: Insulin tolerance test for rodents
11:00-12:00	Lecture 6: Consciousness and neural correlates
12:00-13:00	Lunch
13:00-13:30	Technical lecture 6: Cell viability assay
13:30-14:00	Technical lecture 7: Nitric oxide determination
14:00-14:15	Tea break
14:15-15:00	Visit to the start-up companies of MNUMS–Entrepreneurship
15:00-17:00	Group Discussion 1: Poster presentations
17:00-18:00	Dinner & back to hotel

Day 4 : Thursday, September 20th		
9:00-10:00	Invited lecture 1: Psychoneuroimmunology	
10:00-10:15	Tea break	
10:15-12:00	Invited lecture 2: Diffusion-weighted magnetic resonance imaging	
12:00-13:00	Lunch	
13:00-14:00	Technical lecture 8: Primary neuronal cell culture	
14:00-14:15	Tea break	
14:15-15:00	Group discussion 2: Oral presentations (selected students)	
15:00-17:00	Lab training course 3: Experiments based on the cell cultures	
17:00-18:00	Dinner & back to hotel	
Day 5 : Friday, September 21st -The 5th Annual Meeting of MNS		
9:00-10:30	IBRO lectures	
10:30-10:45	Coffee break	
10:45-12:00	IBRO lectures	
12:00-13:00	Lunch break	
13:00-13:30	Opening Ceremony of The 5th Annual Meeting of MNS	
13:30-13:40	Meeting memorial photography	
13:40-16:00	Plenary lectures	
16:00-17:30	Introductory lectures	
18:00-21:00	Welcome reception	
Day 6 : Saturday, September 22nd- The 5th Annual Meeting of MNS		
	Hall A	Hall B
9:00-10:30	Neuroscience	Neuroimaging
10:30-10:45	Coffee break	
10:45-12:00	Neurology	Neurosurgery
12:00-13:00	Lunch break	
13:00-14:45	Psychiatry	Satellite event 1
14:45-15:00	Coffee break	
15:00-16:30	Social Psychology	Satellite event 2
16:30-17:00	Closing remarks& Award Ceremony	
17:00-18:00	Dinner & back to hotel	

LECTURES, EXPERIMENTAL MODULES, AND GROUP DISCUSSIONS

- Proposed lectures and technical lectures:
  Invited Lecture 1: Psychoneuroimmunology (Tetsuya Hiramoto, Kyushu University, Japan)

  Invited Lecture 2: Diffusion-weighted magnetic resonance imaging of brain metastasis. (Ivan A. Stepanov, Irkutsk State Medical University, Russia)

  Invited Lecture 3: Language Organization in the Human Brain (Prof. Nansalmaa, National University of Mongolia, Mongolia)

  Lecture 1: Cell signalling in the brain (Bilegtsaikhan Ts, MNUMS)

  Lecture 2: Molecular cloning and applications of CRISPR-CAS systems in neurosciences (Tsevelmaa N, MNUMS)

  Lecture 3: Neuroendocrinology (Damdindorj B, MNUMS)

  Lecture 4: Neuroinflammation (Enkhsaikhan L, MNUMS)

  Lecture 5: Gene sequencing in neurodegenerative disorders (Sevjidmaa B, MNUMS)

  Lecture 6: Consciousness and neural correlates (Battuvshin L, MNUMS)

  Technical lecture 1: Cell culture using ENDD cell line (Baljinnyam T, IMS)

  Technical lecture 2: DNA extraction protocols (Enkhsaikhan L, MMUMS)

  Technical lecture 3: Essentials in RT-qPCR (Jambaldorj J, MNUMS)

  Technical lecture 4: Genotyping methods (Sevjidmaa B, MNUMS)

  Technical lecture 5: Insulin tolerance test for rodents (Damdindorj B, MNUMS)

  Technical lecture 6: Cell viability assay (Javkhlan B, MNUMS)

  Technical lecture 7: Nitric oxide determination (Baasansuren E, MNUMS)

  Technical lecture 8: Primary neuronal cell culture (Darambazar G, MNUMS)

  IBRO Lectures: (The 5th Annual Meeting of MNS)

- Experimental modules:
  Lab training course 1: Preparation for cell culture (Tsevelmaa N, MNUMS)

  Hands-on session 1-1: Aliquoting of penicillin streptomycin mixture (Javkhan B, MNUMS)

  Hands-on session 1-2: Heat inactivation of fetal bovine serum (Uranbileg U, MNUMS)

  Hands-on session 1-3: Nucleic acid isolation (Khulan, MNUMS)

  Lab training course 2: Culturing cell lines (Tsevelmaa N, MNUMS)

Hands-on session 2-1: Refreshing cell line (Baasansuren E, MNUMS)

Hands-on session 2-2: Cell counting method (Baljinnyam T, MNUMS)

Hands-on session 2-3: Nucleic acid quantification (Batkhishig M, MNUMS)

Lab Training Course 3: Experiments based on the cell cultures (Tsevelmaa N, MNUMS)

Hands-on session 3-1: Changing medium and passaging cell line (Khulan U, MNUMS)

Hands-on session 3-2: Preparation of nitrite standard reference curve (Batkhishig M, MNUMS)

Hands-on session 3-3: Determination to nitric oxide (Baasansuren E, MNUMS)

- Group discussions
  Group discussion 1: Poster presentations (all students)

  Group discussion 2: Oral presentations (selected students)

- Entrepreneur tour
  Video introduction to start-up companies of MNUMS

  Visit to Erdem Pharma start-up pharmaceutical company of MNUMS

Tetsuya Hiramoto

M.D., PhD.

Department of Psychosomatic Medicine, National Hospital Organization, Fukuoka Hospital. 811-1394 Fukuoka, Japan

✉: tetsuhcephalmed@yahoo.co.jp

EDUCATION AND DEGREES

Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan: PhD 2009

Medical Sciences Hiroshima University, Hiroshima, Japan: BA 1994, MA 1996 Medical Sciences

CURRENT POST

Chief Doctor of the Department of Psychosomatic Medicine, National Hospital Organization, Fukuoka Hospital, Fukuoka, Japan (since 04/2013)

BOARD CERTIFICATION

Board Certified Specialist of the Japanese Society of Internal Medicine

Board Certified Specialist of the Japanese Society of Psychosomatic Medicine

Board Certified Specialist of the Japanese Society of Oriental Medicine

CAREER HISTORY

04/2005~03/2009: PhD course of Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

04/2003~03/2005: Internal Medical Doctor of Tubu Rousai Hospital, Aichi, Japan

04/2002~03/2003: Internal Medical Doctor of Kyushu University Hospital, Fukuoka, Japan

OTHER QUALIFICATIONS AND DISTINCTIONS

Medical Doctor License: since 04/1998

PUBLICATIONS

1. Hiramoto T, Yoshihara K, Asano Y, Sudo N. (2017) Protective Role of the Hepatic Vagus Nerve against Liver Metastasis in Mice. Neuroimmunomodulation. 24:341-7.

2. Sawamoto R, Nagano J, Kajiwara E, Sonoda J, Hiramoto T, Sudo N. (2016) Inhibition of emotional needs and emotional wellbeing predict disease progression of chronic hepatitis C patients: an 8-year prospective study. Biopsychosoc Med. 10:24.
3. Yoshihara K, Hiramoto T, Oka T, Kubo C, Sudo N. (2014) Effect of 12 weeks of yoga training on the somatization, psychological symptoms, and stress-related biomarkers of healthy women. Biopsychosoc Med. 8:1.
4. Nishino R, Mikami K, Takahashi H, Tomonaga S, Furuse M, Hiramoto T, Aiba Y, Koga Y, Sudo N. (2013) Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. Neurogastroenterol Motil. 25:521-8.
5. Zhao P, Hiramoto T, Asano Y, Kubo C, Sudo N. (2012) Chronic psychological stress exaggerates the compound 48/80-induced scratching behavior of mice. Pharmacology, Biochemistry and Behavior. 105:173–6
6. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, Koga Y, Sudo N. (2012) Role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. American Journal of Physiology Gastrointesitinal and Liver Physiology. 303:G1288-95.
7. Yoshihara K, Hiramoto T, Sudo N, Kubo C. (2011) Profile of mood states and stress-related biochemical indices in long-term yoga practitioners. Biopsychosocial medicine 5:6.
8. Hiramoto T, Oka T, Yoshihara K, Kubo C. (2009) Pyrogenic cytokines did not mediate a stress interview-induced hyperthermic response in a patient with psychogenic fever: a case report. Psychosomatic Medicine 71:932-936.
9. Hiramoto T, Chida Y, Sonoda J, Yoshihara K, Sudo N, Kubo C. (2008) The hepatic vagus nerve attenuates Fas-induced apoptosis in the mouse liver via α7 nicotinic acetylcholine receptor. Gastroenterology 134:2122-2131.
10. Chida Y, Sudo N, Sonoda J, Hiramoto T, Kubo C. (2007) Childhood psychological stress exacerbates adult mouse asthma by hypothalamus-pituitary-adrenal axis. American Journal of Respiratory Critical Care Medicine 175: 316-322.
11. Chida Y, Hiramoto T, Sudo N, Kubo C. (2007) The modulation of central nervous system on Fas-induced liver injury. Chapter 5 In Johansson LM (ed) Neuroimmunology Research Perspective. Hauppauge, NY: Nova Science Publishers, Inc., p131-147.

HONORS:

1. Best Presentation Award at the 26th Congress of Japanese Association of Stress Science (Fukuoka, Japan, November 5-6, 2010)

Invited Lecture 1

Title

Psychoneuroimmunology

Abstract

The brain reciprocally communicates with the peripheral organs to keep homeostasis. Psychoneuroimmunology includes disciplines, such as, the behavioral sciences, the neurosciences, the endocrinology, and the immunology. Stress information is gathered and processed in the brain and brain regulate the peripheral organ-functions through the complex networks of the endocrine, immune, and nervous system.

In this presentation, we intend to achieve a more complete understanding of the interactions among these systems serving homeostasis and influencing health and disease.

In this presentation, I would like to present the outline of psychoneuroimmunology with showing our experimental data and introducing other principal papers.

1. Neurosciences & Immunology:
   1-1) the role of the parasympathetic (vagus) nerve in regulating immunity
   1-2) the role of the sympathetic (vagus) nerve in regulating immunity
   1-3) the role of the autonomic nervous system in cancer regulating
2. Stress & Neurosciences (Behavioral sciences)
   2-1) what is stress
   2-2) psychological stress and symptoms
3. Stress & Psychoneuroimmunology (Respiratory Disease)
   3-1) Effects of comorbidities on physical activity in chronic obstructive pulmonary disease

Ivan Andreevich Stepanov
Neurosurgeon, PhD

Researcher, Neurosurgery Department of Irkutsk State Medical University, Irkutsk, Russia

✉: edmoilers@mail.ru, stepanovivanneuro@gmail.com;
☎: + 7-(964)-410-32-52.

Invited Lecture 2

Vadim A. Byvaltsev[1, 2, 3, 4, 5], Ivan A. Stepanov[1]

[1] Irkutsk State Medical University, Irkutsk, Russian Federation
[2] Railway Clinical Hospital on the station Irkutsk-Passazhirskiy of Russian Railways Ltd., Irkutsk, Russian Federation
[3] Irkutsk Scientific Center of Surgery and Traumatology, Irkutsk, Russian Federation
[4] Irkutsk State Academy of Postgraduate Education, Irkutsk, Russian Federation
[5] Institute of Nuclear Physics n.a. G.I. Budker of the SB RAS, Novosibirsk, Russian Federation

Diffusion-Weighted Magnetic Resonance Imaging of Brain Metastases

Introduction. Brain metastases are observed in up to 40% of all intracranial tumors. Some types of metastatic tumors cause difficulties in differential diagnosis, since they have similar signal characteristics with other pathological entities in neuroimaging. Obviously, the additional diagnostic methods to determine the prognosis and tactics of further management of this group of patients should be implemented.

The purpose. To study the role of diffusion-weighted magnetic resonance imaging (MRI) in differential diagnostics and predicting the survival rate in patients with brain metastases. Methods. The study included data from MRI and morphological studies of 23 patients with brain metastases. The obtained values of the apparent diffusion coefficient (ADC) of tumors were compared with their histological type, cell density, and the index of proliferative activity Ki-67. In addition, the influence of ADC values on the overall survival rate was assessed.

Results. A reliable inverse correlation of ADC values and the index of proliferative activity for various types of brain metastases (r=-0.74, p=0.014) was established. The dependence of ADC values and overall survival rate of patients with metastases in the brain is presented. The overall survival rate in patients with an ADC value greater than 947.2 mm2/sec was 9.8 months (95% CI: 8.6−11.3), and with ADC value less than 947.2 mm2/sec — 6.4 months (95% CI: 3.7−9.1).

Conclusions. The technique of diffusion-weighted MRI plays an important role in the differential diagnosis of brain metastases; it can be used as a tool of comprehensive preoperative assessment when planning the surgery and as a prognostic factor of overall survival rate for this group of patients.

Nansalmaa Nyamjav

Doctor of philosophy in linguistics (PhD), Professor

School of Sciences, Faculty of Humanities
Department of European Studies
NATIONAL UNIVERSITY OF MONGOLIA

☎: (976)-99842699
✉: nansalmaa@mail.ru, nansalmaanum@gmail.com

Academic Titles

Associate professor in 2004

Professor in 2012

Awards

- "Best Practitioner of Education" award in 2004
- "Best Practitioner of Science" award in 2010
- "Teacher of the Year" award in 2002, 2004, 2009 and 2013 at Faculty of Foreign Relations and Culture, NUM
- Second place in "Teacher of the Year" competition in 2009, 2013 at Faculty of Socio-Humanity, NUM
- "Teacher of the Year" in 2015 at Faculty of Socio-Humanity, NUM

Education

1983

Graduated from Ural Federal University, Russian Federation. Qualified to teach in Russian language, literature and linguistics.

1997

Ph.D in linguistics. Thesis: Semantical and Lexicographical Research of Metaphors on Model "Animal - Human" in Russian and Mongolian.

Employment

1983 – Present

Lecturer at NUM

Professional and Research Interest:

Modern theories of language: lexicology, lexicography, cognitive linguistics, neurolinguistics, sociolinguistics, psycholinguistics, intercultural communications

Publications and Academic Researches:

Author of 17 monographs and textbooks for university and college students. Wrote 40 research papers, 28 of which were published locally and 12 were published abroad. 50 presentations were discussed at international and local academic conferences.

Led 49 research works, 4 dissertations of doctorate students, 27 diploma thesis of graduate students, and 6 diploma thesis of undergraduate students. Currently leading 10 doctorate students' research works on linguistics and translation and 3 graduate students' diploma thesis researches.

Edited 14 monographs, textbooks, handbooks, dictionaries, translated books and wrote academic reviews on 12 dissertations and 49 graduate students' diploma thesis.

Invited Lecture 3

Title

Language Organization in The Human Brain

Abstract

Where is language located in the brain? What kind of structure or organization does the language have in the brain? These are truly intriguing questions we have. Finding the answers for said questions is likely to be critical for us to know who we really are. Researchers and scientists tried different hypotheses and conducted many observations and experiments until present to find those answers. Thanks to modern scientific achievements of studying human brain and its roles or functions, now we know more about how think and speak.

Today we are partaking in a rapidly progressing race for revealing the secrets of human existence and unlocking the mysteries of the extraordinary human ability that is speaking. New discoveries in cognitive studies make it possible for us to understand more of the complexity of functions of thinking and speaking. In this lecture, we will explore about language organization in the human brain in two main sections:

1. Neuropsychology and neurophysiology methods for studying the organization in the human brain
2. The modern studies of language organization in the human brain.

Bilegtsaikhan Tsolmon /MD, PhD/

Core Laboratory, Science and Technology Center,
Mongolian National University of Medical Sciences (MNUMS)

☎: (976)-9988-9925
✉: bilegtsaikhan@gmail.com

Current Position:

November 2016 - Present
Head of Professional Board for Bio-product and Reagents,
Ministry of Health

October 2014 - Present
Specialist of Core Laboratory
Mongolian National University of Medical Sciences (MNUMS), Science and Technology Center, Ulaanbaatar, Mongolia

October 2015 - Present
Board Member of Mongolian Neuroscience Society

Education:

April 2011 - March 2014
Doctor of Philosophy (Ph.D.)
Department of Microbiology and Immunology, School of Medicine, Aichi Medical University, Japan

October 2006 - June 2009
Master of Science (M.Sc.)
Department of Molecular Biology and Genetics, School of Biomedicine, Health Sciences University, Mongolia (HSUM)

September 1997 - May 2003
Degree of Medical Doctor (M.D.)
School of Medicine, HSUM

Research experience:

October 2013 - September 2014
Post-Doctoral Fellow
University of Oklahoma Health Sciences Center, Stephenson Cancer Center, Oklahoma, USA

December 2010 - April 2011
Foreign Visiting Researcher
Department of Microbiology and Immunology, School of Medicine, Aichi Medical University, Japan

June 2003 - December 2010

Scientific Researcher

Central Scientific Research Laboratory, Institute of Medical Sciences, Mongolia (IMSM)

Research interests:

Signal Transduction, Neuroimmunology, Molecular Biology and Molecular Genetics

Publications and presentations:

Peer reviewed full articles-27 (17+13) (Cumulative IF - 54.18)
Presentations-40

Lecture 1

IBRO-School Associate, School on Basic Techniques in Neuroscience
The 1st Ulaanbaatar School
Sep 17-20, 2018

Cell Signaling in the Brain

Tsolmongyn Bilegtsaikhan, MD, PhD
Core Laboratory, STC, MNUMS

Why is it Worthwhile Study Cell Signaling ?

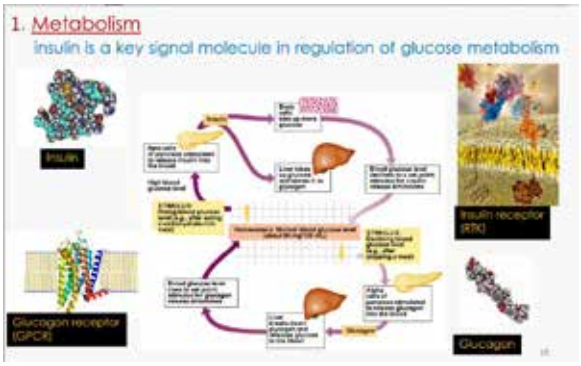
Most pharmaceuticals (and many environmentally hazardous substances) act on signaling pathways

Understanding cell communication can (and have) in many cases explain the basis for diseases and thus allowed the development of new therapeutic strategies

Many of the breakthrough discoveries in medical sciences has come from studies of signaling molecules

Outline

What type of processes are regulated by cell-cell signaling



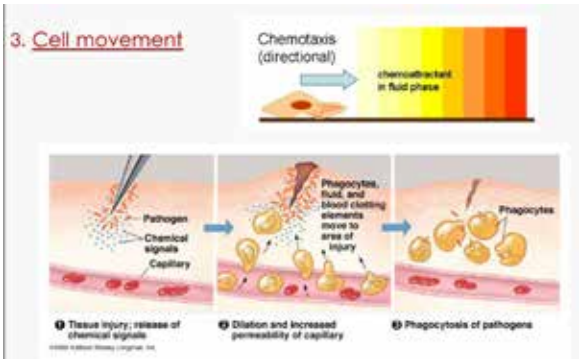
Ref: Molecular Cell Biology (2014)

Outline

What type of processes are regulated by cell-cell signaling

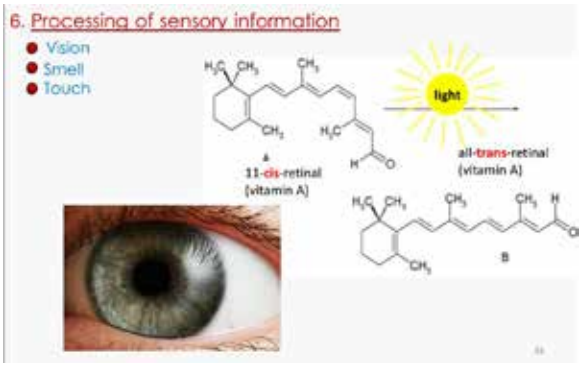
Ref: Molecular Cell Biology (2014)

What type of processes are regulated by cell-cell signaling



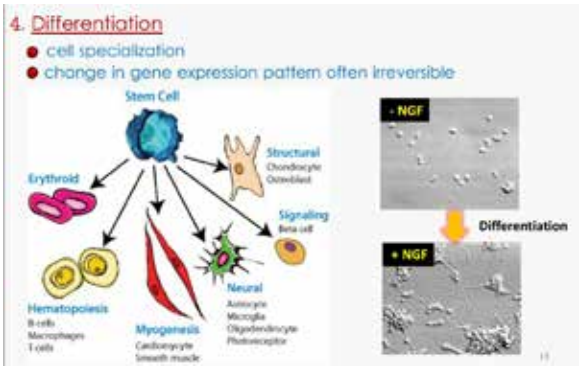
Ref: Molecular Cell Biology (2014)

What type of processes are regulated by cell-cell signaling



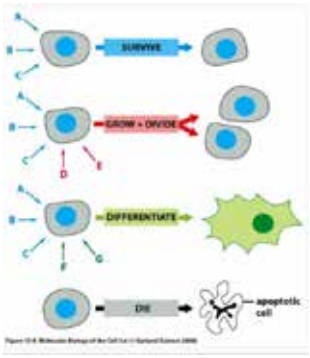
Ref: Molecular Cell Biology (2014)

What type of processes are regulated by cell-cell signaling



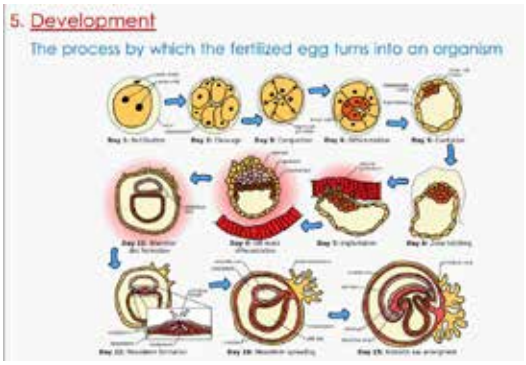
Ref: Molecular Cell Biology (2014)

Cell Behavior is Controlled Signal Molecules in a Combinatorial Manner



Ref: Molecular Cell Biology (2014)

What type of processes are regulated by cell-cell signaling



Ref: Molecular Cell Biology (2014)

Cell - Cell Communication Can Occur over Small or Large Distances

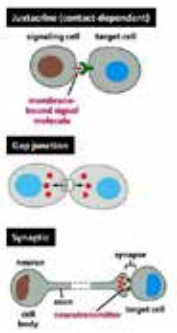
Endocrine signaling:
Blood stream distributes hormone throughout the body
Place of action determined by receptor expression of target cells

Paracrine signaling:
Signal molecule diffuse to close neighbours (mm-mm range)

Autocrine signaling:
Signal molecule acts back on the secreting cell
Self-reinforcement of proliferation or differentiation

Ref: Molecular Cell Biology (2014)

Cell - Cell Communication Can Occur over Small or Large Distances



Juxtacrine signaling:
Signal molecule located on surface of signaling cells, or on extracellular matrix
No diffusion of signal molecule

Gap junctions:
Open channels through the plasma membrane
Signaling molecule passes directly between cytoplasms of two adjacent cells

Synaptic signaling:
Extremely precise delivery of signal molecule
Short diffusion distance (20 nm), resulting in very high speed of signaling

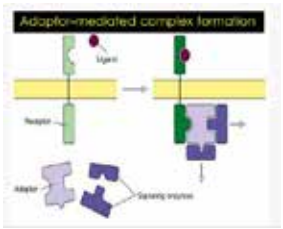
Ref: Molecular Cell Biology (2014)

Signal Transduction Proteins: Adaptors

Lack intrinsic enzymatic activity

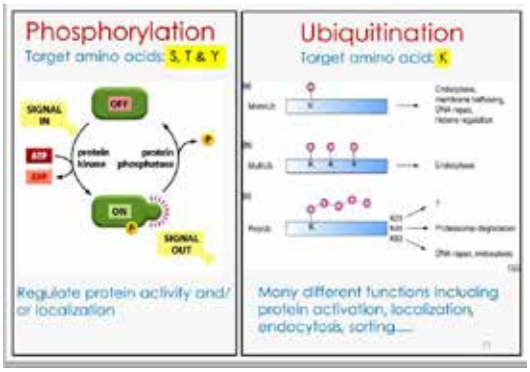
Contain two or more interaction domains

Mediate specific protein-protein interactions and thereby drive formation of protein complexes



Ref: Molecular Cell Biology (2014)

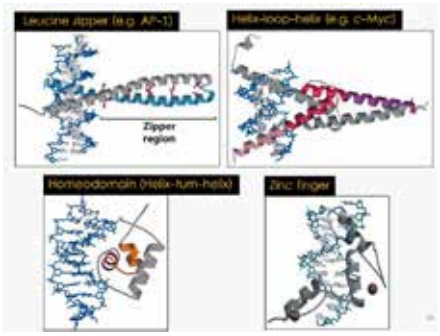
Post-translational Modifications



Ref: Molecular Cell Biology (2014)

Signal Transduction Proteins: Transcription Factors

Common DNA Binding Motifs



Ref: Molecular Cell Biology (2014)

Signal Transduction Proteins: Enzymes

Kinases:
add phosphate groups

Phosphatases:
remove phosphate groups

Phospholipases:
hydrolyze membrane phospholipids

Ubiquitin-ligase:
add ubiquitin moities to target proteins

Acetyltransferases:
add acetyl-groups

Proteases:
hydrolyze peptide bonds

GTPases:
hydrolize GTP to GDP

Ref: Molecular Cell Biology (2014)

Cell-Cell Communication at the Synapse

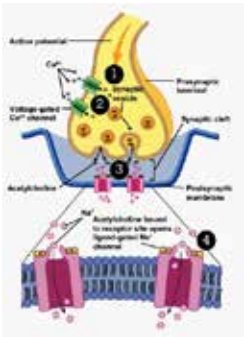
Action potentials cause voltage gated Ca2+ channels to open.

Ca2+ diffuse into the cell and cause synaptic vesicles to release acetylcholine.

Acetylcholine diffuses across the synaptic cleft.

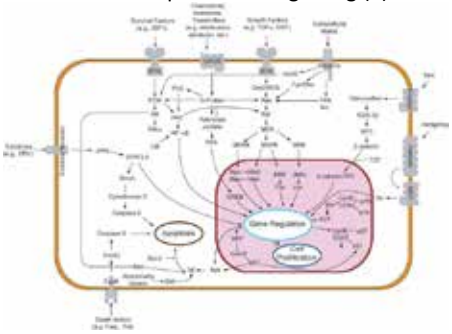
Acetylcholine binds their receptor sites and cause Na+ channels to open, causing depolarization.

If depolarization reaches threshold, an action potential is produced in the postsynaptic cell.



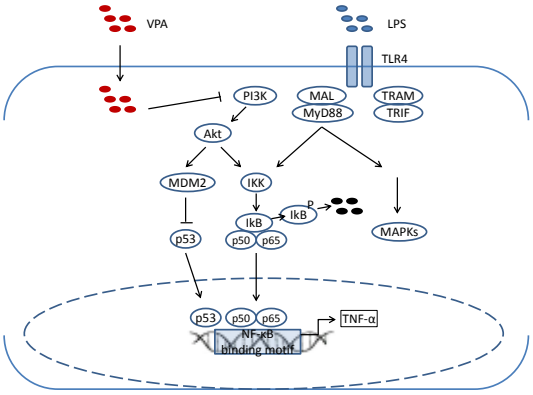
Ref: Molecular Cell Biology (2014)

Basic Concepts on Cell Signaling (1)



Cellular Process/ Signal Molecules/Cell Communication/Post-translational Modification/Enzyme-Proteins/Adaptor-Proteins/Transcription Factors/DNA Motifs/Gene Expression/ Protein Expression

Inflammation immunology (Gram neg. bacteria) (2-1)



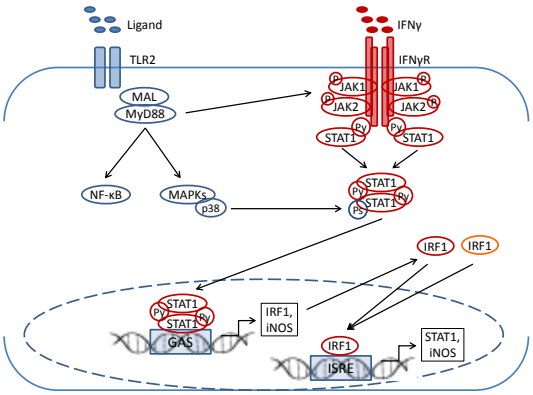
Outline

Inflammation immunology (Gram neg. bacteria) (2-1)

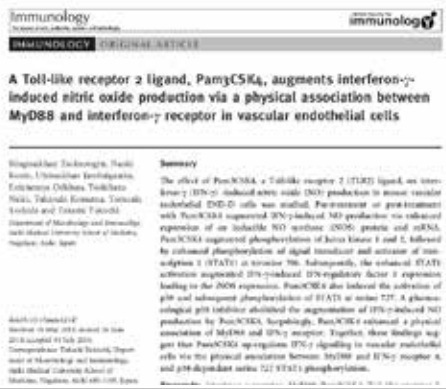
Our capacity on Cell Signaling (2)

Inflammation Immunology	Anti-Inflammatory Reactions
Cancer Immunology	Anti-Cancer Activity
Cancer Biology	Anti Cancer Metastasis Approach

Inflammation immunology (Gram pos. bacteria/Fungus) (2-2)



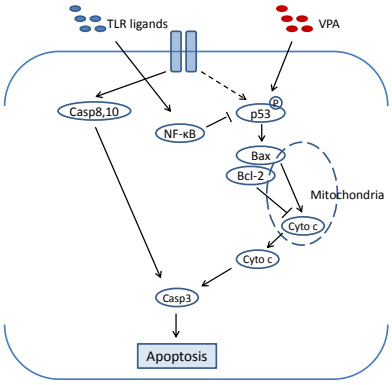
Inflammation immunology (Gram pos. bacteria/Fungus) (2-2)


Immunology

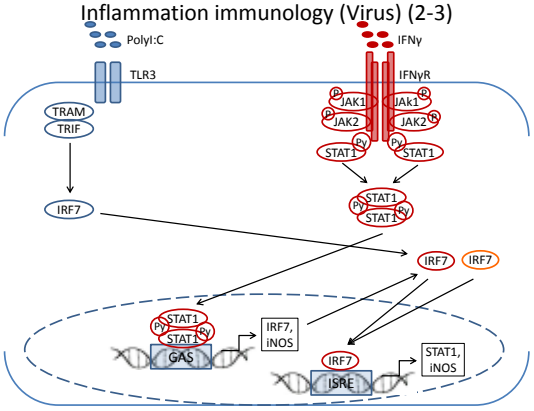
IMMUNOLOGY ORIGINAL ARTICLE

A Toll-like receptor 2 ligand, Pam3CSK4, augments interferon-γ-induced nitric oxide production via a physical association between MyD88 and interferon-γ receptor in vascular endothelial cells

Cancer immunology (2-4)



Inflammation immunology (Virus) (2-3)



Cancer immunology (2-4)


Cellular Immunology

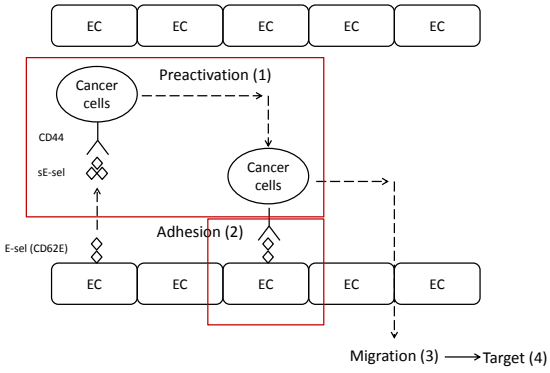
Lipopolysaccharide prevents valproic acid-induced apoptosis via activation of nuclear factor-κB and inhibition of p53 activation

Inflammation immunology (Virus) (2-3)


Microvascular Research

Poly I:C enhances production of nitric oxide in response to interferon-γ via upregulation of interferon regulatory factor 7 in vascular endothelial cells

Cancer biology (2-5)



Cancer biology (2-5)

Soluble E-selectin

ESTA nanoparticle

Basic Techniques on Cell Signaling (3)

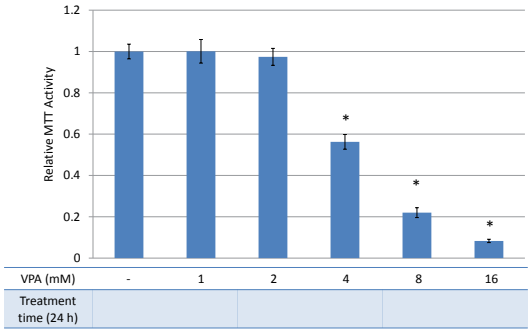
Inflammation Immunology
(Gram neg. bacteria)

1. Cell Culture
2. MTT Assay
3. TUNEL Assay
4. Immunoblotting (IB)
5. Immunoprecipitatiion (IP)
6. Laser flow cytometric analysis (FACS)
7. Determination of TNF-α, IL-6
8. TransAM Assay
9. Luciferase reporter gene assay for NF-κB activation
10. Transfection of small interfering RNA (siRNA)

Our capacity on Cell Signaling (2)

Inflammation Immunology	Anti-Inflammatory Reactions
Cancer Immunology	Anti-Cancer Activity
Cancer Biology	Anti Cancer Metastasis Approach

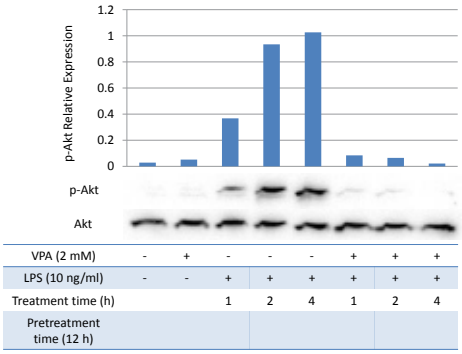
Cytotoxic action of VPA on RAW 264.7 cells (MTT Assay)



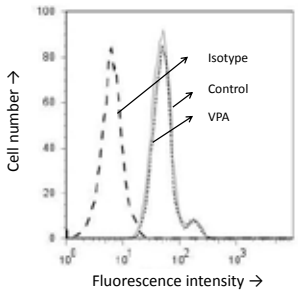
Outline

Basic Concepts on Cell Signaling	(1)
Our Capacity on Cell Signaling	(2)
Basic Techniques on Cell Signaling	**(3)**
Cell Signaling and Neuroscience	(4)

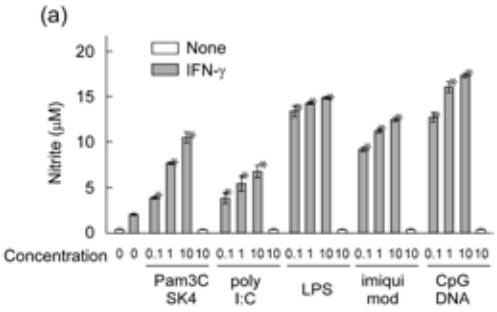
Effect of VPA pretreatment on LPS induced Akt activation (IB)



Effect of VPA treatment on cell surface expression of TLR4 (FACS)

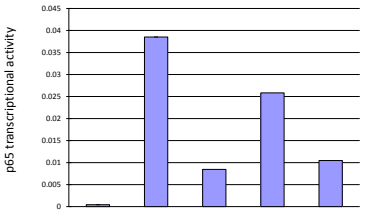


The effect of TLR ligands pretreatment on IFN-γ-induced NO production (Determination of nitrite concentration)



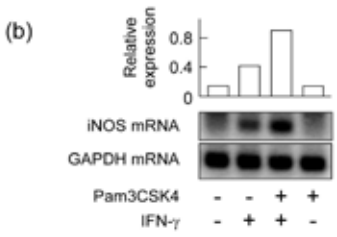
All TLR ligands up-regulate IFN-γ-induced NO production in a roughly concentration dependent manner

Effect of p53 siRNA on LPS induced NF-κB p65 transcriptional activation in pretreatment with or without VPA (TransAM Assay & Transfection of siRNA)



siRNA				p53	Cont
VPA (2 mM)	-	-	+	+	+
LPS (10 ng/ml)	-	+	+	+	+
Trans/Pre/ Treatment time (24h/12h/2h)					

The effect of Pam3CSK4 pretreatment on IFN-γ-induced iNOS mRNA expression (RT-PCR)



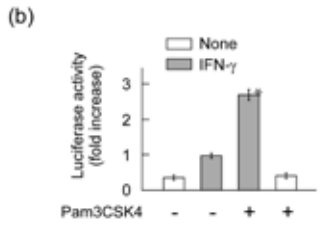
Pam3CSK4 enhances IFN-γ-induced iNOS mRNA expression

Basic Techniques on Cell Signaling (3)

Inflammation Immunology
(Gram pos. bacteria/Fungus)

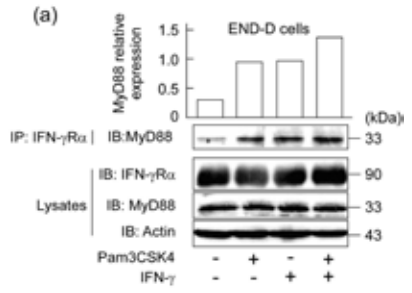
1. Cell Culture
2. Cell viability assay
3. Immunoblotting (IB)
4. Immunoprecipitation (IP)
5. Determination of nitrite concentration
6. Luciferase reporter gene assay
7. Semi-quantitative reverse transcription (RT)-polymerase chain reaction (PCR)
8. Laser flow cytometric analysis (FACS)

The effect of Pam3CSK4 pretreatment on IFN-γ-induced iNOS transcription activity (Luciferase reporter gene assay)



Pam3CSK4 elevates the luciferase activity in response to IFN-γ

The effect of Pam3CSK4 on the interaction between MyD88 and IFN-γRα (IP)



The combined treatment with Pam3CSK4 and IFN-γ augments the physical association between MyD88 and IFN-γRα

Basic Techniques on Cell Signaling (3)

Cancer Biology

1. Animal experiments
2. Blood Cell Counts
3. Blood Chemistry
4. Cytokines Analysis
5. Histological Analysis

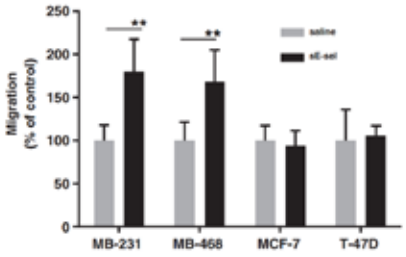
6. Cell Culture
7. In vitro Cell Adhesion Assay
8. In vitro Permeability Assay
9. Cell Migration Assay
10. Anoikis Assay
11. Animal Models
12. Flow Cytometry (FACS)

Basic Techniques on Cell Signaling (3)

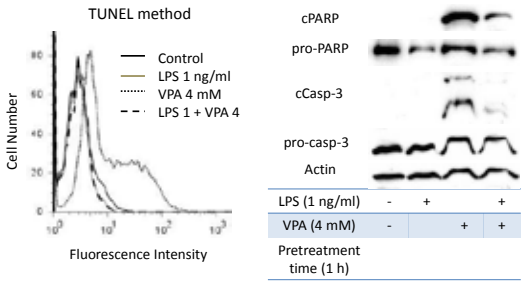
Cancer immunology

1. Cell Culture
2. MTT Assay
3. TUNEL Assay
4. Immunoblotting (IB)

Effect of soluble E-selectin on Trans Endothelial Migration (TEM) of breast cancer cells (Cell Migration Assay)

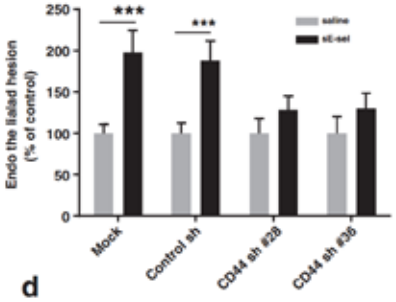


The effect of LPS pretreatment on VPA induced apoptosis (TUNEL Assay & IB)

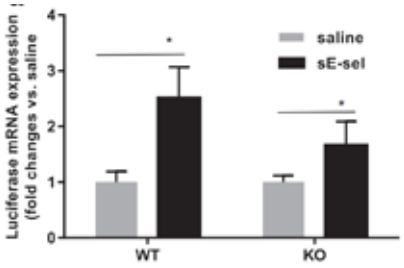


LPS inhibits VPA induced apoptosis

Effect of soluble E-selectin on adhesion of MDA-MB-231 cells to endothelial cells (In vitro Cell Adhesion Assay)



Effect of soluble E-selectin on in vivo lung metastasis using luciferase-expressing 4T1 murine breast cancer cells
(Animal Models)



Possibility or Capacity of the Core Lab ?

ELISA

RT-PCR

IB/IP

FACS

Core Laboratory

- HR - 8
  - Head - 1
  - Specialist - 5
  - Consultant - 2
- Location, structure
  - West campus of MNUMS, Core Lab (400 м^2)
  - 6 laboratories
- Source of funding (2015)
  - MECS (5.3 billion MNT)
  - MNUMS (2 billion MNT)

  (Research & Training Center, Immunology lab)

Opening Ceremony,
January 28, 2015

Outline

Possibility or Capacity of the Core Lab ?

Cellular reaction	Properties	Methods	Available at Core Lab
Final production	Cytokines (NO, TNF-α, IL-6)	ELISA	Yes
Transcription level	mRNA expression (iNOS mRNA)	RT-PCR	Yes
Protein level	Protein expression (p53, NF-κB)	IB, Immunobloting	Yes
Protein interaction	Physical interaction (MyD88, NF-κB)	IP, Immunoprecipitation	Yes
Expression of extracellular receptors	Receptor expression (TLR, IFNγRα)	FACS	Yes

Cell Signaling and Neuroscience (4)

Alzheimer's Disease

Parkinson's Disease

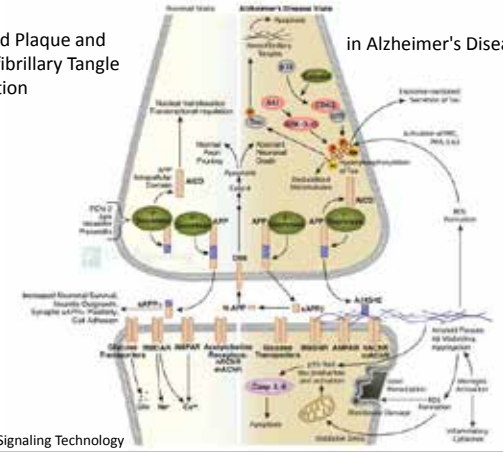
Presynaptic Signaling

Signaling in Neural Crest

Signaling in Immature Neurons

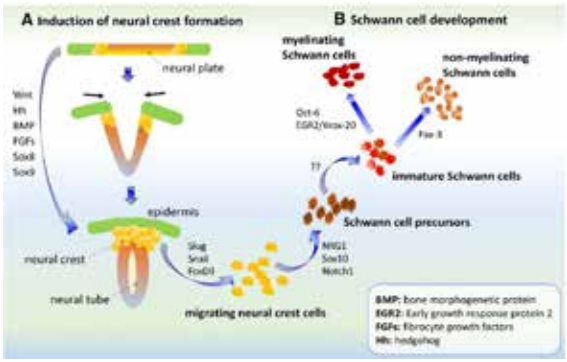
Neurohereditary Disease (CMT)

Amyloid Plaque and Neurofibrillary Tangle Formation in Alzheimer's Disease



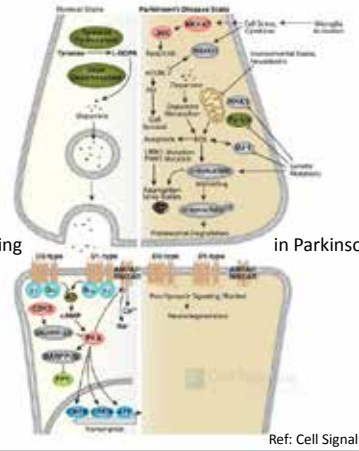
Ref: Cell Signaling Technology

Neural Crest and Schwann Cells (4)



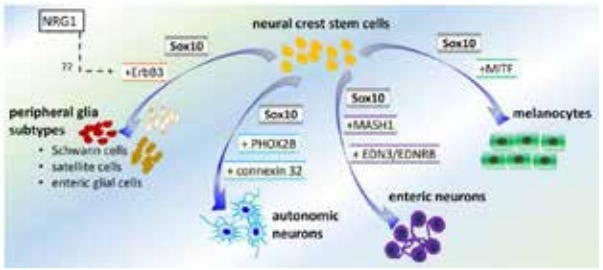
Ref: M.J.Kipanyula et al./Cellular Signailing 26 (2014) 673-682

Dopamine Signaling in Parkinson's Disease



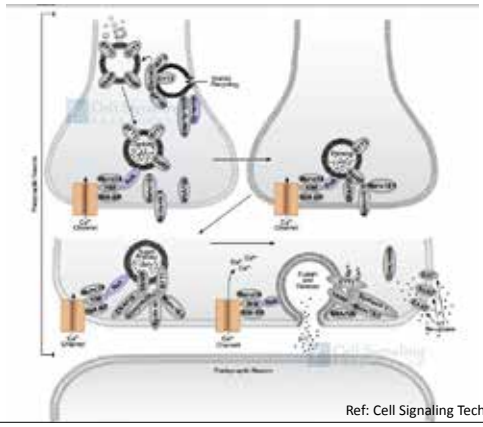
Ref: Cell Signaling Technology

Neural Crest Stem Cells and Sox10



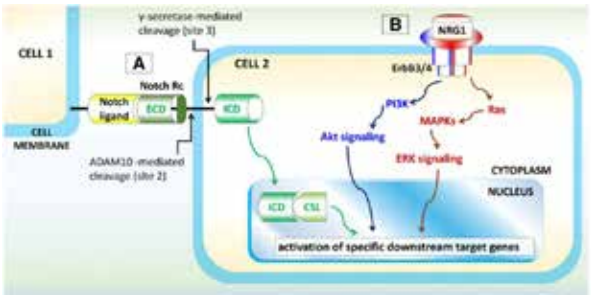
Ref: M.J.Kipanyula et al./Cellular Signailing 26 (2014) 673-682

Vesicle Trafficking Presynaptic Signaling



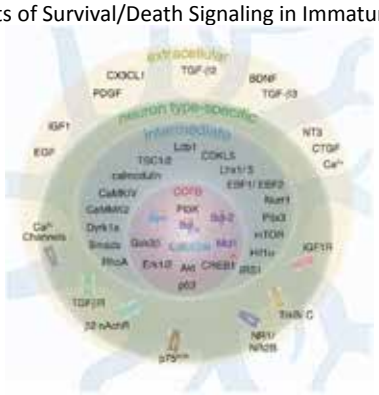
Ref: Cell Signaling Technology

Notch and Neuregulin1 (NRG1) Signaling Pathways



Ref: M.J.Kipanyula et al./Cellular Signailing 26 (2014) 673-682

Components of Survival/Death Signaling in Immature Neurons



Ref: U.Pfisterer et al./Cell Death and Disease (2017) 8, e2643

Cell Signaling under Hypoxic (purple arrows) and Hyperoxic (blue arrows) Conditions in Immature Neurons

Ref: U.Pfisterer et al./Cell Death and Disease (2017) 8, e2643

Examples of Neuron Type-Specific Pro-Survival Genes

Factors[a]	Embryonic						Adult			References
	CX EX	CB GC	MB DA	CB PC	ST MS	CX IN	OB PG	OB GC	DG GC	
BDNF/TrkB[b]	-	+	+	+	+	+	+[c]	+	+	[d]
NGF/TrkA[b]	-	-	-	-	+	-	+	+	+	[d]
NT-3/TrkC	-	+	-	+	+	+	+	+	-	[d]
IGF1/ IGF1R	+[c]	+	+	+	?	?	-	-	+	[d]
β2 nAChR	-	?	?	?	-	-	+	+	+	[d]
TGFβ/TGFβR	+	?	+	?	?	?	+	-	+	[d]
CTGF	-	-	-	-	-	-	+	+	+	[d]
p75[NTR][b]	-	-	-	-	?	-	+	+	?	[d]
Akt1[b]	-	-	-	-	-	-	+	+	?	[d]
NR2B[b]	-	-	-	-	-	-	+	+	?	[d]

Ref: U.Pfisterer et al./Cell Death and Disease (2017) 8, e2643

Introduction: CMT Classification

CMT

Demyelinating (mNCV < 38m/s)

Axonal (mNCV > 38m/s)

CMT1

CMT2

AD CMT1 → 4

AR CMT1 → 11

AD CMT2 → 14

AR CMT2 → 4

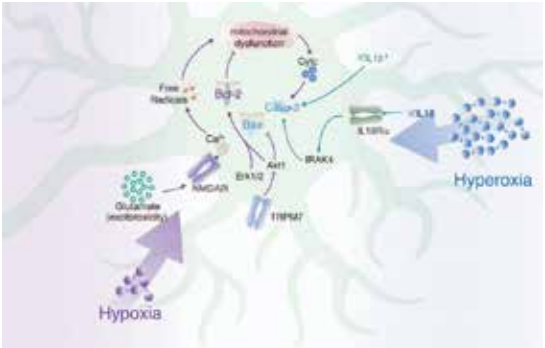
Clinical sign

Electrophysiological sign

Hereditary type

Susceptibility gene & loci

Cell Signaling under Hypoxic (purple arrows) and Hyperoxic (blue arrows) Conditions in Immature Neurons



Ref: U.Pfisterer et al./Cell Death and Disease (2017) 8, e2643

Introduction: CMT Classification

CMT1

AD CMT1 → 4 → AD CMT1A (Dornod) → PMP22 gene

AR CMT1 → 11

CMT2

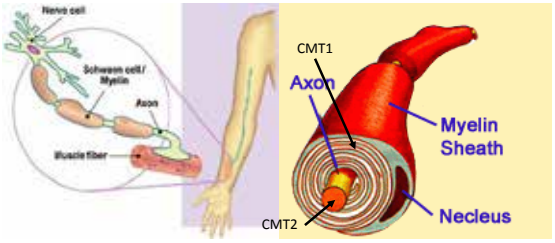
AD CMT2 → 14 → AD CMT2D (Arkhangai) → GARS gene

AR CMT2 → 4

Hereditary type

Susceptibility gene & loci

Introduction: Peripheral Nerve



Compact myelin sheath which is surrounded axon created by Schwann cell differentiation. It is called myelination process.

Outline

Autosomal Dominant Charcot Marie Tooth Disease (AD CMT)

Genetic Subtype	Locus	Gene	Protein	Protein function
CMT1A	17p11.2	*PMP22*	Peripheral myelin protein 22	Its structure protein of Myelin Sheets due to involve myelination
CMT2D	17p14	*GARS*	Glycyl tRNA synthetase	Regulate on tRNA binds its amino acids

Thank you for your attention

Tsevelmaa Nanjidsuren /PhD/

Core Laboratory, Science and Technology Center,
Mongolian National University of Medical Sciences
Ulaanbaatar, Mongolia

☎: (976)-88310330
✉: tsevelmaa.n@mnums.edu.mn

Current Position:

Scientist at Core Laboratory, Science and Technology Center, Mongolian National University of Medical Science. .

Education:

2011-2014
Ph.D., Animal Biotechnology, Hankyong National University, Republic of Korea

2009-2011
M.Sc., Cell Biochemistry, Hankyong National University, Republic of Korea

2004-2008
B.Sc., Biotechnology and Biochemistry, National University of Mongolia, Mongolia

Research Experience:

2017-Present
Scientist, Core Laboratory of Mongolian National University of Medical Science, Mongolia

2014-2017
Scientist and Innovation Project Manager, Laboratory of Bio-organic Chemistry and Pharmacognosy, National University of Mongolia

2009-2014
Graduate Research Assistant (M.Sc and Ph.D candidate), Laboratory of Cell Biochemistry and Molecular Biology, Hankyong National University, Republic of Korea

2004-2008
Research Student, Laboratory of Plant Biotechnology, National University of Mongolia

Research interest:

Genetic engineering, animal reproduction, research of biological active compounds

International Publications:

Tsevelmaa Nanjidsuren, Purevjargal Naidansuren, Chae-Won Park, Jong-Ju Park, Seong-Jo Yun, BoWoong Sim, Myung-Hwa Kang,Sang-Rae Lee, Kyu-Tae Chang, Kwan-Sik Min “Expression and localization of the 20α-hydroxysteroid dehydrogenase (HSD) enzyme in the reproductive tissues of the cynomolgus monkey Macaca fascicularis” The Journal of Steroid Biochemistry and Molecular Biology 127, 337-344, 2011.

Purevjargal Naidansuren, Cha-Won Park, Sang-Hwan Kim, Tsevelmaa Nanjidsuren, Jong-Ju Park,Seong-Jo Yun, Bo-Woong Sim, Seongsoo Hwang, Myung-Hwa Kang, Buom-Yong Ryu Sue-Yun Hwang1, Jong-Taek Yoon, Keitaro Yamanouchi and Kwan-Sik Min "Molecular characterization of bovine placental and ovarian 20a-hydroxysteroid dehydrogenase" Reproduction 142 723-731, 2011.

Kyeong-Seok Seo, Purevjargal Naidansuren, Sang-Hwan Kim, Seong-Jo Yun, Jong-Ju Park, Bo-Woong Sim, Cha-Won Park, Tsevelmaa Nanjidsuren, Myung-Hwa Kang, Heewon Seo, Hakhyun Ka, NamHyung Kim, Sue-Yun Hwang, Jong-Taek Yoon, Keitaro Yamanouchi and Kwan-Sik Min "Expression of aldo-keto reductase family 1 memberC1 (AKR1C1) gene in porcine ovary and uterine endometrium during the estrous cycle and pregnancy " Reproductive Biology and Endocrinology 9:139, 2011.

Seong-Jo Yun, Purevjargal Naidansuren, Bo-Woong Sim, Jong-Ju Park, Cha-Won Park, Tsevelmaa Nanjidsuren, Myung-Hwa Kang, Sue-Yun Hwang, Jong-Taek Yoon and Kwan-Sik Min "Aberrant phenotypes of transgenic mice expressing dimeric human erythropoietin" Reproductive Biology and Endocrinology 10:6, 2012.

P.J. Naidansuren, C.W. Park, T.M. Nanjidsuren, J.J. Park, S.J. Yun, M.H. Kang, K. Yamanouchi, K.S. Min "Ovarian and placental expression of 20a-hydroxysteroid dehydrogenase during pregnancy in deer" Animal Reproduction Science 130, 63–73, 2012.

Tsevelmaa Nanjidsuren and Kwan-Sik Min "The transcription factor Ap-1 regulates monkey 20α-hydroxysteroid dehydrogenase promoter activity in CHO cells" BMC Biotechnology 14:71, 2014.

T. Nanjidsuren, S.J. Yun, C.W. Park, M.S. Kim, M.H. Kang, K.S. Min "Expression and localization of 20a-hydroxysteroid dehydrogenase (20a-HSD) in porcine reproductive tissues during pregnancy" Animal Reproduction Science 148, 63–71, 2014.

Tsevelmaa Nanjidsuren, Chae-Won Park, Bo-Woong Sim, Myung-Hwa Kang, Kyu-Tae Chang and Kwan-Sik Min "Distribution of G-protein-couple receptor kinase (GRK) 5 in the mice tissues" Academia Journal of Biotechnology 4(1): 037-044, 2016.

Tsevelmaa Nanjidsuren, Chae-Won Park, Bo-Woong Sim, Sun-Uk Kim, Kyu- Tae Chang, Myung-Hwa Kang & Kwan-Sik Min "GRK5-Knockout Mice Generated by TALEN- Mediated Gene Targeting" Animal Biotechnology, 27:4, 223-230. 2016.

Tsevelmaa. N, Narangerel.B, Dariimaa.D, Odgerel.O, Batkhuu.J "Anti Brucella activity of Caryopteris mongolica Bunge root extract against Brucella melitensis infection mice". BMC Complementary and Alternative Medicine, 18:144, 2018.

Lecture 2

Molecular Cloning And Applications Of CRISPR-Cas Systems In Neuroscience

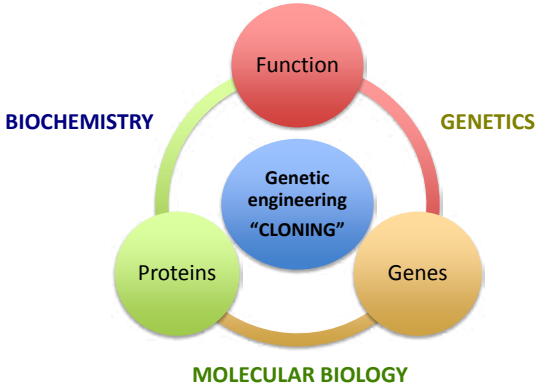
N.Tsevelmaa (Scientist, Ph.D)
Core laboratory of Mongolian National University of Medical Science

IBRO-APRC ASSOCIATE SCHOOL
2018.09.17
ULAANBAATAR, MONGOLIA

Molecular cloning

Recombinant technology

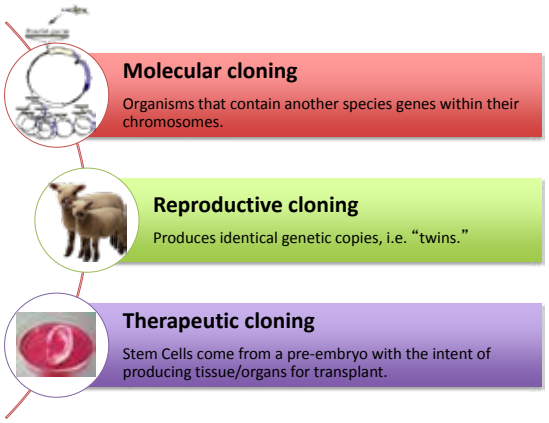
Applications



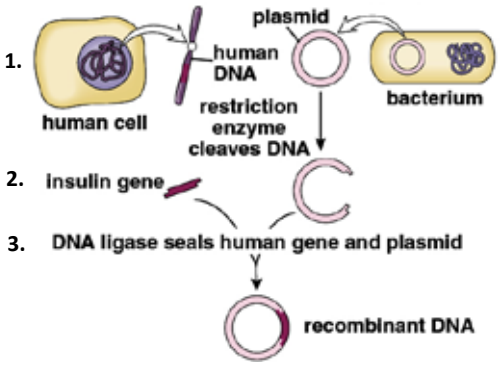
Recombinant DNA: (rDNA) is a form of artificial DNA that is created by combining two or more sequences that would not normally occur together through the process of gene splicing.

Recombinant DNA technology: is a technology which allows DNA to be produced via artificial means. The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.

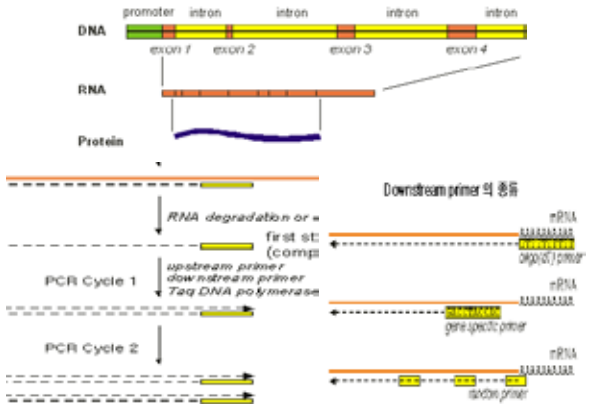
Isolating (vector and target gene)
↓
Cutting (restriction enzyme)
↓
Joining (Ligase)
↓
Transforming (Recipient cell)
↓
Propagation (bacteria, animal cell, plant and animal)
↓
Selecting (Screening methods)

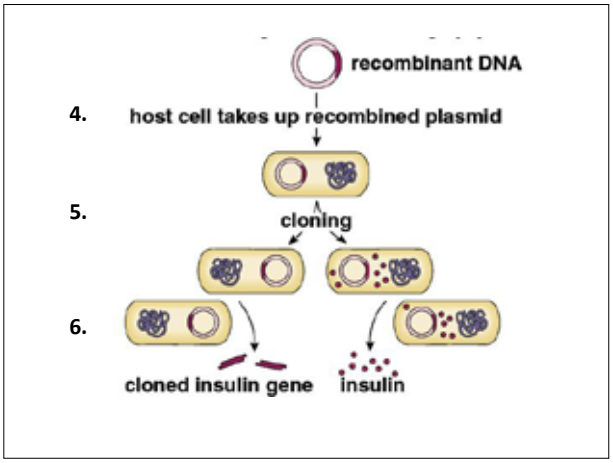


Recombinant technology steps



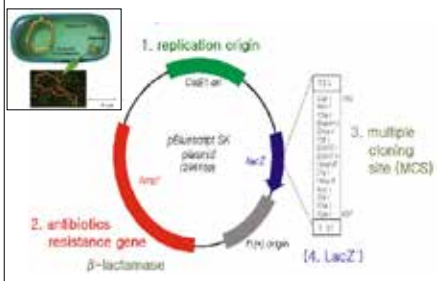
1. Isolating





Vector system

Vectors are circular, double-stranded DNA (dsDNA) molecules that are separate from a cell's chromosomal DNA.



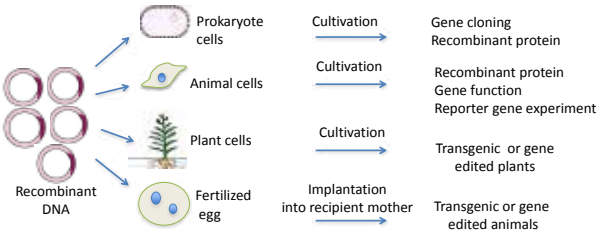
By Dedication
- ✧ Cloning
- ✧ Expression
- ✧ Reporter
- ✧ Gene targeting

By Insert DNA size

Yac > Bac > Bacteriophage > Cosmid > Plasmid

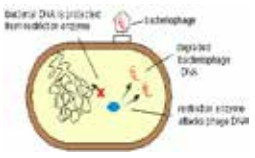
up to 3000kb 350kb 53kb 28-45kb 15kb

4.5.Transforming and cloning



Recipient Cell	Method	Term
Prokaryote	Chemical	Transformation
	Physical	Electroporation
Animal cell	Chemical	Transfection
Plant cell	Biological	Transfection
Fertilized egg	Chemical	Linker Based Sperm-Mediated Gene Transfer
	Physical	Microinjection
	Virus	Retrovirus-Mediated gene transfer

2.3. Cutting and ligase



- ✧ **Hinf III : *Hemophilus influenza* type f, (III) enzyme**
- ✧ **Specific site**
- ✧ **Palindrome**
- ✧ **3 type end**

5'-overhang cohesive (sticky) end - ex) EcoR I

5'--GAATTC--3' 5'--G AATTC--3'

3'--CTTAAG--5' 3'--CTTAA G—5

3'-overhang cohesive (sticky) end - ex) Sac I

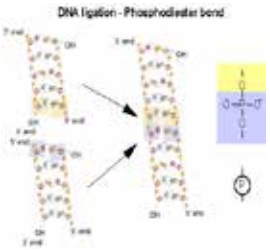
5'--GAGCTC--3' 5'--GAGCT C--3'

3'--CTCGAG--5' 3'--C TCGAG--5'

blunt end - ex) Sma I

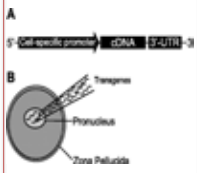
5'--CCCGGG--3' 5'--CCC GGG--3'

3'--GGGCCC--5' 3'--GGG CCC--5'



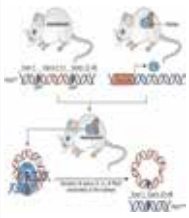
Vectors used to producing of Transgene and Knock-out organism

Transgene vector
- Bac transgenes
- Promoter transgenes



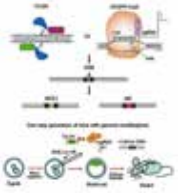
- Position dependent or independent expression

Conditional vector
- Cre-loxP
- Flp-FRT



- Gene deletion
- Gene inversion

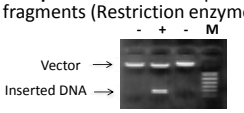
Gene targeting vectors
- HR
- ZFN
- TALEN
- CRISPR-Cas9



- -Gene deletion
  -Point mutation
  -Insertion

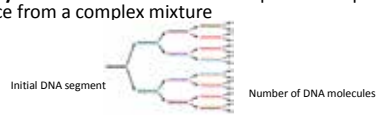
6.Selecting

✧ **Gel Electrophoresis** allows separation of vector DNA from cloned fragments (Restriction enzyme cleavage)



✧ Cloned DNA molecules are **Sequenced** rapidly by the Dideoxy Chain-Termination Method

✧ The **Polymerase Chain Reaction** amplifies a specific DNA sequence from a complex mixture

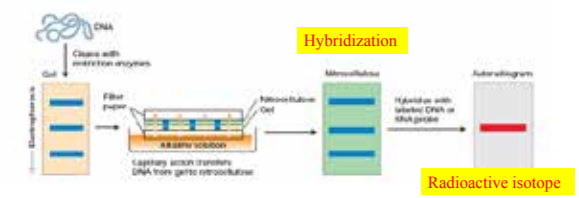


SOME PROTEIN PRODUCTS OF RECOMBINANT DNA TECHNOLOGY

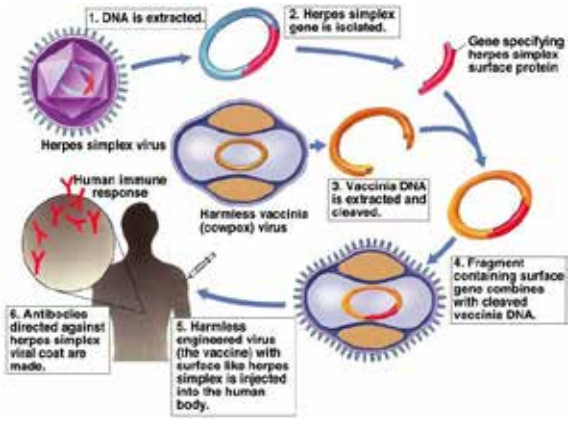
Product	Made In	Use
Human insulin	*E. coli*	Treatment for diabetes
Human growth hormone (GH)	*E. coli*	Treatment for growth defects
Epidermal growth factor (EGF)	*E. coli*	Treatment for burns, ulcers
Interleukin-2 (IL-2)	*E. coli*	Possible treatment for cancer
Bovine growth hormone (BGH)	*E. coli*	Improving weight gain in cattle
Cellulase	*E. coli*	Breaking down cellulose for animal feeds
Taxol	*E. coli*	Treatment for ovarian cancer
Interferons (alpha and gamma)	*S. cerevisiae*; *E. coli*	Possible treatment for cancer and viral infections
Hepatitis B vaccine	*S. cerevisiae*	Prevention of viral hepatitis
Erythropoietin (EPO)	Mammalian cells	Treatment for anemia
Factor VIII	Mammalian cells	Treatment for hemophilia
Tissue plasminogen activator (TPA)	Mammalian cells	Treatment for heart attacks

✧ **Blotting Techniques** Permit Detection of Specific DNA Fragments and mRNAs with DNA Probes

Term	Detection molecule	Probe type
Southern blot	DNA	DNA
Northern blot	RNA	cDNA or RNA
Western blot	Protein	Antibody

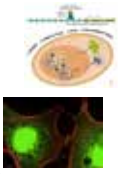


Subunit Herpes Vaccine



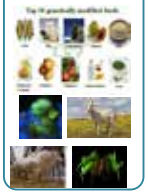
Molecular cloning: Applications

Analysis gene structure and expression
- Gene cloning
- Promoter analysis
- Lineage tracing
- etc



Pharmaceutical products
- Drug
- Vaccines

Genetical modified organism
- Transgenic and KO plant
- Transgenic and KO animal



Application in medicine
- Human gene therapy
- Diagnosis of genetic disorders
- Forensic evidence

Genetically modified organisms (GMO)

Plants with genetically desirable traits

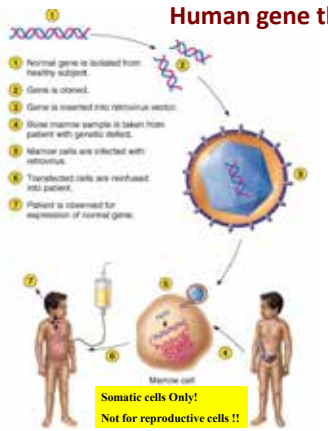
- **herbicide or pesticide resistant corn & soybean**
  - Decreases chemical insecticide use
  - Increases production
- **"Golden rice" with beta-carotene**
  - Required to make vitamin A, which in deficiency causes blindness

- **Disease resistance**
  - There are many viruses, fungi, bacteria that cause plant diseases
  - "Super-shrimp"
- **Cold tolerance**
  - Antifreeze gene from cold water fish introduced to tobacco and potato plants
- **Drought tolerance & Salinity tolerance**
  - As populations expand, potential to grow crops in otherwise inhospitable environments

Genetically Modified Crops

Altered Plant	Effect
Rice with beta carotene and extra iron	Added nutritional value
Canola with high-laurate oil	Can be grown domestically; less costly than importing palm and coconut oils
Delayed ripening tomato	Extended shelf life
Herbicide-resistant cotton	Herbicide kills weeds without harming crop
Minipeppers	Improved flavor, fewer seeds
Bananas resistant to fungal infection	Extended shelf life
Delayed-ripening bananas and pineapples	Extended shelf life
Elongated sweet pepper	Improved flavor, easier to slice
Altered cotton fiber	"Plasticized" fabric
Altered paper pulp trees	Paper component (lignin) easier to process
High-starch potatoes	Absorb less oil when fried
Pest-resistant corn	Can resist European corn borer
Seedless minimelons	Single serving size
Sweet peas and peppers	Retain sweetness longer
Sugarcane with corn gene	Resists bacterial and fungal toxins

Human gene therapy



- *ADA- SCID* (severe combined immunodeficiency due to adenosine deaminase [ADA] deficiency),
- Neuroblastoma
- Cystic fibrosis
- Treat cancer through expression of the E1A and p53 tumor suppressor genes.

Genetically modified organisms (GMO)

Farm animals and "Pharm" animals

- **Smart mouse**
  - Biological model engineered to overexpress NR2Breceptor in the synaptic pathway.
  - This makes the mice learn faster like juveniles throughout their lives
- **Transgenic goats**
  - Goats that could express tissue plasminogen activator, anti thrombin III, spider silk etc in milk

Medical importance	Agricultural importance	Industrial importance
• Disease model • Bioreactors for pharmaceutical • Xenotransplantation	• Disease resistance animal • For improving quality and quantity of milk, meat, eggs and wool production	• Toxicity sensitive transgenic animals to test chemicals • Spider silk in milk of goat

Applications of CRISPR-Cas systems in neuroscience

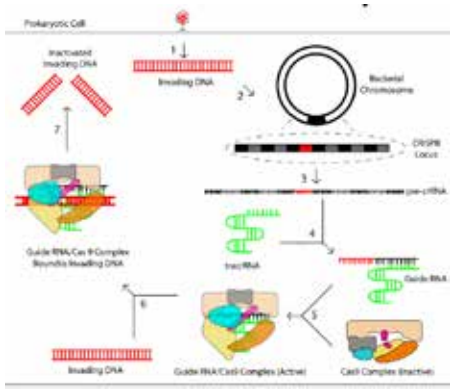
Principle of CRISPR-Cas system

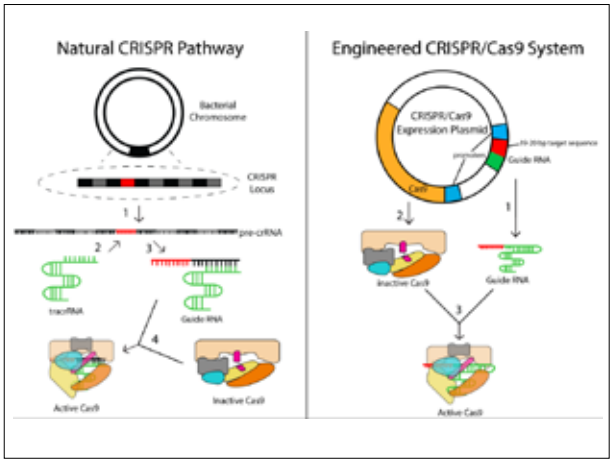
Applications in neuroscience

Disadvantages of Molecular cloning

- **Introduce allergens?**
- **Pass trans-genes to wild populations?**
  - **Pollinator transfer**
- **R&D is costly**
  - **Patents to insure profits**
    - **Patent infringements**
    - **Lawsuits**
    - **potential for capitalism to overshadow humanitarian efforts**

Natural CRISPR Pathway
(Clustered Regularly Interspaced Short Palindromic Repeat)

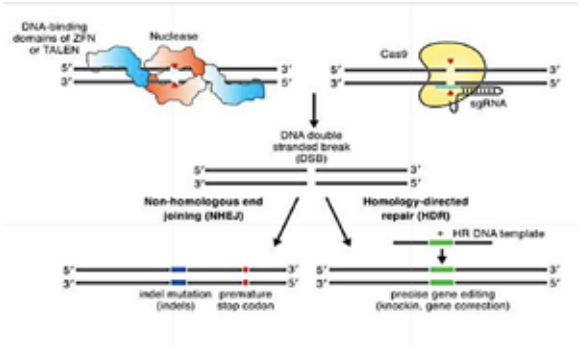




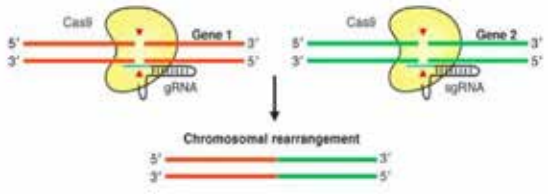
Comparison of approaches for gene knockout

	ZFN	TALEN	CRISPR-Cas
Molecular target	DNA	DNA	DNA
Result of targeting	Irreversible knockout	Irreversible knockout	Irreversible knockout
Ease of generating target specificity	Difficult: substantial cloning and protein engineering required	Moderate: substantial cloning steps required	Easy: simple oligo synthesis and cloning steps
Off-target activity	Moderate	Low	Low
Ease of multi-plexing	Low	Moderate	High
Transcriptional and epigenetic control	DNA binding ZF domains can be fused to new functional domains	DNA binding domains can be fused to new functional domains	Enzymatically inactive Cas9 can be fused to new functional domains
Ease of delivery into the mammalian CNS	Moderate: delivered by viral vectors	Moderate: delivered by viral vectors but large size makes packaging into viral vectors challenging	Moderate: Delivered be electroporation, PEI-mediated transfection, nanoparticles and viral vectors
Ease of generating large scale libraries	Low: complex protein engineering required for each gene	Moderate: technically challenging cloning steps	High: simple oligo synthesis and cloning required
Costs	High	Moderate	Low

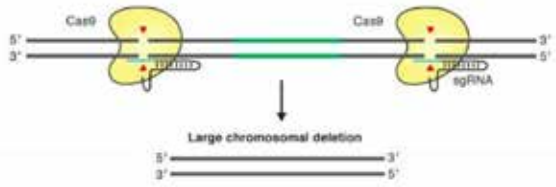
a. Precise gene editing



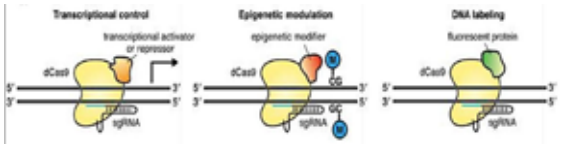
b. Chromosomal rearrangement



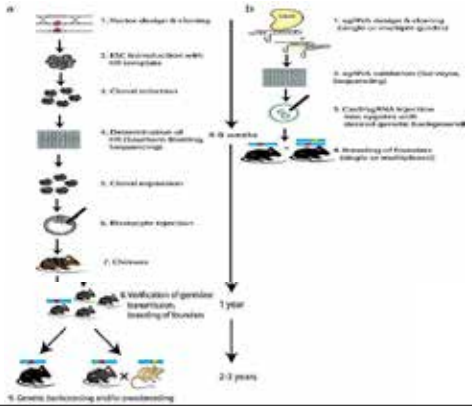
c. Large chromosomal deletion



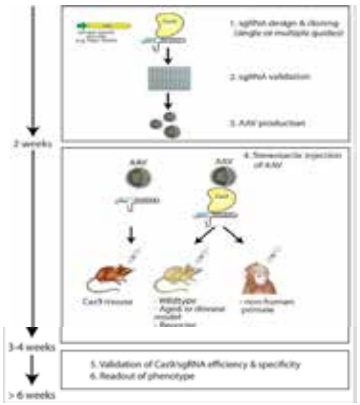
d. Applications of inactivated Cas9



Using Cas9 to generate genetically modified rodents and for in vivo genome editing



Delivery to the specific cells of organ

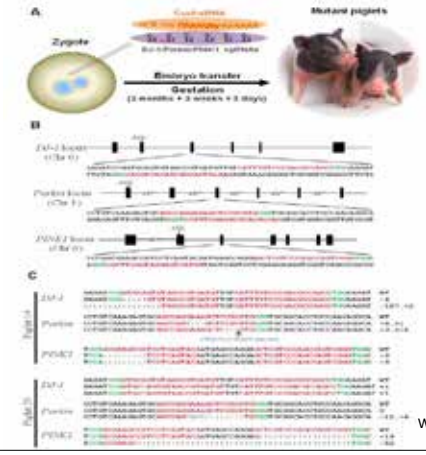


Examples of CRISPR as a tool to model aspects of neurological disorders in animals

Neurological disorder or disorder groups	Gene or Chromosome target	Animal	Editing process with CRISPR	Reference
Dystonia, TSC, BN	Th, Rheb, Mc4r	Mouse, rat	Zygote injection of Cas 9 mRNA and sgRNA (KO)	Li et al, Nat.Biotechnol. (2013)
MS, AD, PD, ALS, and glioma	PPARG,	Cynomolgus monkey	Zygote injection of Cas 9 mRNA and sgRNA (KO)	Niu et al. Cell (2014)
Motoneuron disease	miR-218-1 miR-218-2	Mouse	Zygote injection of Cas 9 mRNA and sgRNA (mutant)	Amin et al, Science (2015)
Parkinson disease	Parkin, DJ-1, PINK1	Mini pig	Zygote injection of Cas 9 mRNA and sgRNA (mutant)	Wang et al, Nat Publ GR (2016)

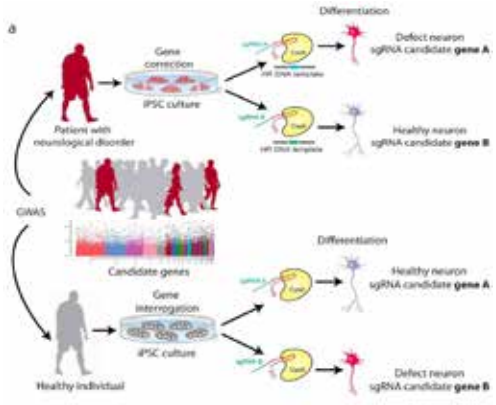
Example of CRISPR as a tool to model aspects of neurological disorder in specific cell type

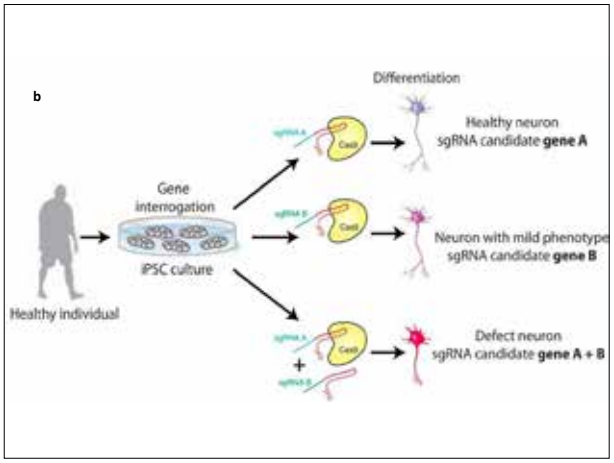
Neurological disorder or disorder groups	Gene or Chromosome target	Editing process with CRISPR	Reference
Rett syndrome and synaptic plasticity	Mecp2, Dnmt1, Dnmt3a, Dnmt3	AAV-mediated delivery into adult mouse brain and KO	Sweich. L et al. Nat. Biotechnol.33 102-106 (2015)



Wang et al, Nat Publ GR (2016)

In vitro applications of Cas9 in human iPSCs





GRK5-KO mice generated by TALEN-mediated gene targeting

Academic Advisor: Prof. Kwan-Sik, Min

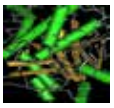
Major in Animal Biotechnology
Graduate School of Future Convergence Technology
Hankyong National University

Examples of CRISPR as a tool to model aspects of neurological disorders in iPSCs

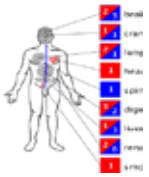
Neurological disorder or disorder groups	Gene or Chromosome target	Editing type with CRISPR	Reference
Major mental illness	DISC1	Frame shift mutation in exon 2 (homozygous), frame shift mutation in exon 8 (homozygous and heterozygous)	Srikanth P et al. Cell Rep.12 (2015)
Autism	CHD8	Knock out (heterozygous)	Wang P et al. Mol. Autism 6:55 (2015)
Hungtington's disease	HTT	Insertion of 97 CAG repeats into exon 1	An MC et al. PLoS Curr. 6 (2014)
Recurrent microdeletion and microduplication syndromes	16p11.2 and 15q13.3 copy number variants	575 kb deletion, 740 kb deletion 740 kb insertion	Tai DJC et al. Nat. Neurosci.19 (2016)
Epilepsy	SCN1A	Insertion of tdTomato into GAD67 to fluorescently label GABAergic neurons	Liu J et al. Transl. Psychiatry.12 (2016)
Fragile X syndrome	FMR1	Deletion of CGG repeats at the 5'-UTR of FMR	Park CY et al. Cell Rep. 13 (2015)

G-protein coupled receptor kinase 5 (GRK5)

Rhodopsin kinase	GRK1; GRK7
B-adrenergic receptor kinase	GRK2; GRK3
GRK4 subfamily	GRK4; GRK5; GRK6



GRK5, 590 a.a
catalytic RH domain 50-176 a.a

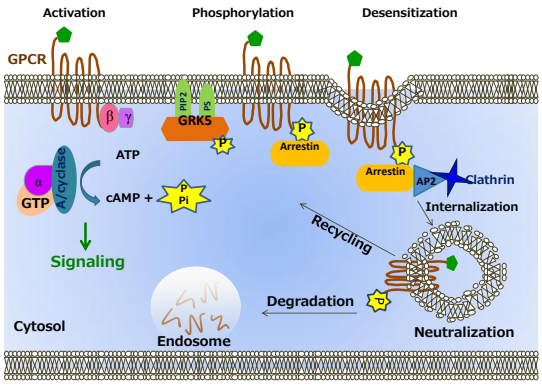


Future perspectives

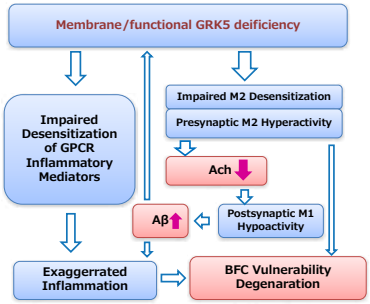
- Existing methods for delivering Cas proteins and RNA guides to the brain must be optimized and new methods must be developed to achieve sufficient levels of specificity and efficiency.
- New methods for stimulating efficient gene insertion and and correction in post mitotic cells have to be established.
- Safety and ethical concerns have to be carefully addressed.

Nevertheless, novel genome-editing technologies, together with powerful readout methods will help us better understand the logic of neuronal circuits and unravel some of mysteries of complex neurological disorders in the near future.

GRK5 functions in GPCR desensitization

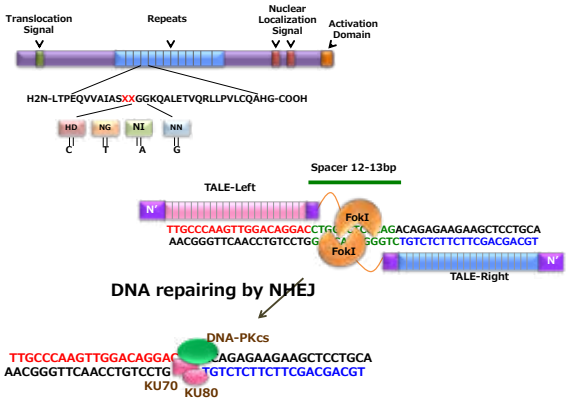


Why need GRK5-KO model organism?

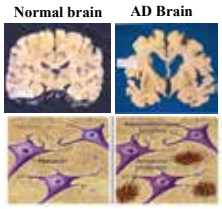


William et al., 2010 (Review)

DNA-recognition specificity of TALENs



Alzheimer's disease and model animals



Amyloid protein-TG

BRI-Aβ42A (McGrown et al., 2005)
PDAPP (Morgan et al., 2003)
J20 (Mucke et al., 2000)
Tg2576 (Westerna et al., 2002)
APP23 (Lalonde et al., 2003)
TgCRND8 (Hyde et al., 2005)
TASD-41 (Rockenstein et al., 2001)
R1.40(Lamb et al., 1997)

Tau protein-TG

TAPP (Lewis et al., 2001)
3xTg (Oddo et al., 2003)
H tau (Polydora et al., 2009)

GRK5-KO

GRK5 –KO (Gainetdinov et al., 1999)

Confirmation methods of AD model animals

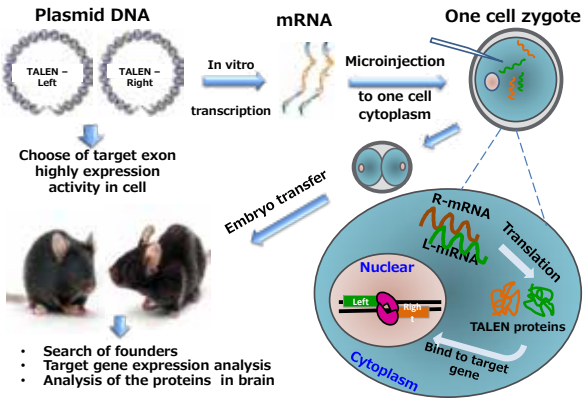
- b-amyloid plaque and tau aggregation staining (antibody, thioflavin,-S, Congo red and Gallyas stain)
- Ach secretion test
- Positron Emission Tomography
- Cerebrospinal fluid analysis
- Morris water maze test
- Mirror test

TALEN system designed for exon 1, 3 and 5 mice GRK5 gene

Exon	Left 1-DAS 5′-3′	Spacer 5′-3′	Right 1-RR 5′-3′
E1	TCCCTCGCTGACTGCCGACT	GTCAATGGAGCT	GGAAAACATCGTGGCCAACA
E3	TGCTTTTTCGACAGTTCTGT	GAAACCAGGCCT	GGGCTGGAGTGCTACATTCA
E5	TTGCCCAAGTTGGACAGGA	CTGGTCTCCCAG	ACAGAGAAGAAGCTCCTGCA

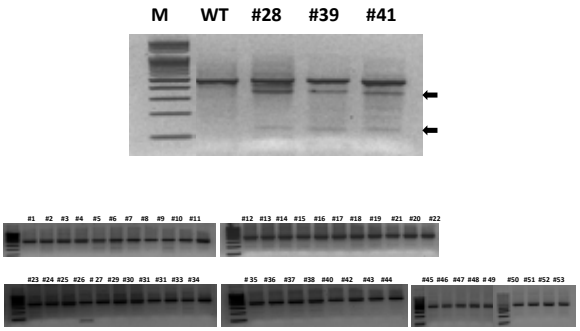
Materials and methods

Overview of the research



Results

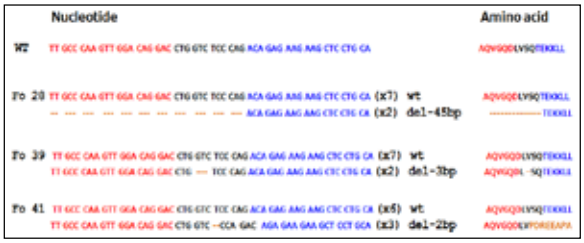
T7 Endonuclease I assay for founders



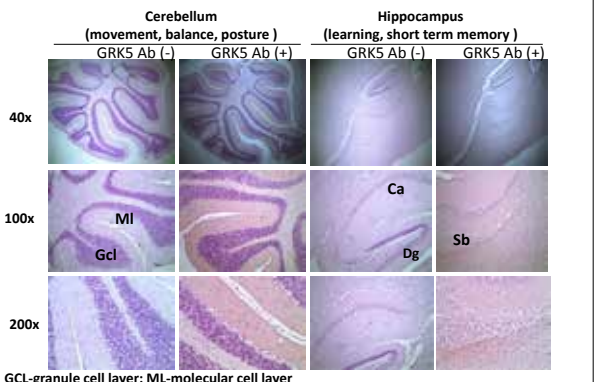
Production of GRK5-KO founder by TALEN-mediated in C57/BL mice

Dose	Injected zygotes	Survived zygotes	Recipient mother	Newborns	Survived fetus	Founders
4 ng/μl	180	135 (75%)	7	60	53 (88.3%)	3 (5.6%)

Sequence information of TALEN-mediated GRK5 mutant alleles



GRK5 localization on brain of C57/BL mouse



GCL-granule cell layer; ML-molecular cell layer

DG-dentate gyrus ; Sb-Subiculum; Ca-hippocampus

Conclusions

- We designed the TALEN vectors and mRNA was injected to 180 one-cell embryos. And then embryos was transferred into oviduct of seven pseudopregnant C57BL/6 mice. The mutated mice was found 3 lines (5.6%).
- We also mated 3 Fo GRK5 mutant lines with wild type mice and confirmed the genotype of F1 progenies. All the mutations observed in Fo mice were transmitted through the germline but not all progenies (37.5%, 37.5%, 57.1%).
- We generated of 10 homozygote GRK5-KO mice for 28 and 41 lines in F3 and F2 progenies.
- Taken together, TALEN-mediated mutagenesis might accelerate the creation of genetically engineered mouse models and elucidate the mechanism of AD pathogenesis using GRK5 knock out mice.
- Next, we will analysis the AD mechanism and AD pathogenic cause using the mutant mice.

Damdindorj Boldbaatar
/MD, PhD/

Mongolian National University of Medical Sciences
Zorig street, Ulaanbaatar-14210, Mongolia

☎: (976)-99996522, (976)-11-311561
✉: damdindorj@mnums.edu.mn

Current Position:

Chair, Department of Physiology, Mongolian National University of Medical Sciences

Academic Career

Chair
Department of Physiology, Mongolian National University of Medical Science
Since. 2013

Postdoctor (Neurophysiology)
Graduate school, Jichi Medical University, Japan 2012.Sep

Doctor (Medicine)
Graduate school, Jichi Medical University, Japan 2010.Jan

Master (Medicine)
Graduate school, Health Sciences University of Mongolia 2005.May

Bachelor (Medicine)
School of Medicine, Health Sciences University of Mongolia 2003.May

Employment

Sep, 2004
lecturer, Department of Physiology, School of Bio-Medicine, Health Sciences University of Mongolia

Mar, 2013
Chair, Department of Physiology, Department of Physiology, Mongolian National University of Medical Science

Jan, 2014
President, Mongolian Neuroscience Society

Publication

(Books)

1. “Ghrelin Health and Desease”. Library of Congress Control Number: 2012956723. Springer Science, Business Media New York 2012. Chapter 3: Ghrelin’s Novel Signaling in Islet β-cells to Inhibit Insulin secretion and Its Blockade As a Promising Strategy to Treat Type 2 Diabetes. B.Damdindorj co-author

2. Interactive effects of ghrelin and GLP-1 on islet β-cell signaling, insulin release and glycemia. PhD dissertation 2010, Jichi Medical University, B.Damdindorj.
3. “Монголын физиологчдын түүхэн шастир” УБ 2013, 300х Г.Сүхбат, Г.Батмөнх, Ц.Лхагвасүрэн, Б.Дамдиндорж.

(Articles)

4. “The Regulation of Energy Metabolism: An Important Facet of P53 Function” Marc Gilbert1, Enkhsaikhan Lkhagvasuren2, Damdindorj Boldbaatar3, Christophe Magnan1 Cent Asian J Med Sci 2017;3:106-115
5. Assessing risk of sleep apnea in obese and non obese adults, a hospital based case-control study. Renchindorj E, Norov T, Munkhtulga G, Adiyakhuu O, Damdindorj B Divcovery-2015. 2015 May; 8; 20-22, Ulaanbaatar Mongolia
6. Postprandial insulinostatic ghrelin level in the serum for type 2 diabetic patients Battulga. Kh1,2, Altanzul.A1,3, Enkhchimeg.Ts1, Enkhsaikhan.L4, Bayasgalan.T3, Damdindorj. B1 Mongolian Journal of Health Science 2016
7. Ghrelin signaling in β-cells regulates insulin secretion and blood glucose. Yada T, Damdindorj B et all Diabetes Obes Metab. 2014 Sep;16 Suppl 1:111-7.
8. Exogenous and endogenous ghrelin counteracts GLP-1 action to stimulate cAMP signaling and insulin secretion in islet b-cells. Boldbaatar Damdindorj et all. FEBS letters. 06/2012; 586(16):2555-62.
9. Ghrelin attenuates cAMP-PKA signaling to evoke insulinostatic cascade in islet b-cells. Katsuya Dezak, Boldbaatar Damdindorj et all. Diabetes. 07/2011; 60(9):2315-24.
10. Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Kunitosh Uchida, Katsuya Dezaki, Boldbaatar Damdindorj et all. Diabetes. 1/2011; 60(1):119-26.
11. グレリンによるインスリン分泌制御と膵β細胞シグナル伝達機構の解明. Boldbaatar Damdindorj. Jichi Medical University (33): 201-202
12. Stressor-responsive central nesfatin-1 activates corticotropin-releasing hormone, noradrenaline and serotonin neurons and evokes hypothalamic-pituitary-adrenal axis. Natsu Yoshida, Yuko Maejima, Udval Sedbazar, Akihiro Ando, Hidahiru Kurita, Boldbaatar Damdindorj et all Aging (Albany NY). 2010 Nov;2(11):775-84.
13. Reconstruction-dependent recovery from anorexia, ghrelin-refractory period, and compensatory ghrelin production in duodenum and pancreas in gastrectomized rats. Masaru Koizumi, Katsuya Dezaki, Hiroshi Hosoda, Boldbaatar Damdindorj et all. Int J Pept. 2010;2010
14. 食欲制御における視床下部，幹脳の役割．前島裕子，Darambazar Gantulga, Boldbaatar Damdindorj, 矢田俊彦．肥満研究 2010.16(3):125-130.
15. Ghrelin regulates insulin release and glycemia: Physiological role and therapeutic potential. Toshihiko Yada, Katsuya Dezaki, Hideyuki Sone, Masaru Koizumi, Boldbaatar Damdindorj et all. Curr. Diab. Rev. Feb;4(1):18-23, 2008

Lecture 3

THE ENDOCRINE SYSTEM

Boldbaatar Damdindorj

2017.3.16.

Дааврын үүрэг:

- Биеийн, бэлгийн болон оюуны хөгжлийг хангана.
- Хэрэгцээнд тохируулж эрхтэн болон эрхтний тогтолцооний идэвхийг тохируулж дасан зохицлыг хангана
- Цусны зарим үзүүлэлтийн хэвийн түвшинд барьж байх дотоод орчны тогтмол байдлыг хангах үүрэгтэй

PRINCIPLES OF ENDOCRINE FUNCTION

1. The **nervous system** integrates tissue functions by a network of cells.
2. The **endocrine system** integrates organ function through chemicals that are secreted from **endocrine tissues or "glands"** into the extracellular fluid. These chemicals, called **hormones**, are then carried through the blood to distant target tissues, where they are **recognized by specific, high-affinity receptors.**
3. These receptors located on the **surface of the target tissue**, within the **cytosol**, or in the **target cell's nucleus.**
4. Hormones **very low concentration (10^{-9} to 10^{-12} M)** in blood.

Дааврын физиологийн ангилал:

- Шууд гүйцэтгэгч эрхтэндээ нөлөөлж буй дааврыг **үйлчлэгч даавар** гэнэ.Үүнд: өсөлтийн даавар, пролактин даавар
- Үйлчлэгч дааврын нийлэгжил ялгаралтыг зохицуулдаг дааврыг **идэвхижүүлэгч даавар**
- Гипоталамусын мэдрэлийн эсээс ялгараад, аденогипофизын шүүрлийн ялгаралтанд нөлөөлдөг дааврыг **рилизинг даавар**
- Өнчин тархины идэвхжүүлэгч дааврын ялгарлыг нэмэгдүүлдэг дааврыг либерин саатуулдаг дааврыг **статин даавар**

Chemical signaling pathways:

- **endocrine**
- **paracrine**
- **autocrine**

Endocrine Glands

1. **The pituitary**
2. **The thyroid**
3. **The parathyroids**
4. **The testes**
5. **The ovary**
6. **The adrenal (cortex and medulla),**
7. **The endocrine pancreas.**

Part of the endocrine system produce hormones and play a v role in endocrine regulation **central nervous system (CNS)**, particularly **the hypothalamus**, **the gastrointestinal tract, liv heart, kidney, and others.**

Paracrine Factors

- **Interleukins,**
- **Lymphokines,**
- **Growth factors,**
- **Platelet-derived growth factor**
- **Fibroblast growth factor**

Chemical Classification of Selected Hormones

Hormones:

I. Peptides

II. Metabolites of single amino acids

III. Metabolites of cholesterol

Peptide Hormones
ACTH
Atrial natriuretic peptide (ANP)
AVP (ADH)
Calcitonin
Cholecystokinin (CCK)
CRH
FSH
Glucagon
GnRH
GH
GHRH
Inhibin
Insulin
IGFs
LH
Oxytocin
PTH
PRL
Secretin
Somatostatin
TSH
TRH
Vasoactive intestinal peptide (VIP)

Amino Acid–Derived Hormones
DA
Epinephrine (adrenaline)
Norepinephrine (noradrenaline)
Serotonin (5-HT)
T_4
T_3

Steroid Hormones
Aldosterone
Cortisol
Estradiol (E2)
Progesterone
Testosterone

Differences Between Steroid and Peptide Amine Hormones

Property	Steroid Hormones	Peptide Amine Hormones
Storage pools	None	Secretory vesicles
Interaction with cell membrane	Diffusion through cell membrane	Binding to receptor on cell membrane
Receptor	In cytoplasm or nucleus	On cell membrane
Action	Regulation of gene transcription (primarily)	Signal transduction cascades affecting a variety of cell processes
Response time	Hours to days (primarily)	Seconds to minutes

Peptide Hormones and Their Signal Transduction Pathways

Agonists	Receptor	Linked Enzyme	Second Messenger
PTH	Coupled to $G\alpha_s$	Adenylyl cyclase	cAMP
ANG II	Coupled to $G\alpha_i$	Adenylyl cyclase (inhibited)	cAMP
AVP, ANG II, TRH	Coupled to $G\alpha_q$	PLC	IP_3 and DAG
ANG II	Coupled to G_i/G_o	PLA_2	Arachidonic acid metabolites
ANP	Guanylyl cyclase	Guanylyl cyclase	cGMP
Insulin, IGF-1, IGF-2, EGF, PDGF	Tyrosine kinase	Tyrosine kinase	Phosphoproteins
GH, erythropoietin, LIF	Associated with tyrosine kinase	JAK-STAT family of tyrosine kinases	Phosphoproteins

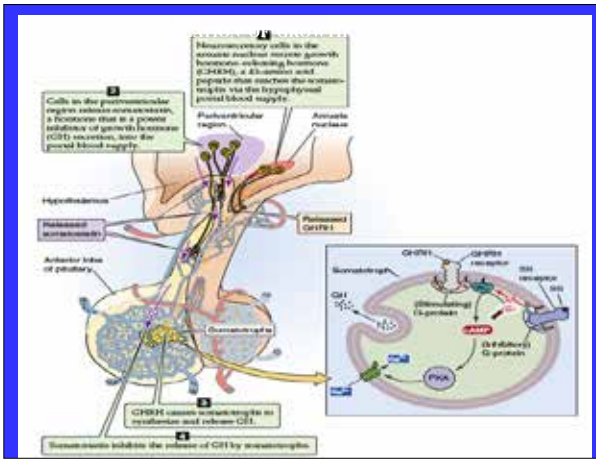
ANG II, angiotensin II; ANP, atrial natriuretic peptide; EGF, epidermal growth factor; JAK-STAT, Janus kinase/signal transducer and activator of transcription; LIF, leukemia inhibitory factor.

Дааврын зөөвөрлөлт:

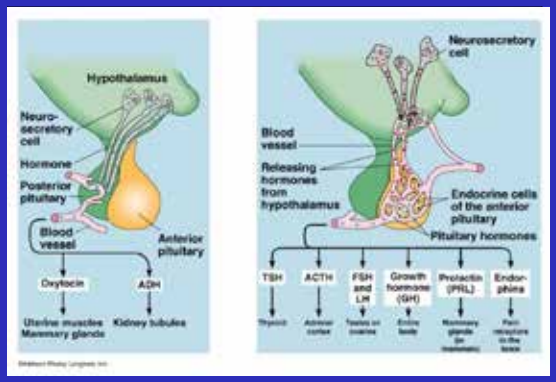
<u>Дааврын зөөвөрлөлт нь гипоталамусын удирдлаганд</u>

- Дотоод шүүрлийн булчирхайн эсийн ялгаруулдаг даавар нь **<u>цусаар дамжин бай эсэд очдог</u>**.
- **<u>Стероид даавар</u>** нь эсийн липидэн мембранаар амархан нэвтэрдэг учир эдгээр дааврууд нь булчирхайн эсэд нөөцлөгддөггүй.

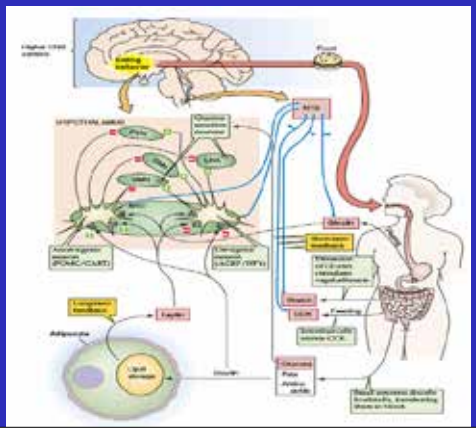
Дааврууд хоёр хэлбэрээр зөөвөрлөгдөнө.
- чөлөөт хэлбэр (дааврын 5-10%)
- холбоот



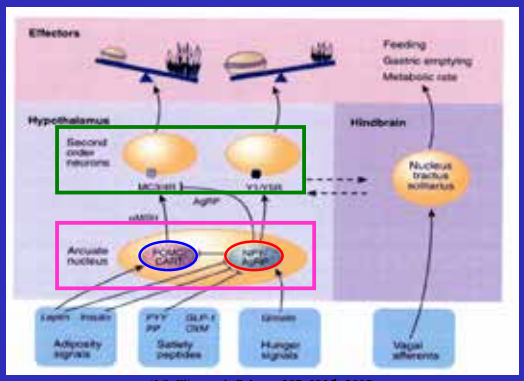
Hypothalamic and Pituitary Hormones



ENDOCRINE R EGULATION OF FEEDING AND BODY MASS

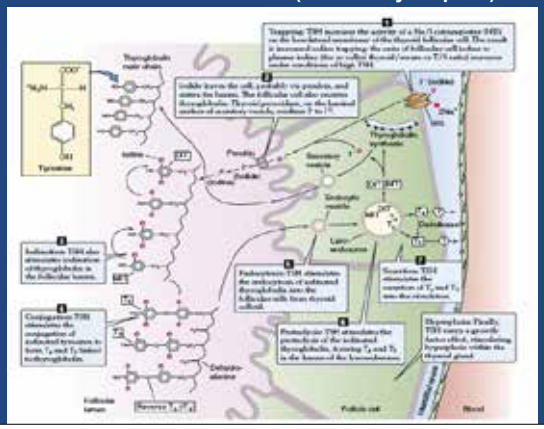


Gastro intestinal hormones role in feeding



(J.S. Flier et al. *Science 307, 1911- 2005*)

TH E THYROID GLAND (Бамбай булчирхай)



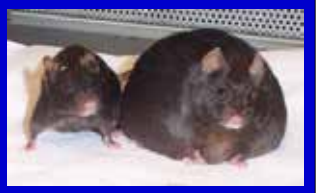
What is ob/ob mice?

In 1994, *ob* gene discovered by Friedman , is located in 7 chromosome.
Ob gene is produced new hormone leptin in adipose tissue. (Ob-obese)

Leptin plays a key role in regulating energy intake and expenditure, including feeding, appetite and hunger, metabolism and feeding behavior.

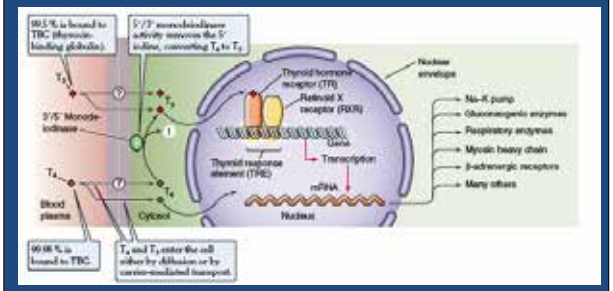
Lep^{ob} or *ob/ob* is not produced leptin *db/db* is not expressed leptin receptor

ob/ob mice exhibit :
- obesity
- hyperphagia
- hyperglycemia
- glucose intolerance
- elevated plasma insulin
- subfertility
- hypometabolic
- hypothermic



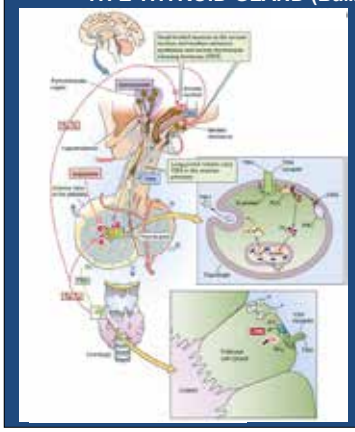
Wild type mice ob/ob mice

Action of thyroid hormones on target cells.



- Acts by nongenomic signaling pathways
- Increases basal metabolic rate

TH E THYROID GLAND (Бамбай булчирхай)

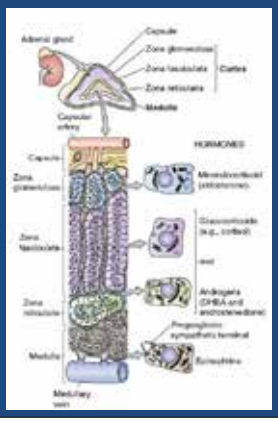


TRH
⬇
TSH
⬇
T_3 and T_4
⬇
Target tissue

Action of thyroid hormones on metabolism

Basal metabolic rate	↓	↑
Carbohydrate metabolism	↓ Gluconeogenesis ↓ Glycogenolysis Normal serum [glucose]	↑ Gluconeogenesis ↑ Glycogenolysis Normal serum [glucose]
Protein metabolism	↓ Synthesis ↓ Proteolysis	↑ Synthesis ↑ Proteolysis Muscle wasting
Lipid metabolism	↓ Lipogenesis ↓ Lipolysis ↑ Serum [cholesterol]	↑ Lipogenesis ↑ Lipolysis ↓ Serum [cholesterol]
Thermogenesis	↓	↑
Autonomic nervous system	Normal levels of serum catecholamines	↑ Expression of β adrenoceptors (increased sensitivity to catecholamines, which remain at normal levels)

THE ADRENAL GLAND

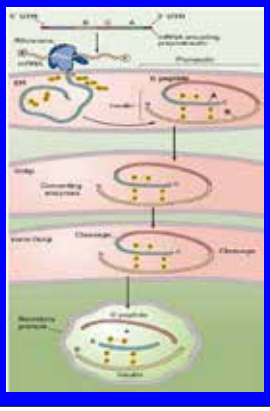


Steroid hormones:

- Glucocorticoids
- Mineralocorticoids
- Sex steroids.

Cortisol is glucocorticoid.
plasma glucose inceases,
stress hormone

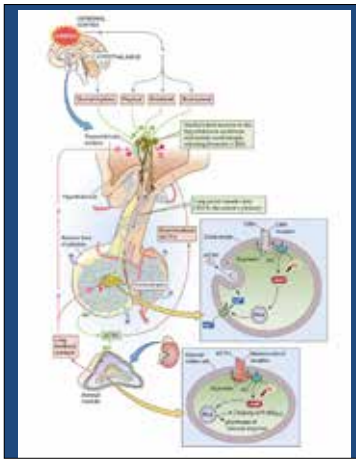
Synthesis of insulin in islet beta cells



C peptide

Amilin

Insulin



Synthesis of adrenal hormones

CRH

⬇

ACTH

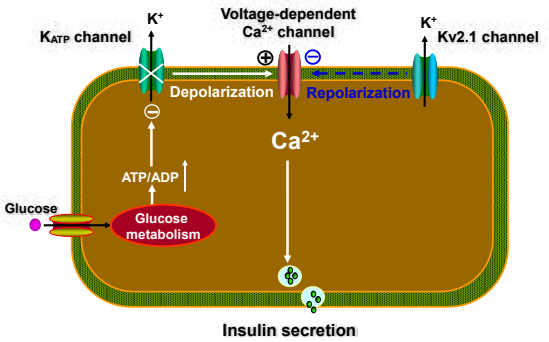
⬇

Cortisol e.t.,

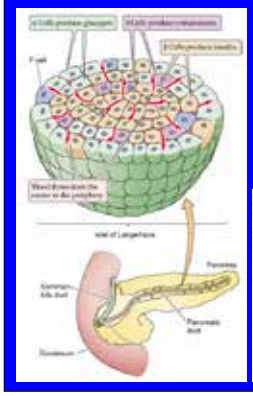
⬇

Target tissue

Glucose-induced insulin secretion in islet beta cell



THE ENDOCRINE PANCREAS

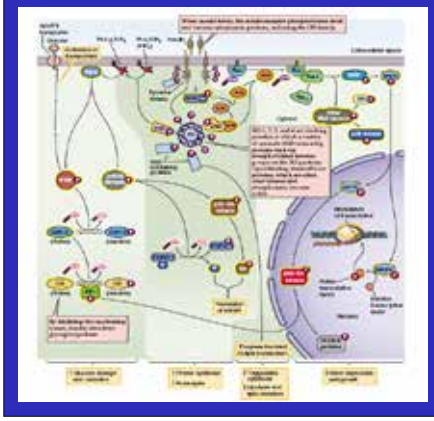


The human pancreas contains between 500000 and several million islets.

Islets diameter is 50 - 300 µm

Cell Type	Product
α	Glucagon
β	Insulin Proinsulin C peptide Amylin
δ	Somatostatin
F	Pancreatic polypeptide

Insulin action in target tissue



In liver:

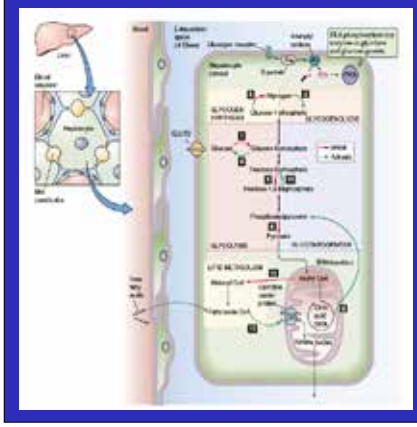
Glycogen synthesis ⬆

Glycogenolysis ⬇

Gluconeogenesis ⬇

Glycolysis ⬆

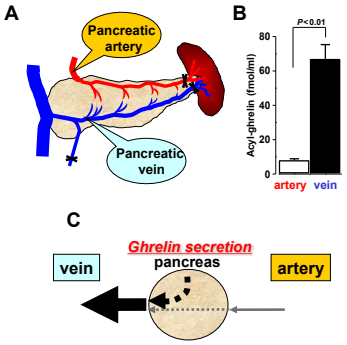
Glucagon action in liver



In liver:

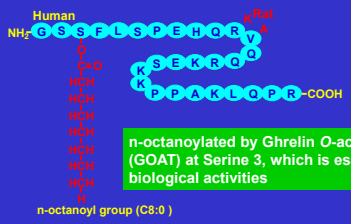
- Glycogen synthesis ↓
- Glycogenolysis ↑
- Gluconeogenesis ↑
- Glycolysis ↓

Ghrelin secreted from pancreas



Introduction: Ghrelin

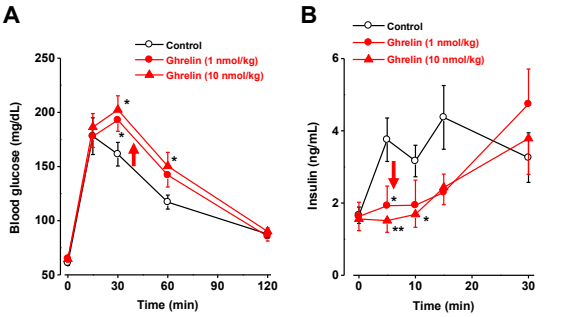
Ghrelin, a 28-amino acid peptide, is the **endogenous ligand for growth hormone-secretagogue receptor** (GHS-R) and predominantly produced in the stomach *(Nature 1999)*.



n-octanoylated by Ghrelin *O*-acyl transferase (GOAT) at Serine 3, which is essential for biological activities

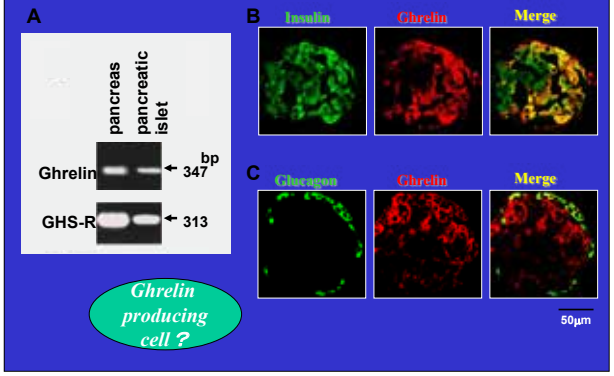
1. Stimulates growth hormone release
2. Activates feeding

Ghrelin administration inhibits plasma insulin and elevates blood glucose levels *in mice*



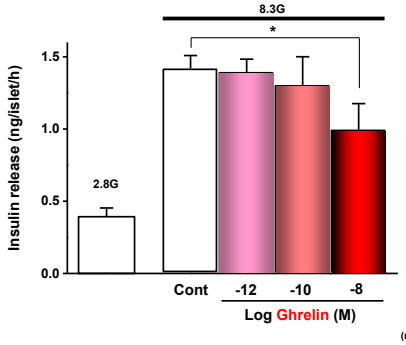
(Diabetes 53:3142-3151, 2004)

Ghrelin and GHSR are expressed in rat pancreatic islets of rat

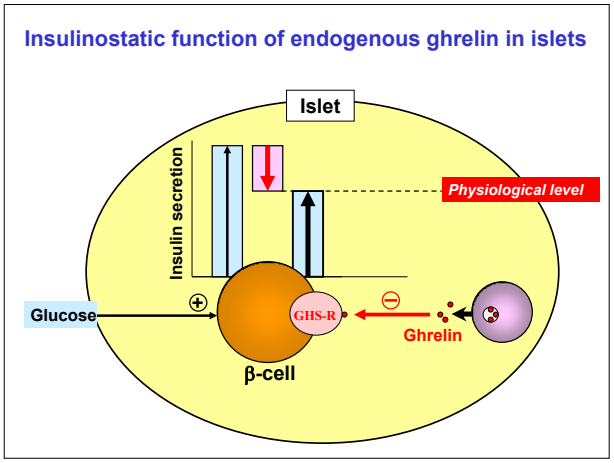


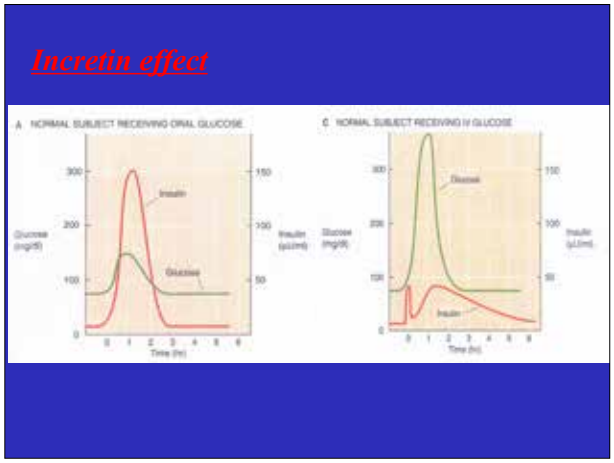
Ghrelin producing cell ?

Ghrelin inhibits glucose-induced insulin release in isolated islets of Wistar rat



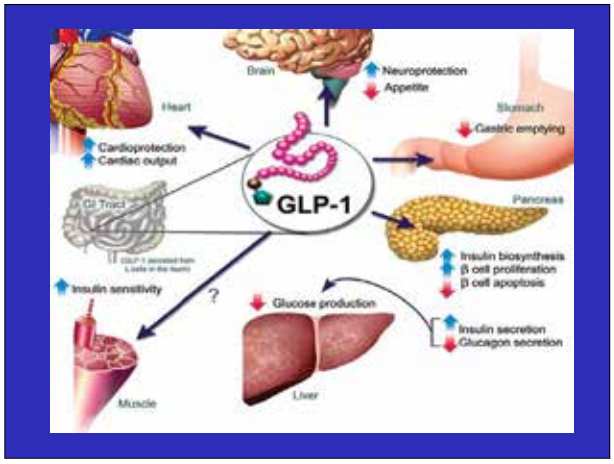
(n=10) (*P<0.05)

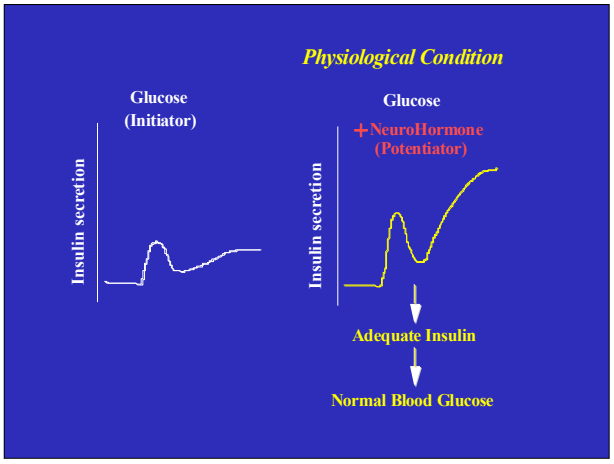


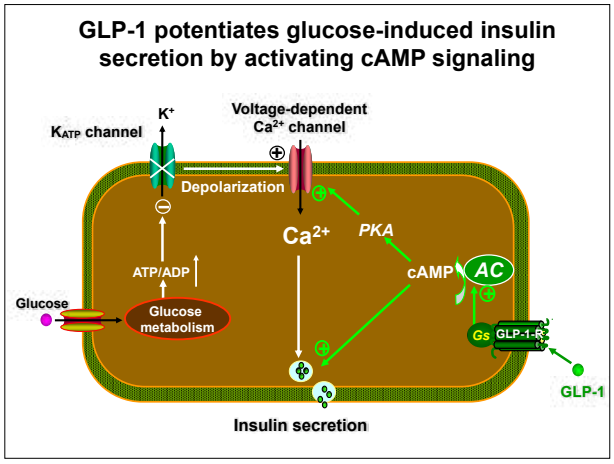


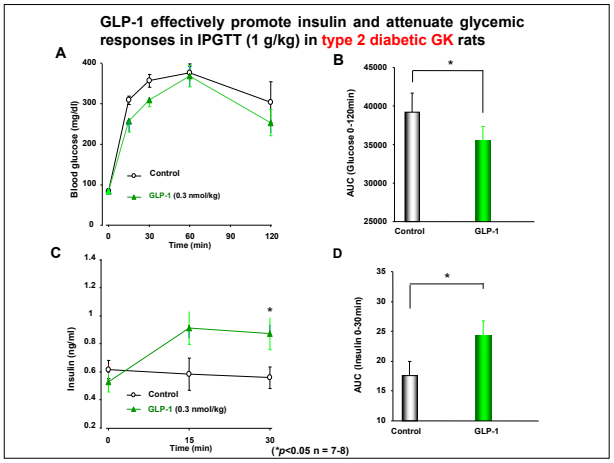
What is GLP-1 ?

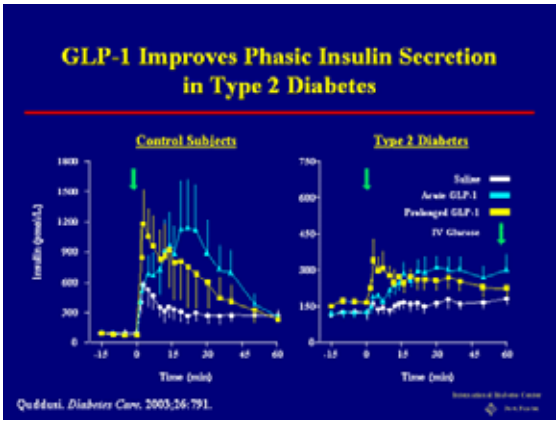
GLP-1 is incretin hormone.

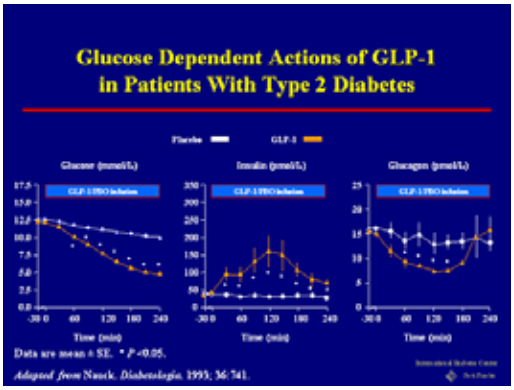


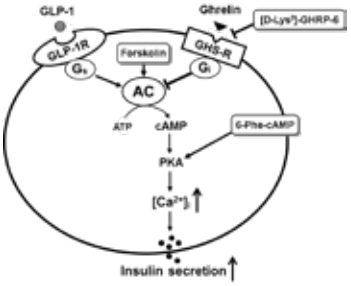




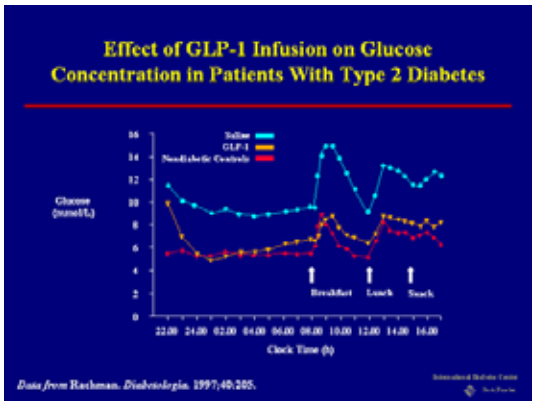








(B.Damdindorj et all. *Febs letters 2012*)



Enkhsaikhan Lkhagvasuren /MD, PhD/

Mongolian National University of Medical Sciences (MNUMS)

☎: (976)-89981001
✉: enkhsaikhan@mnums.edu.mn

Current Position:

2015-current
Senior Lecturer, Department of Microbiology and Immunology, School of Pharmacy and Biomedicine, Mongolian National University of Medical Sciences

Education:

2009-2013
Doctor of Philosophy, Tokushima University
Japan, June 2013

2007-2009
Residence course of Internal Medicine, Health Sciences University of Mongolia, 2009

2003-2005
Master of Science Degree, Health Sciences University of Mongolia, June 2005

1997-2003
Bachelor of Science Degree, Health Sciences University of Mongolia, May 2003

International Publication:

1. Takeshi Nitta, Lkhagvasuren Enkhsaikhan, Ohigashi Izumi, Takahama Yousuke. Effects of RANKL on the thymic medulla. Eur J Immunol. 2011 Jul;41(7):1822-7.
2. Lhagvasuren Enkhsaikhan, Sakata Mie, Ohigashi Izumi, Takahama Yousuke. Lymphotoxin β receptor regulates the development of CCL21-expressing subset of postnatal medullary thymic epithelial cells. Journal of Immunology, 2013 190:5110-5117;
3. Enkhsaikhan Lkhagvasuren. Janeway's Immunology, 9th Edition // Central Asian Journal of Medical Sciences, Vol. 3, No. 1, 2017, p.100-101 (Book review/
4. Marc Gilbert, Enkhsaikhan Lkhagvasuren, Damdindorj Boldbaatar, Christophe Magnan, The regulation of energy metabolism: An important Facet of p53 function // Central Asian Journal of Medical Sciences, Vol. 3, No. 2, 2017, p.106-115 (Review)
5. Enkhsaikhan Lkhagvasuren, The future of biomedical specialist in Mongolia // Central Asian Journal of Medical Sciences, Vol. 3, No. 3, 2017, p.1-4 (Editorial)
6. Dolgorsuren Sandagdorj, Batkhishig Munkhjargal, Baasansuren Enkhjargal, Baljinnyam Tuvdenjamts, Enkhjin Zorigt, Budjav Jadamba, Altanshagai Chinbat, Khongorzul Samdankhuu, Ulziisaikhan Jambalganii, Batsuren Boldbaatar, Tsogtsaikhan Sandag,

Bilegtsaikhan Tsolmon, Enkhsaikhan Lkhagvasuren. “Regulating action of in vitro hepatitis C virus infection on type II interferon-induced interferon stimulating genes in murine macrophages”. “Central Asian Journal of Medical Sciences, Vol. 4, No. 1, 2018, p. 25-34

Domestic publication

1. Soodoi Chimidtseren, Lkhagvasuren Enkhsaikhan, Gunchin Batbaatar. The status of cellular immunity in children with acute leukemia. Mongolian Medical Sciences. 2005 №2, 6-9
2. Soodoi Chimidtseren, Lkhagvasuren Enkhsaikhan, Gunchin Batbaatar. The status of humoral immunity in children with acute leukemia. Mongolian Medical Sciences. 2005 №1, 10-12
3. Б.Батсүрэн, Л.Энхсайхан, С.Цогтсайхан нар. Чихрийн шижингийн нефропатийн оношилгоонд ийлдсийн цистатин С-ийн ач холбогдол. Эрүүл мэндийн шинжлэх ухаан. 2016;16(3):
4. Ганцэцэг Г, Дариймаа Г, Энхсайхан Л, Ганмаа Д, Мөнхзол М. Сүрьеэгийн далд халдварын тархалтыг тодорхойлж, түүнд нөлөөлөх зарим хүчин зүйлийн эрсдэлийг үнэлсэн нь, Innovation сэтгүүл, Vol.11, No.2. 05 сар, 2017, х.33-37, (Өгүүлэл)
5. Ганцэцэг Г, Дариймаа Г, Энхсайхан Л, Ганмаа Д, Мөнхзол М. “Сүрьеэгийн далд халдварын тархалтыг тодорхойлж, түүнд нөлөөлөх зарим хүчин зүйлийн эрсдэлийг үнэлсэн нь Монголын анагаах ухаан сэтгүүл, №2, 2017, х.26-33, (Өгүүлэл)
6. Долгорсүрэн С, Батхишиг М, Баасансүрэн Э, Балжинням Т, Алтай Э, Өлзийсайхан Ж, Хонгорзул С, Эрхэмбаяр Ш, Гантулга Г, Лхагвасүрэн Д, Билэгтсайхан Ц, Цогтсайхан С, Энхсайхан Л. RAW-264.7 болон HUH-7 шугаман эсийн өсгөвөрт гепатитын С вирусын халдварын загвар үүсгэсэн нь // Эрүүл Мэндийн Шинжлэх Ухаан Сэтгүүл, Vol.13, №3, (43) 2017, х.160-163 (Өгүүлэл)

Organized conference:

1. Organization: Mongolian Society of Immunology and Microbiology
   Conference name: Current Advances of Microbiology and Immunology-CAMI biannual meeting /CAMI-2008, CAMI-2014, CAMI-2016, CAMI-2018/
   Role: membership
   Period: since 2012
2. Organization: Mongolian Neuroscience society
   Conference name: Multidisciplinary Brain Science annual meeting /2013, 2014, 2015, 2016, 2017/
   Role: Board member
   Period: since 2013

Activities in specialist bodies over the last 5 years

1. “World AIDS Day” cooperation with Ministry of Health, Mongolia
2. “World TB day”
3. “National Olympic on Microbiology and Immunology”, Quiz champion for Medical Students in Mongolia

Lecture 4

NEUROINFLAMMATION
Мэдрэлийн үрэвсэл

https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-7-74

Лхагвасүрэнгийн Энхсайхан
Бичил амьд, дархлаа судлалын тэнхим,
Био-Анагаахын сургууль, АШУҮИС

Enkhsaikhan Lkhagvasuren
Department of Microbiology and Immunology
School of Biomedicine
Mongolian National University of Medical Sciences
enkhsaikhan@mnums.edu.mn

COMMON CAUSES OF NEUROINFLAMMATION

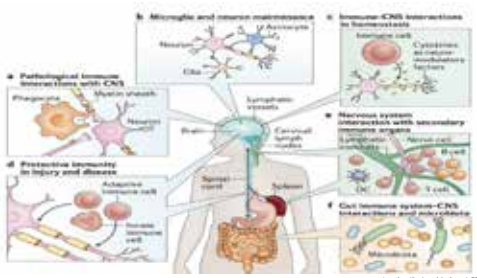
- Хортой метаболитууд
- Өөрийн дархлаа
- Хөгшрөлт
- Бактери
- Вирус
- Тархины гэмтэл
- Агаарын бохирдол
- Дам тамхидалт

- Toxic Metabolites
- Autoimmunity
- Aging
- Microbes
- Viruses
- Traumatic brain injury
- Air pollution
- Passive smoke

Overview

1. Neuro-imflammation
2. Immune system activation
3. PAMPs, DAMPs – PRRs
4. TLR signaling, Low grade inflammation

Six of the neuroimmunology research areas



Jonathan Kipnis and Anthony J. Filiano
Nature Immunology, 2017. Year in review

Мэдрэлийн үрэвсэл

- Мэдрэлийн үрэвсэл нь мэдрэл сөнөрлийн өвчүүдийн үеийн хамгийн онцлог шинж юм.

Neuroinflammation

- Neuro inflammation is an important feature of many neurodegenerative diseases

- Multiple sclerosis
- Alzheimer's disease
- Parkinson's disease
- Narcolepsy
- Autism etc

The central nervous system privileged by immune connections

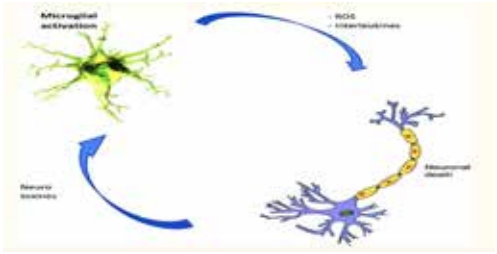
Jonathan Kipnis and Anthony J. Filiano
Nature Immunology, 2017. Year in review

Microglia: Receptors and Secreted Factors

Molecule Class	Examples	Function
Scavenger receptors	CD36	Uptake of apoptotic cells
Ig Receptors Complement receptors	Fcg RI CR3	Uptake of opsonized particles
Growth factor receptor	M-CSF R, GM-CSF R	Proliferation and survival
TLR	TLR1-9	Pathogen and damage associated activation
Antigen presenting molecules	MHC II	Presentation of phagocytosed material
Cytokines	TNF, IL-6, IL10, M-CSF	Immunostimulation
Neurotransmitters Neurotoxins	Glutamate ROS	Cellular toxicity
Neurotrophins	BDNF, NGF	Neurotrophic signals

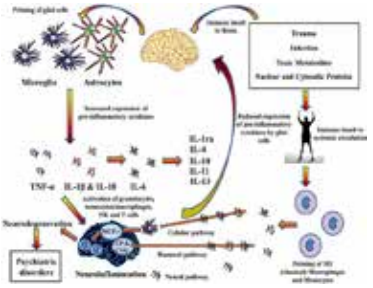
From Langmann 2007

Relationship between microglial activation and neuronal death



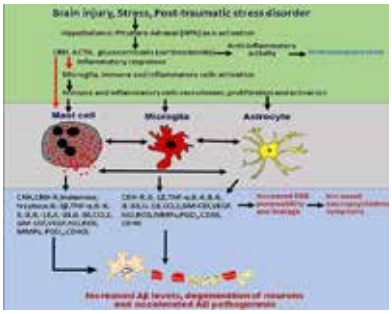
Chen, W.W., Zhang, X.I.A. and Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases. *Molecular medicine reports*, 13(4), pp.3391-3396.

Cytokines hypothesis of neuroinflammation



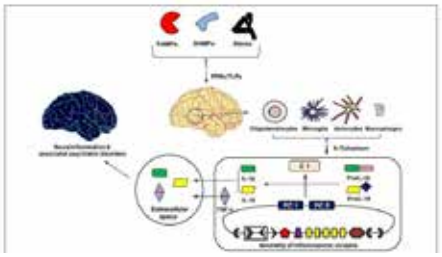
- Inflammation – The deep cause of so much chronic illness and even dementia

Gaurav Singhal et al, Front. Neurosci., 07 October 2014 | https://doi.org/10.3389/fnins.2014.00315



Color atlas of immunology page 76-77

Inflammasomes Cascade in Brain



Gaurav Singhal et al, Front. Neurosci., 07 October 2014 | https://doi.org/10.3389/fnins.2014.00315

There are two types of Neuroinflammation

- Мэдрэлийн цочмог үрэвсэл

(Мэдрэлийн цочмог үрэвсэл нь ихэвчлэн төв мэдрэлийн тогтолцоонд шууд гэмтэл учруулдаг ба үрэвслийн молекул, эндотелийн эсийн идэвхжил, ялтсын бөөгнөрөл болон эдийн хаван зэргээр тодорхойлогдоно)

- Мэдрэлийн архаг үрэвсэл

(Мэдрэлийн архаг үрэвсэл нь мэдрэлийн глиал эсийг идэвхжүүлж үрэвслийн болон дархлааны бусад эсийг тархины эд рүү нүүн шилжиж, мэдрэлийн эдийг сөнөрөл үүсэхэд нөлөөлнө)

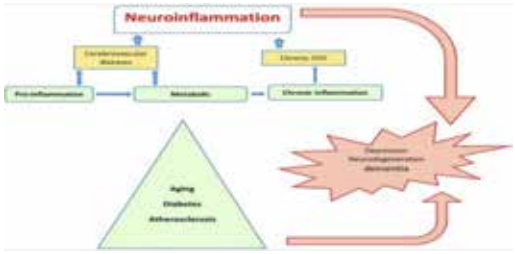
- Acute Neuroinflammation

(Acute neuroinflammation usually follows injury to the central nervous system immediately, and is characterized by inflammatory molecules, endothelial cell activation, platelet deposition, and tissue edema)

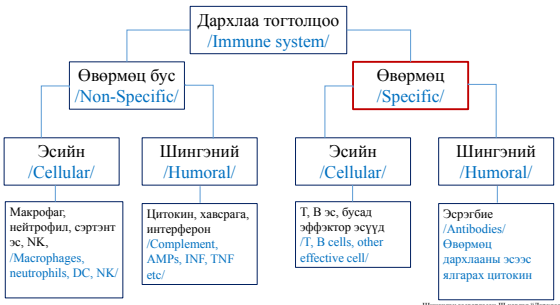
- Chronic Neuroinflammation

(Chronic Neuroinflammation is the sustained activation of glial cells and recruitment of other immune cells into the brain. It is chronic inflammation that is typically associated with neurodegenerative diseases.)

Sources of neuroinflammation

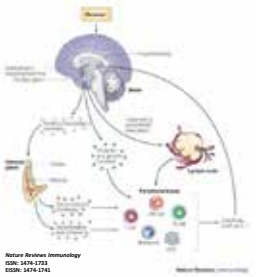


Chen, W.W., Zhang, X.L.A. and Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases. *Molecular medicine reports*, *13*(4), pp.3391-3396.



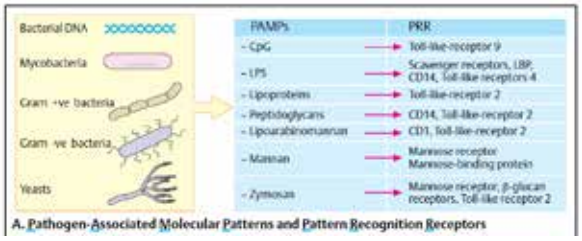
Шингэний хамаарсан III хэлэл "Дархлаа судлал "

Cytokine HPA Axis



Nature Reviews Immunology
ISSN: 1474-1733
EISSN: 1474-1741

Pathogen-associated Molecular Patterns and Pattern Recognition Receptors



A. Pathogen-Associated Molecular Patterns and Pattern Recognition Receptors

Color atlas of immunology page 76-77

Мэдрэлийн үрэвсэл

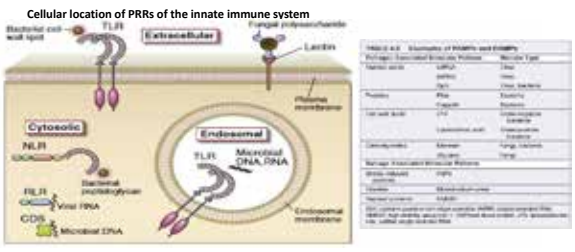
- Нейрон, макроглиа, микроглиа
- Эмгэгтөрөгч болон бусад гадаад хүчин зүйлсийн нөлөөгөөр микроглиа эс идэвхжин, мэдрэлийн үрэвслийг өрнүүлэх замыг идэвхжүүлнэ.
- ЛПС нь микрогли эсийн гадаргуугийн TLR4-тэй холбогдсоноор эсэд дохио дамжих хэд хэдэн замыг идэвхжүүлдэг.

Neuroinflammation

- Neurons, macroglia, microglia
- Antigens and some factors activate microglia and complex neuroinflammation pathways.
- LPS binding to TLR-4 on the microglia surface activates signal transduction pathways

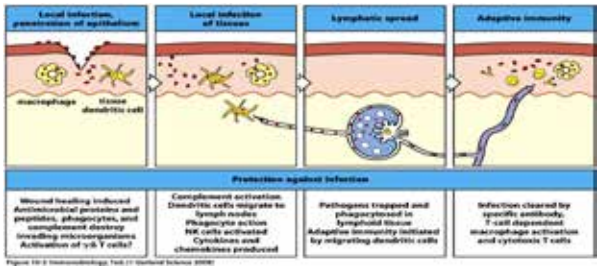
https://www.researchgate.net/publication/305344224

Cellular location of PRRs of the innate immune system



Abul K.Abbas.,Celullar and molecular immunology, 8th edition, page 54, 56

Phases of the immune response



Өвөрмөц бус хамгаалах тогтолцоо

- Эсийн хүчин зүйл
- Эмгэг төрөгч төст бүтцийн загвар /PAMPs/ - таньж идэвхждэг.
- Эзэн эсэд тодорхойлогддоггүй.
- Эмгэгтөрөгчдөд тодорхойлогддог.
- Харьцангуй тогтвортой бүтэц
  - Бактер пептидогликан, липопротейн
  - Гарам сөрөг нян – липополисахарид
  - Вирусийн хос уталсат РНХ
  - Метилжээгүй ДНХ

Non-specific immune system

- Pathogen-Associated Molecular Pattern /PAMPs/
- Absent in host
- Present in diverse organisms
  - dsRNA, ssRNA
  - unmethylated DNA
  - LPS
  - Peptidoglycan etc

Шинэчлэн засварласан III хэвлэл "Дархлаа судлал "

Үрэвсэл, эдгэрэлтийн гол шинжүүд

Maria C.Basil & Bruce D.Levy (2016) Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. *Nat Reviews Immunol.* **16**: 51–67 (2016) doi:10.1038/nri.2015.4

Эмгэгтөрөгчийг таних рецептор

- Дархлааны эсүүдэд PAMPs-г таних PRR /Pattern Recognition Receptor/ илэрдэг.

- PRR - 3 төрөл
1. Ялгардаг PRR /опсонин, хавсрага, манноз холбох лептин/
2. Фагоцитозын рецептор
3. Цитокины ялгаралтыг өдөөх рецептор /TLR – Toll Like Receptor/

PRR

- PRR /Pattern Recognition Receptor/

- 3 types of PRR
1. Secreted /mannose-binding lectin/
2. Endocytic /Scavenger receptor/
3. Signaling /TLR/

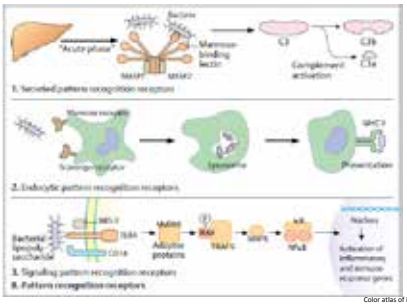
https://www.researchgate.net/publication/305344224

Үрэвслийг намдаагч медиатор
Specialized pro-resolving mediators (SPMs)

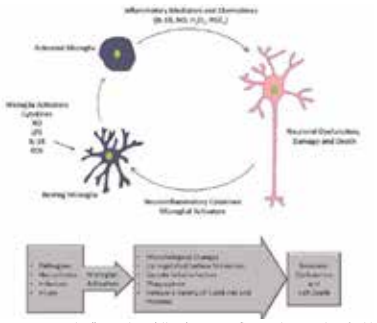
- Үрэвслийг намдаагч медиатор
- Өөхний хүчлээс ферментын задралаар үүсдэг
- Липид медиаторууд
- Халдварыг бууруулна.
- Иммуносуппресантаас ялгаатай
- Төрөлхийн дархлааны үрвалыг дэмждэг.
- Эхийн хөхний сүүнд ихээр агуулагддаг нь түүний хамгаалах үйлдлийг илтгэж байна.

Maria C.Basil & Bruce D.Levy (2016) Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. *Nat Reviews Immunol.* **16**: 51–67 (2016) doi:10.1038/nri.2015.4

Эмгэгтөрөгчийг таних рецептор - PRRs



Color atlas of immunology page 76-77



https://www.researchgate.net/publication/30534424_Neuroinflammation_Pathways_a_General_Review?enrichId=rgreq-58781d888038fa737682eb70bb382317-XXX&enrichSource=Y292XUQ7WdlOxMwNTM0NDIyNDdBUzo0MjExNDAwMzcxNjA5NjBAMTQ3NzQxOTAxNzcyNg%3D%3D&el=1_x_2&_esc=publicationCoverPdf

Several receptors on microglia contribute to cellular activation by the various forms of Ab

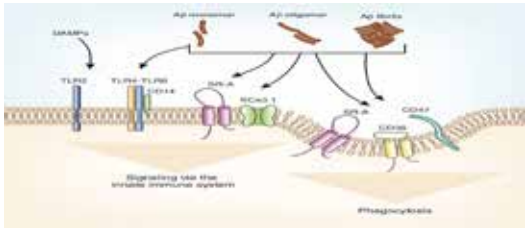
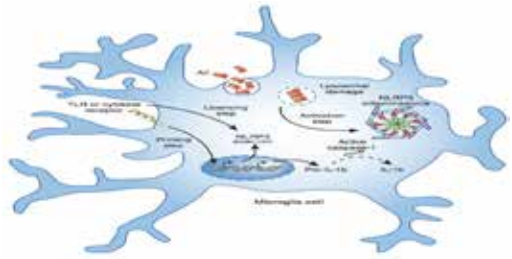


Figure TLR2, TLR4 and TLR6 and their co-receptors can be triggered by fibrillar and aggregated Ab to induce signaling via the innate immune system. Furthermore, the scavenger receptor SR-A and the ion channel KCa3.1 have been linked to microglial activation by Ab. SR-A, CD36 and CD47 promote the phagocytosis of Ab. DAMPs, damage-associated molecular patterns.

Heneka, M.T., Golenbock, D.T. and Latz, E., 2015. Innate immunity in Alzheimer's disease. *Nature immunology*, *16*(3), p.229.

Inflammasomes and the production of active IL-1b



Heneka, M.T., Golenbock, D.T. and Latz, E., 2015. Innate immunity in Alzheimer's disease. *Nature immunology*, *16*(3), p.229.

Innate immunity in Alzheimer's disease

Only recently has neuroinflammation emerged as an important component of AD pathology

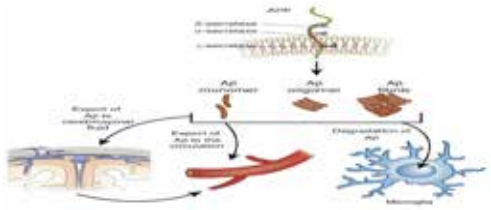


Figure 1 Clearance of Aβ.

Heneka, M.T., Golenbock, D.T. and Latz, E., 2015. Innate immunity in Alzheimer's disease. *Nature immunology*, *16*(3), p.229.

External and internal influences on AD

Risk factors for the development of AD include advanced age, sterile damage or infectious challenges. Furthermore, lifestyle factors and metabolic changes contribute to the risk of developing AD.

Heneka, M.T., Golenbock, D.T. and Latz, E., 2015. Innate immunity in Alzheimer's disease. *Nature immunology*, *16*(3), p.229.

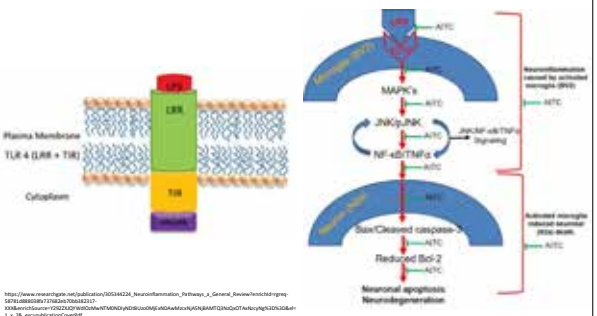
Дохио дамжих зам

- TLR нь нянгийн PAMPs-тай холбогдон NF-kB транскрипцийн хүчин зүйл идэвхжих дохио дамжин үлмаар цитокин, хемокиныг нийлэгшүүлэлт идэвхжинэ.
- Мөн бусад замуудыг идэвхжүүлдэг.

Signal transduction pathways

- Phosphoinositol 3-kinase/protein kinase B (PI3K/AKT)
- Mitogen- activated protein kinase (MAPK)
- NF-kB activation

Pamps and damps signal 0s that spur autophagy and immunity.Daolin tang et al



https://www.researchgate.net/publication/305344224_Neuroinflammation_Pathways_a_General_Review?enrichId=rgreq-58781d888038b737682eb70bb382317-XXX&enrichSource=Y292ZXJQYWdlOzMwNTM0NDIyNDtBS1oo0MjEzNDAwMzcxNjA5NjBAMTQ2NzQxOTAwNzcyNg%3D%3D&el=1_x_2&_esc=publicationCoverPdf

NF-kB

- Идэвхжсэн В эсийн иммуноглобулины хөнгөн-гинж-чангалах хэсгийн кВ хэсэгтэй холбогддог.
- NF-kB үүргийн нэгдэл нь
  - ДНА транскрипци
  - Цитокины ялгаралт
  - Эсийн амьд үлдэх чадвар
  - Үрэвсэл, апоптоз, дархлааны үйл ажиллагааг зохицуулдаг.
- NF-kB идэвхжихэд нейронуудын амьд үлдэх чадвар, уян хатан чанарыг идэвхжүүлдэг.
- Глиал эсүүд мэдрэл сөнөрөлийн үеийн үрэвсэлд чухал үүрэгтэй.

NF-kB

- Nuclear Factor kappa-light-chain-enhancer of activated B cell.
- Protein complex that controls:
  - Transcription of DNA
  - Cytokine production
  - Cell survival
  - Inflammation, apoptosis, immunity
- Regulating immune response to infection.
- Activation of neurons promotes survival and plasticity.
- Glial cells plays a major role in inflammation process which is neurodegenerative.

https://www.researchgate.net/publication/305344224

Pro - inflammatory cytokines and neuroinflammation pathway
Үрэвслийн цитокин ба мэдрэлийн үрэвслийн зам

- Апоптоз, үрэвсэл болон TNF-α -ийн дохио дамжуулалт нь төрөлхийн дархлаа хариу үрвал хамааралт микроглио эсийн үйл ажиллагаатай хамааралтай
- TNF-α ба IL-1β –ийн түвшин ихсэхэд нейроны үхдэг нь өмнө ажиглагдсан.

- Microglia functions related to an innate immune response are associated withTNF-α signaling and its regulation of both inflammation and apoptosis
- Increases in TNF-α and IL-1β have been observed prior to neuronal death

https://www.researchgate.net/publication/305344224_Neuroinflammation_Pathways_a_General_Review?enrichId=rgreq-58781d888038b737682eb70bb382317-XXX&enrichSource=Y292ZXJQYWdlOzMwNTM0NDIyNDtBS1oo0MjEzNDAwMzcxNjA5NjBAMTQ3NzQwOTAxNzcyNg%3D%3D&el=1_x_2&_esc=publicationCoverPdf

NF-kB идэвхжлийн зам

- Гадны эмгэгтөрөгчийг TLR танина. (TLR 1 - 5)
- TLR нь
  - эсийн гаднах LRR /лейцин баялаг рецептор/
  - Цитоплазм дах TIR /Toll/IL-1 рецептор/
- My88/миелойд ялгаран хөгжлийн фактор/ тохируулагч үүраг – TLR холбогдон (TLR3-с бусад) дохио дамжих зам идэвхжинэ.
- ТМС халдвар, астроцит идэвхжлийн үед My88 үүсэх зам чухал үүрэг гүйцэтгэдэг.

NF-kB activation pathway

- TLR contain:
  - an extracellular leucine-rich repeat domain /LRR/
  - Toll/IL-1 receptor /TIR/ domain in cytoplasmic.
- Myeloid differentiating factor 88 /My88/, adaptor protein, bind to TLRs via their TIR domain, which activates several signal transduction pathways and NF-kB activation and inflammation.
- My88 pathways plays a role in CNS infection and consequent astrocyte activation.

Pro - inflammatory cytokines and neuroinflammation pathway
Үрэвслийн цитокин ба мэдрэлийн үрэвслийн зам

- IL-1β ба TNF-α нь өвчний хүрд болон үрэвслийн эмгэг жамд голлох үүрэг гүйцэтгэдэг
- Цус тархины хоригийн (ЦТХ) бүрэн бүтэн байдал алдах, адгезив-молекулын нийлэгжлийн зохицуулга ихсэх, азотын оксид (NO) зэрэг хорт бодисын нэвчитгий чанар ихсэх шалгаан нь TNF-α болон IL-1β байдаг

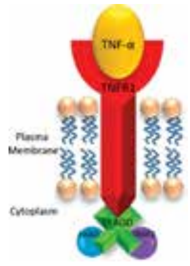
- IL-1β and TNF-α play an integral role in pathological inflammation and the acceleration of disease
- TNF-α and IL-1β can cause blood–brain barrier(BBB) breakdown, up-regulate adhesion-molecule expression and stimulate diffusion of toxic substances such as nitric oxide (NO)

Pro - inflammatory cytokines and neuroinflammation pathway

Үрэвслийн цитокин ба мэдрэлийн үрэвслийн зам

- Харвалт, ишеми, тархины гэмтэл зэрэг цочмог мэдрэлийн үрэвсэлт нөхцөл мөн түүнчлэн Альцхаймерийн өвчин, Паркинсоны өвчин зэрэг архаг мэдрэлийн сөнөрөлт өвчний даамжиралтанд IL-1*β* хамгийн чухал голлох үүрэгтэй оролцдог.

- IL-1*β* plays a crucial role in the progression of chronic neurodegenerative diseases such as AD and PD as well as acute neuroinflammatory conditions including stroke, ischemia and brain injury.



TNFR1 дохио дамжуулалтын эмгэгжам

- (TNFR1) рецептортэй TNF-*α* холбогдоно
- TNF-ийн рецептор-1 нь апоптоз өдөөгч уургийн хэсэгтэй (TRADD) холбоотой
- Мэдрэлийн үрэвсэл болон апоптозын эмгэг жам идэвхижсэн байдал

TNFR1 signaling pathway

- TNF-*α* binds to its receptor (TNFR1), and following tumor necrosis factor receptor 1-associated death domain protein (TRADD), binding, neuroinflammation and apoptosis pathways can be initiated

https://www.researchgate.net/publication/305344224_Neuroinflammation_Pathways_a_General_Review?enrichId=rgreq-58781d888038fa737682eb70bb382317-XXX&enrichSource=Y292ZXJQYWdlOzMwNTM0NDIyNDtBUzo0MjExNDAwMzcxNjA5NjBAMTQ3NzQxOTAxNzcyNg%3D%3D&el=1_x_2&_esc=publicationCoverPdf

Thank you for your attention!

Sevjidmaa Baasanjav /MD, PhD/

Mongolian National University of Medical Sciences (MNUMS)

☎: (976)-99190921
✉: sevjidmaa@mnums.edu.mn

Career Progression:

2001-2002
Scientific Assistant, Healthcare Institute of Mongolia

2004-2006
Medical Genetics Institute at Charitй Berlin (Governmental Scholarship of Mongolia), Scientific Assistant in the subject of Human Genetics in the Medical Genetics Institute at the Charity Berlin (Campus Virchow)

2006-2008
Scientific Assistant at the Nephrological Section, Endocrinological and Nephrological Hospital, Department for Internal Medicine, Neurology and Dermatology, University Medical Center Leipzig АцR

2010-2011
Scientific Assistant at Nephrological Section, Endocrinological and Nephrological Hospital, Department for Internal Medicine, Neurology and Dermatology, University Medical Center Leipzig АцR

2011-2014
Scientific Assistant at the Institute for Human Genetics, Martin-Luther University Halle-Wittenberg

2014-2017
Mongolian National University of Medical, School of Pharmacy and Bio-Medicine, Department of Physiology

Since 2017
Head of Department of Physiology

Education:

1995-2001 Study of Human Medicine, Medical State University of Mongolia

Nov. 2007 Diploma Recognition of Medical State University of Mongolia by the Charity

Awards and Scholarships:

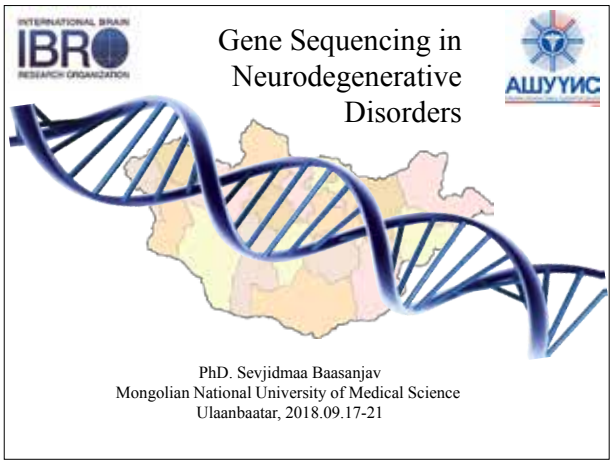
Governmental Scholarship of Mongolia

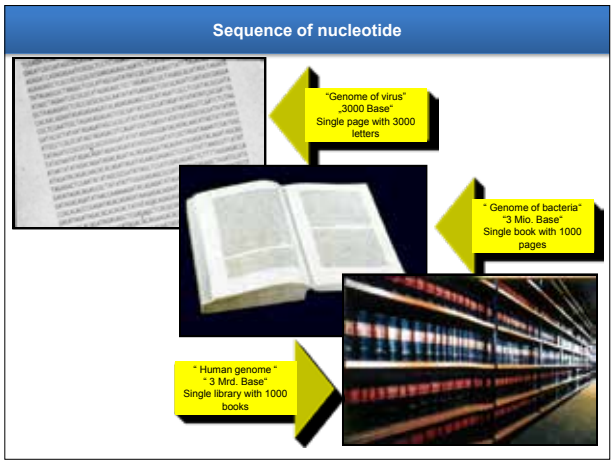
Scholarship for Completion of the Dissertation at the Charitй Berlin

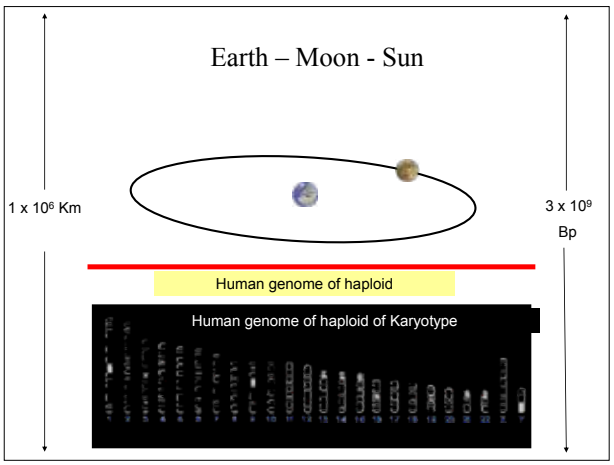
Research Experience:

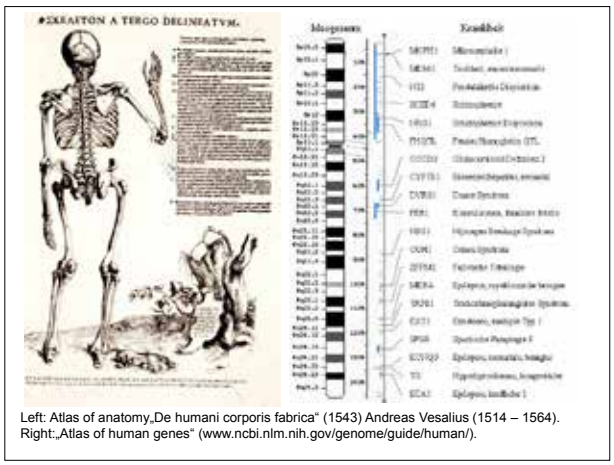
1. Faulty initiation of proteoglycan synthesis causes cardiac and joint defects.
   Baasanjav S, Al-Gazali L, Hashiguchi T, Mizumoto S, Fischer B, Horn D, Seelow D, Ali BR, Aziz SA, Langer R, Saleh AA, Becker C, Nьrnberg G, Cantagrel V, Gleeson JG, Gomez D, Michel JB, Stricker S, Lindner TH, Nьrnberg P, Sugahara K, Mundlos S, Hoffmann K.
2. Mutations causing Greenberg dysplasia but not Pelger anomaly uncouple enzymatic from structural functions of a nuclear membrane protein.
   Clayton P, Fischer B, Mann A, Mansour S, Rossier E, Veen M, Lang C, Baasanjav S, Kieslich M, Brossuleit K, Gravemann S, Schnipper N, Karbasyian M, Demuth I, Zwerger M, Vaya A, Utermann G, Mundlos S, Stricker S, Sperling K, Hoffmann K.
3. Marfan syndrome with neonatal progeroid syndrome-like lipodystrophy associated with a novel frameshift mutation at the 3' terminus of the FBN1-gene.
   Graul-Neumann LM, Kienitz T, Robinson PN, Baasanjav S, Karow B, Gillessen-Kaesbach G, Fahsold R, Schmidt H, Hoffmann K, Passarge E.
4. Osteopoikilosis and multiple exostoses caused by novel mutations in LEMD3 and EXT1 genes respectively--coincidence within one family.
   Baasanjav S, Jamsheer A, Kolanczyk M, Horn D, Latos T, Hoffmann K, Latos-Bielenska A, Mundlos S.

Lecture 5

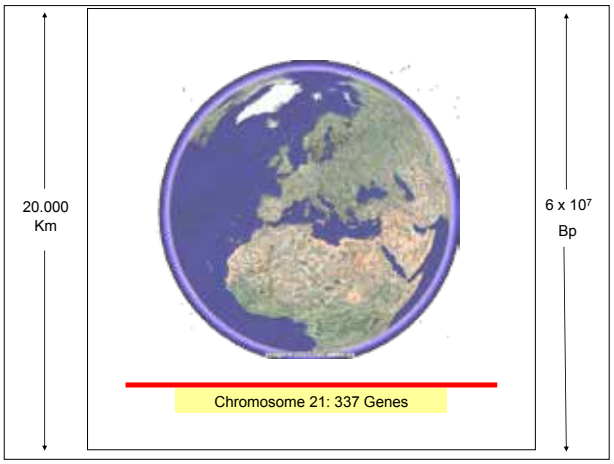


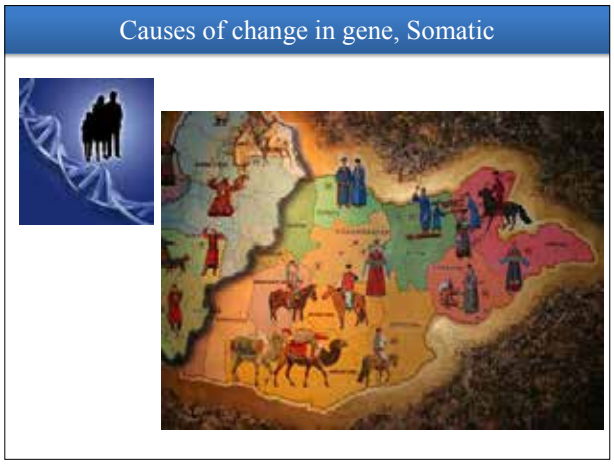






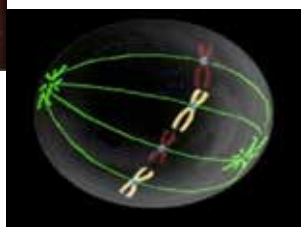
Left: Atlas of anatomy„De humani corporis fabrica" (1543) Andreas Vesalius (1514 – 1564).
Right:„Atlas of human genes" (www.ncbi.nlm.nih.gov/genome/guide/human/).



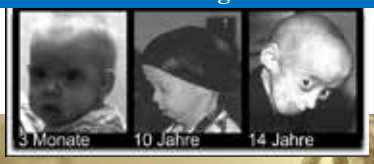


Causes of change in gene, cell division

Mitosis and meiosis



2. Progeria



- **Extending lifespan**
  - limited growth and short stature
  - lack of body fat and muscle
  - generalized atherosclerosis, leading to cardiovascular and heart disease
  - osteoporosis
  - loss of hair, including eyelashes and eyebrows
  - a high-pitched voice
- **Alzheimer's disease**

- Lamin A Receptor
- *ZMPSTE24*-Gene

Causes of change in gene, Spontaneous mutation

2. Progeria

Loss of "Lamin A Receptor"
Loss of nutrients in 34th week of pregnancy

1. Chorea Huntington

- movement disorders
- cognitive and psychiatric disorders

Difficulty organizing, prioritizing or focusing on tasks
Feelings of irritability, sadness or apathy
Mental retardation

- Heredity
  - autosomal dominant
  - Huntington-Gene
  - 67 Exons

Human Genome Project

1990
Exon sequence

SANGER sequencing
99.9%

2008
Exom sequence

NEXT GENERATION SEQUENCING
99.9%

1000 Genome Project

NEXT GENERATION SEQUENCING

2008 он

1000 Genes
1000 candidates
3.8 terabase sequences
11 million (SNP)

2009

1200 Human
Complete Sequencing

1. HGMD-Human Genome Mutation Database

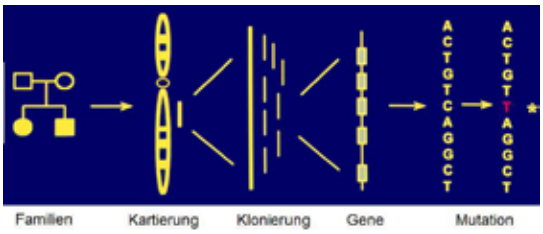
1000 Genome Project

"NEXT GENERATION SEQUENCING" Technology

Map of Human genetic variation

2. Mutation t@ster

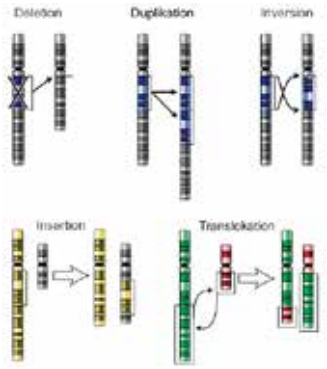
Main scheme of mutation



3. Polyphen

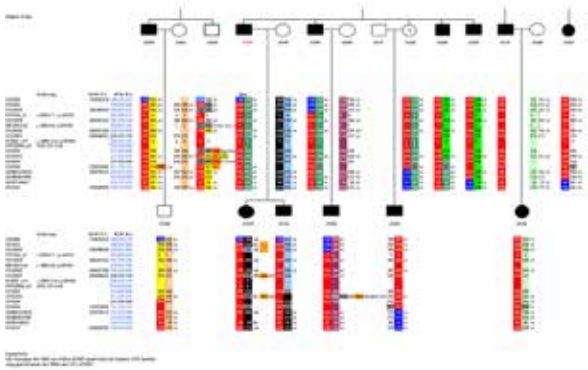
4. NCBI, Population genetic data

Types of mutations

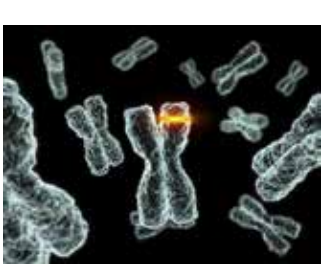


5. Bioinformatic-method of genetic variations

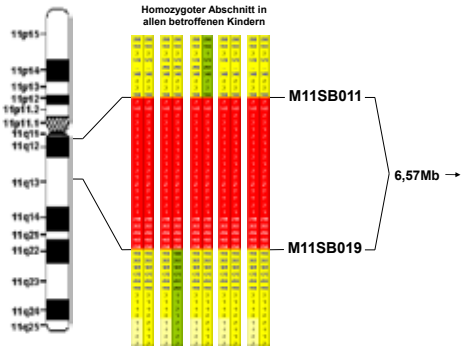
Autosomal dominance of inheritance



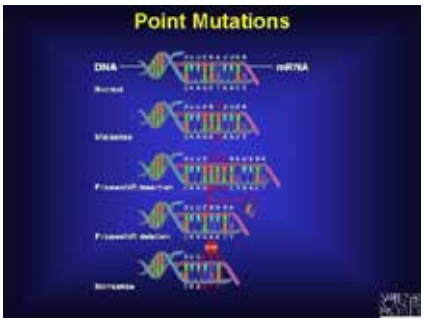
What is Mutation?



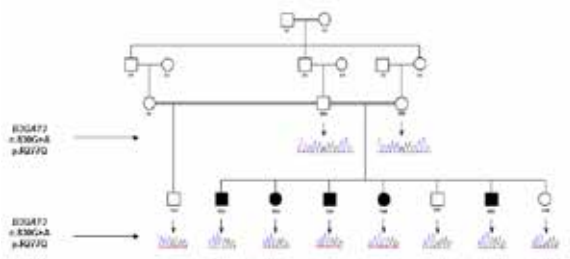
Autosomal recessive of inheritance



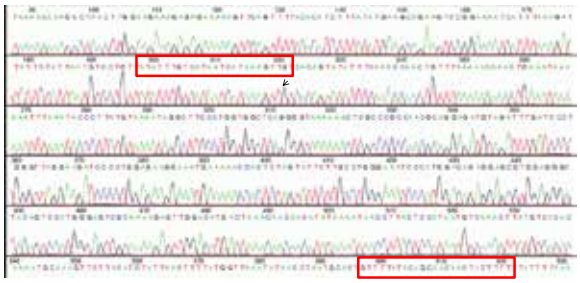
Types of point mutations



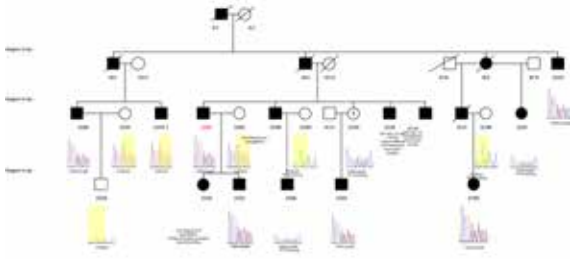
Point Mutation



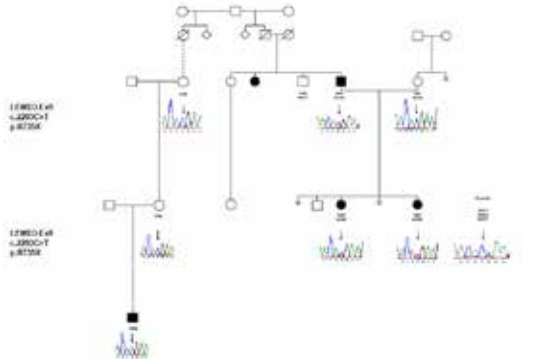
Sequence of nucleotide



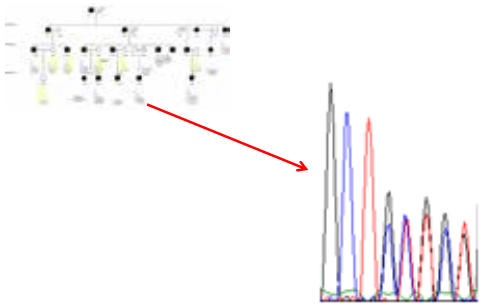
Frameshift mutation
deletion, insertion



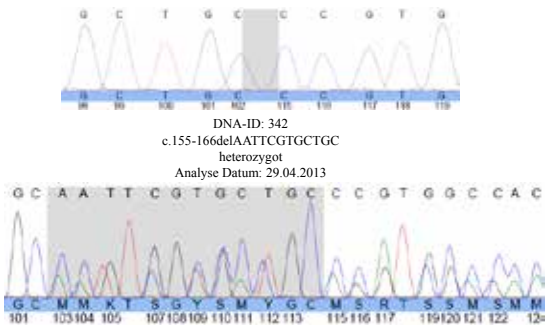
Nonsense, stop Mutation



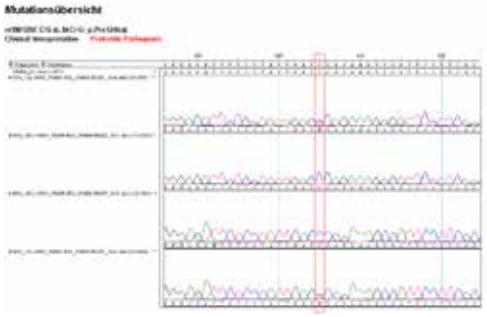
Frameshift mutation
deletion, insertion



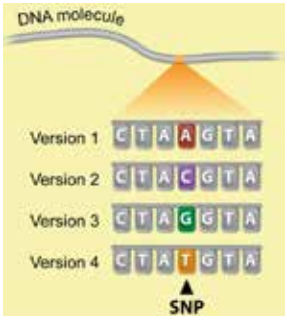
Frameshift mutation
deletion, insertion



Polymorphismus

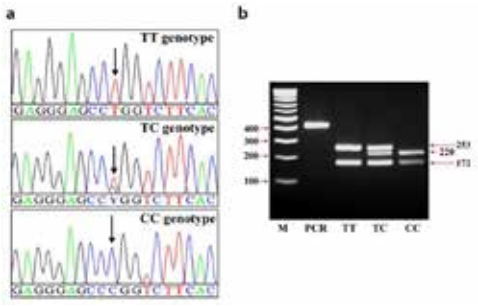


Polymorphismus



Thank you for your attention!

Polymorphismus



Battuvshin Lkhagvasuren /MD, PhD/

Head, Science and Technology Center, MNUMS
Zorig Street 3, Ulaanbaatar, 14210, Mongolia

☎:+976-99192738
✉: battuvshin@neuroscience.mn
🌍: neuroscience.mn/?=180

Education and Qualifications

2013
PhD. in Medicine, Graduate School of Medical Sciences, Kyushu University, Japan
Thesis: Social defeat stress induces hyperthermia through activation of thermoregulatory sympathetic premotor neurons in the medullary raphe region (Supervisor: Prof. Oka T.)

2009
Internship in Psychosomatic Medicine, Kyushu University hospital, Kyushu University, Japan (Supervisor: Prof. Kubo C.)

2006
Residency in Psychiatry, National Center of Mental Health, Mongolia
Clinical psychiatry (Supervisor: Prof. Sandag B.)

2005
Medical Doctor, Mongolian National University of Medical Sciences (MNUMS), Mongolia, & University of Mainz, Germany (1998 – 2004)

Research area

Neuroscience, Psychosomatic Medicine, Psychiatry

Awards & Grants

2014
Cousins Center Global Outreach Awards – by American Psychosomatic Society

2014
IBRO Return Home Program Grant - by International Brain Research Organization

2012
Ikemi Memorial Award – by the Japanese Society of Psychosomatic Medicine

2008 - 2013
Japanese Government Academic Scholarship – by Japanese Government

2007
WHO Research Grant – This grant was supported by World Health Organization

1997
Hasebe Award – by MNUMS

Professional Services

2018 - ... Ad-Interim Board Member of IBRO-APRC

2018 - ... President, Mongolian Neuroscience Society

2015 - ... President, Mongolian Society of Psychiatry

2014 - ... Expert Panel Member, Asian Federation of Psychiatric Associations

2013 - ... Board Member, Mongolian-Japanese Association for Medical Education

Journal review

2015 - ... AdHoc Reviewer, Temperature

2013 - ... AdHoc Reviewer, Psychotherapy & Psychosomatics

2015 - ... AdHoc Reviewer, PloS One

Invited Lectures

August 2017

Thermosensation & Interoception: Limbic system is involved in behavioral thermoregulation, MyNeuro 2017, Kuala Lumpur, Malaysia

August 2016

Behavioral and autonomic thermoregulation of psychological stress-induced hyperthermia, NPAS-IBMS Joint Seminar, Taipei, Taiwan

September 2015

Recent advances in neuroscience, National Center of Mental Health. Ulaanbaatar, Mongolia

April 2014

Neural circuitry of psychological stress-induced hyperthermia, Barrow Neurological Institute, Phoenix, USA

Publications

2007 - ...

Peer-reviewed academic articles in international journals: 16, contributions to academic meetings: 31, textbooks and edited books: 2

Specialized training

Laboratory: Optogenetics, viral tracing, Immunohistochemistry, fluorescent and confocal microscopy, brain lesioning, behavioral testing, electrophysiological testing, RT-PCR, HPLC, Western blot, ELISA.

Lecture 6

Title

Neural Correlates of Consciousness

Abstract

Despite the advances in the research on the neural correlates of consciousness, the mechanisms underlying conscious perception and behavior have not been discovered yet. I would like to describe recent findings referring the anatomical correlates of consciousness are primarily localized to cortical areas that include sensory and fronto-parietal accessory cortices that involved in volunteer monitoring and reporting. Some other candidate neurophysiological markers of consciousness will be also identified to build a physical substrates that offer quantitative indices of consciousness. More about the promising conceptions including interoceptive inference and its relationship to consciousness in the brain will be reviewed to discuss philosophical determinations of consciousness, i.e. arguing the mind and body problem.

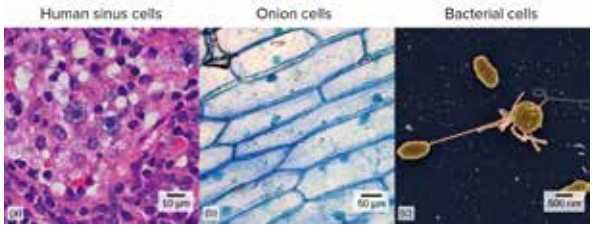
TL 1 – Lecturer: Baljinnyam T, IMS

Cell culture

T.Baljiinyam

- These conditions vary for each cell type, but generally consist of a suitable vessel with a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO2, O2), and regulates the physio-chemical environment (pH buffer, osmotic pressure, temperature).

Cell



- The **cell** is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life.

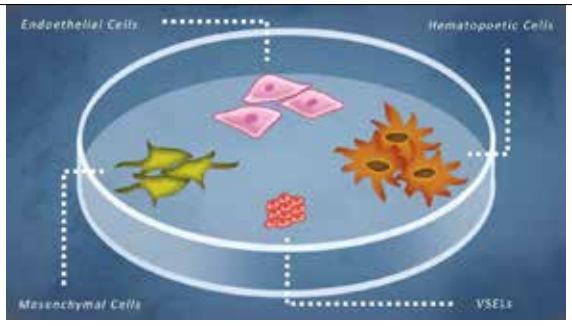
History of Cell Culture


Wilhelm Roux

1885: Wilhelm Roux maintained embryonic chick cells in a saline culture.


Ross Granville Harrison

1907: Ross Granville Harrison cultivated frog nerve cells in a lymph clot held by the "hanging drop" method and observed the growth of nerve fibers in vitro for several weeks.



- Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment. After the cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions.

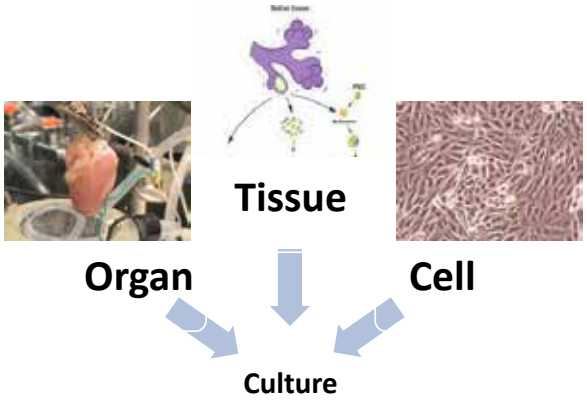
Major development's in cell culture technology

- 1910: Burrows succeeded in long term cultivation of chicken embryo cell in plasma clots. He made detailed observation of mitosis.
- 1911: Lewis and Lewis made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones. They observed limited monolayer growth.
- 1916: Rous and Jones introduced proteolytic enzyme trypsin for the subculture of adherent cells.
- 1940s: The use of the antibiotics penicillin and streptomycin in culture medium decreased the problem of contamination in cell culture.
- 1948: Earle isolated mouse L fibroblasts which formed clones from single cells. Fischer developed a chemically defined medium, CMRL 1066.

- 1955: Eagle studied the nutrient requirements of selected cells in culture and established the first widely used chemically defined medium.
- 1965: Ham introduced the first serum-free medium which was able to support the growth of some cells
- 1965: Harris and Watkins were able to fuse human and mouse cells by the use of a virus.
- 1975: Kohler and Milstein produced the first hybridoma capable of secreting a monoclonal antibody.
- 1985: Human growth hormone produced from recombinant bacteria was accepted for therapeutic use.
- 1990: Recombinant products in clinical trial (HBsAG, factor VIII, HIVgp120, CD4, GM-CSF, EGF, mAbs, IL-2).

B-Tissue Culture

- Fragments of excised tissue are grown in culture media
- a fragment of tissue is placed at a glass (or plastic)–liquid interface, where, after attachment, migration is promoted in the plane of the solid substrate
- Advantages
  - Some normal functions may be maintained.
  - Better than organ culture for scale-up but not ideal.
- Disadvantages
  - Original organization of tissue is lost.

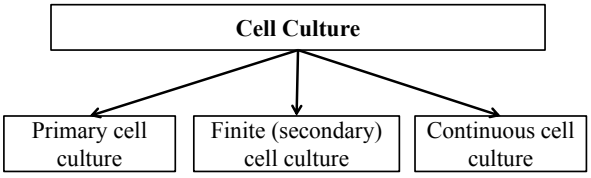


C- Cell Culture

- Implies that the tissue, or outgrowth from the primary explant, is dispersed (mechanically or enzymatically) into a cell suspension, which may then be cultured as an adherent monolayer on a solid substrate or as a suspension in the culture medium
- Advantages
  - Development of a cell line over several generations
  - Scale-up is possible
- Disadvantages
  - Cells may lose some differentiated characteristics.

A- Organ Culture

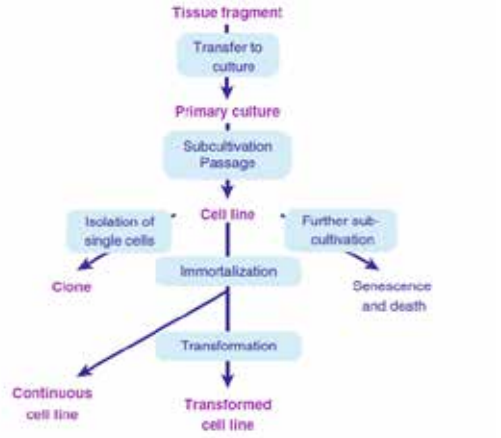
- The entire embryos or organs are excised from the body and culture
- favors the retention of a spherical or three-dimensional
- Advantages
  - Normal physiological functions are maintained.
  - Cells remain fully differentiated.
- Disadvantages
  - Scale-up is not recommended.
  - Growth is slow.
  - Fresh explantation is required for every experiment.



Primary cell culture: Normal cells cultured without any change in their division rate

Finite (secondary) cell culture: Single cell type roughly thirty times of division, enhanced by growth factors

Continuous cell culture: It is nearly the same as finite but the cells here can divide indefinitely by transformation into tumor cells, They are called cell line



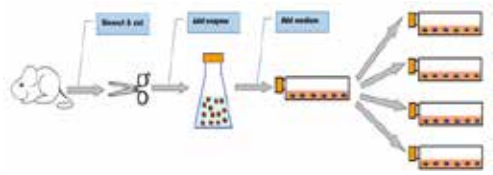
Advantages:

- Usually retain many of the differentiated characteristics of the cell in vivo

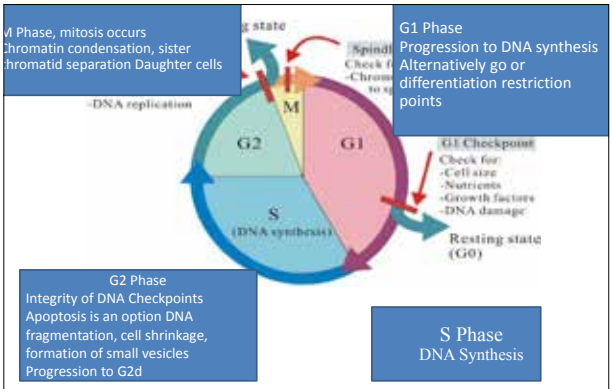
Disadvantages:

- Initially heterogeneous but later become dominated by fibroblasts.
- The preparation of primary cultures is labor intensive
- Can be maintained in vitro only for a limited period of time.

Primary Cell Culture



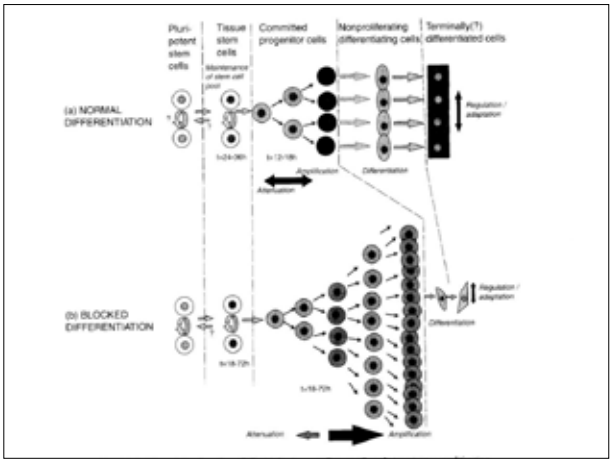
- These are directly derived from tissue explants or disaggregated tissue samples and therefore contain a mixture of cell types. Unlike 'immortalized cell lines', primary cells have not been altered in anyway and have a finite lifespan.



Finite Cell Lines Can Change To Continuous
Often p53 Mutation or Deletion Occurs
Overexpression Of Telomerase

Characteristics of primary culture

- Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow are called as primary culture
- Sub culturing of primary cells leads to the generation of cell lines
- Cell lines have limited life span, they passage several times before they become senescent
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures
- Lineage of cells originating from the primary culture is called a cell strain



Second Cell Lines

- The cells in culture divide only a limited number of times, before their growth rate declines and they eventually die.
- The cell lines with limited culture life spans are referred to as finite cell lines.
- The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction.
- The actual number of doublings depends on the species, cell lineage differences, culture conditions etc.
- The human cells generally divide 50-100 times, while murine cells divide 30-50 times before dying

Characteristics of continuous cell culture

- Smaller, more rounded, less adherent with a higher nucleus /cytoplasm ratio
- Fast growth
- Grow more in suspension conditions
- Ability to grow up to higher cell density
- Stop expressing tissue-specific genes
- Different in phenotypes from donar tissue

Continuous Cell Lines

- A few cells in culture may acquire a different morphology and get altered.
- Such cells are capable of growing faster resulting in an independent culture.
- The progeny derived from these altered cells has unlimited life (unlike the cell strains from which they originated).
- They are designated as continuous cell lines.
- The continuous cell lines are transformed, immortal and tumorigenic.
- The transformed cells for continuous cell lines may be obtained from normal primary cell cultures (or cells strains) by treating them with chemical carcinogens or by infecting with oncogenic viruses

Features	Second	Continuous
ploidy	Diploid	Heteroploid
Transformation	Normal	Transformed
Density limitation og growth	Yes	No
Mode of growth	Monolayer	Monolayer of suspension
Maintenance	Cyclic	Steady state
Serum requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromsomal, Enzymic
Virus susceptibility, diferentation	May be retained	Often lost
Growth rate	Low (24-96) hr	Rapid (12-24) hr
Control features	Generation	Strain

Continuous cell cultures

- Cell lines which either occur spontaneously or induced virally are chemically transformed into continuous cell lines.
- The hallmark of stably transfected cells is that the foreign gene becomes part of the genome and is therefore replicated.
- Descendants of these transfected cells, therefore, will also express the new gene, resulting in a stably transfected cell line.

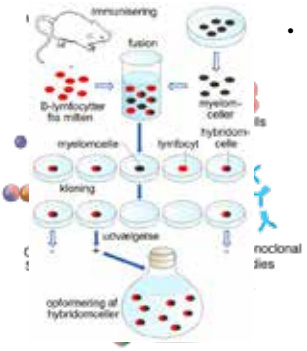
Cell line

1. Normal
- Taken from a tumor tissue and cultured as a single cell type
2. Transformed
- Normal cells underwent a genetic change to be tumor cells
3. Stem cell
- They are Master Cells that generate Other differentiated cell types

Transient cell line

- Transiently transfected cells express the foreign gene but do not integrate it into their genome. Thus the new gene will not be replicated. These cells express the transiently transfected gene for a finite period of time, usually several days, after which the foreign gene is lost through cell division or other factors.

Hybridoma cell culture



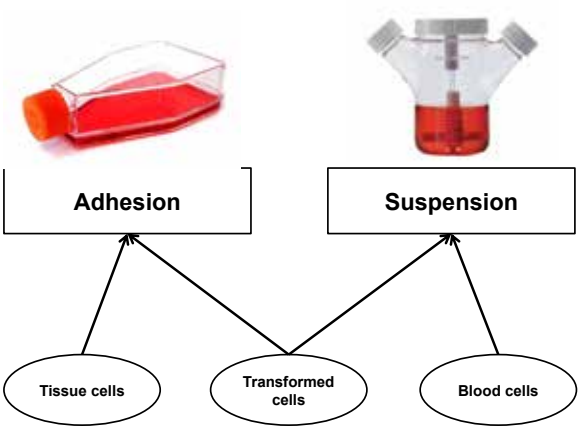
- Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies).

First cancer cell line (HELA)

George Otto Gey

Henrietta Lacks

- In february 1951, Henrietta Lacks was diagnosed with cervical cancer at the John Hopkins Gynecology clinic in Baltimore.
- While Henrietta Lacks was treated at Johns Hopkins, Dr Gey was attempting to fulfill ambitious goals for theTissue Culture Laboratory.
- Cells obtained from the biopsy specimen of Henrietta Lacks into culture by using the roller-tube technique; the cells grew robustly, contrary to the results with previous specimens, becoming the first human cancer cell line immortalized in tissue culture.



Types of cells

On the basis of morphology (shape & appearance) or on their functional characteristics.

They are divided into three.

- **Epithelial like-** attached to a substrate and appears flattened and polygonal in shape
- **Lymphoblast like-** cells do not attach remain in suspension with a spherical shape
- **Fibroblast like-** cells attached to an substrate appears elongated and bipolar

Cell culture applications

Model systems
- Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies
- Study of the function of nerve cells

Toxicity testing
- Study the effects of new drugs

Cancer research
- Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

Virology
- Cultivation of virus for vaccine production, also used to study there infectious cycle.

Genetic Engineering
- Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles

Cell Contamination's

Chemical
- Endotoxin
- Plasticizers
- Metal ions
- Traces of disinfectants

Biological
- Mycoplasma
- Yeast
- Bacteria
- Fungus
- Other cell lines

1. Cell concentration:
- The cultures with high cell concentration utilize the nutrients in the medium faster than those with low concentration; hence the medium is required to be changed more frequently for the former.

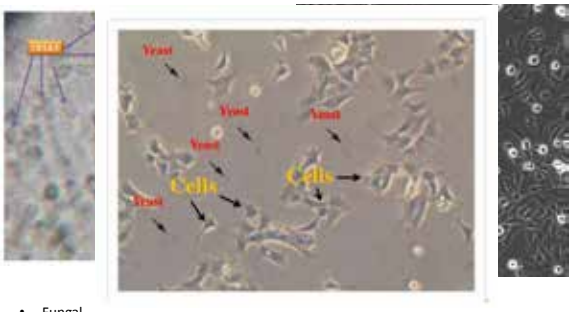
2. A decrease in pH:
- A fall in the pH of the medium is an indication for a change of medium. Most of the cells can grow optimally at pH 7.0, and they almost stop growing when the pH falls to 6.5.

Chemical Contamination's

Nomenclature of Cell Lines

- It is a common practice to give codes or designations to cell lines for their identification.
- For instance, the code **NHB 2-1** represents the cell line from **normal human brain**, followed by **cell strain** (or cell line number) 2 and **clone number** 1.
- The usual practice in a culture laboratory is to maintain a **log book** or computer database file for each of the cell lines.
- While naming the cell lines, it is absolutely necessary to ensure that each cell line designation is unique so that there occurs no confusion when reports are given in literature. Further, at the time of publication, the-cell line should be prefixed with a code designating the laboratory from which it was obtained e.g. NCI for **National Cancer Institute,** WI for Wistar Institute.

Biological Contamination's



- Fungal
- Achromobacter
- Yeast

The best and the oldest way to eliminate contamination is to discard the infected cell lines directly

1. Species:
- In general, non-human cell lines have less risk of biohazards, hence preferred.
- However, species differences need to be taken into account while extrapolating the data to humans.

2. Finite or continuous cell lines:
- Cultures with continuous cell lines are preferred as they grow faster, easy to clone and maintain, and produce higher yield. But it is doubtful whether the continuous cell lines express the right and appropriate functions of the cells. Therefore, some workers suggest the use of finite cell lines, although it is difficult.

3. Normal or transformed cells:
- The transformed cells are preferred as they are immortalized and grow rapidly.

4. Availability:
- The ready availability of cell lines is also important. Sometimes, it may be necessary to develop a particular cell line in a laboratory.

5. Growth characteristics:
- The following growth parameters need to be considered:
- i. Population doubling time
- ii. Ability to grow in suspension
- iii. Saturation density (yield per flask)
- iv. Cloning efficiency.

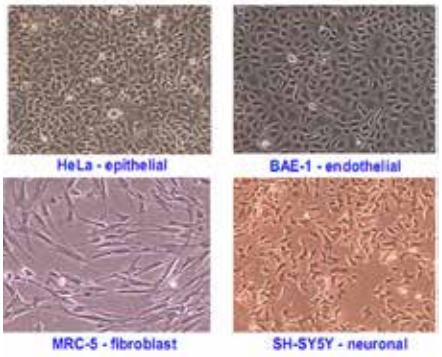
6. Stability:
- The stability of cell line with particular reference to cloning, generation of adequate stock and storage are important.

7. Phenotypic expression:
- It is important that the cell lines possess cells with the right phenotypic expression.

Maintenance of Cell Cultures:
- For the routine and good maintenance of cell lines in culture (primary culture or subculture) the examination of cell morphology and the periodic change of medium are very important.

Common cell lines

- Human cell lines
- -MCF-7 breast cancer
- HL 60 Leukemia
- HEK-293 Human embryonic kidney
- HeLa Henrietta lacks
- Primate cell lines
- Vero African green monkey kidney epithelial cells
- Cos-7 African green monkey kidney cells
- And others such as CHO from hamster, sf9 & sf21 from insect cells



Cell Line: HELA

- Species: Homo Sapiens
- Cell type: Cervix carcinoma
- Origin: established from the epitheloid cervix carcinoma of a 31-year-old black woman in 1951; later diagnosis changed to adenocarcinoma; first aneuploid, continuously cultured human cell line
- Morphology: epithelial-like cells growing in monolayers
- Risk assessment:The cell line is infected with papilloma virus type 18 (HPV-18).The cells contain several copies as proviruses integrated into the eukaryotic genome.The integrated virus genomes are incomplete and exhibit 2-3 kb deletions of the E2-L2 region. An activation and transmission of the HPV-18 during handling of the cell line is improbable.The cell line is categorized biosafety level 1.
- Viruses: ELISA: reverse transcriptase negative; PCR: EBV -, HBV -, HCV -, HHV- 8 -, HIV -, HPV +, HTLV-I/II -, MLV -, SMRV - 10

Neurosciences in cell culture

- The culture of **neuronal cells** is particularly challenging since mature neurons do not undergo cell division.
- One way to overcome this is to establish secondary cell lines that are derived from **neuronal tumors** and have become immortalized.

Cell Line: PC 12

- Species: rat (Rattus norvegicus)
- Cell type: adrenal pheochromocytoma
- Origin: established from a transplantable rat adrenalpheochromocytoma in 1976; cells were described to synthesize catecholamines (dopamine, norepinephrine); in response to nerve growth factor (NGF) a neuronal phenotype could be induced reversibly
- Morphology: small cells growing in clumps in suspension, adhering poorly to plastic;
- Viruses: ELISA: reverse transcriptase negative; PCR: SMRV - 14

Cell refreshing assay

Cell culture preparation medium

- Cell culture media (RPMI 1640, DMEM)
- Antibiotic mix (Penicillin, Streptomycin)
- FBS/FCS (inactivated)

DNA preparation protocol

Enkhsaikhan Lkhagvasuren, MD, PhD
IBRO summer school - 2018
Mongolia

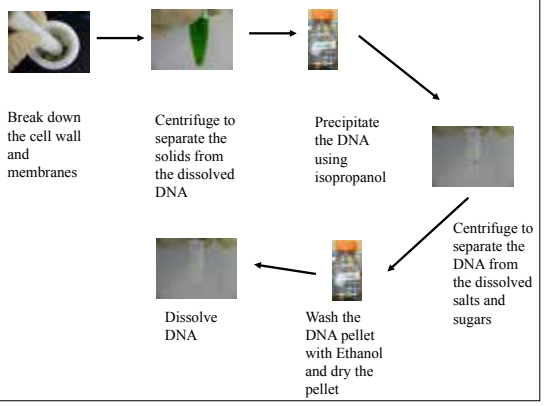
A comparison of DNA extraction methods used in research labs as opposed to classroom labs

Research	Classroom
Lysis: grind in Liquid N_2 and use detergent	***Lysis***: grind in mortar/pestel and use detergent
Precipitation Part I: phenol/chloroform extraction to get rid of proteins	***Precipitation Part I***: NONE (chemical are too dangerous!)
Precipitation Part II: addition of salts to interrupt hydrogen bonding between water and phosphates on the DNA	***Precipitation Part II***: addition of salts to interrupt hydrogen bonding between water and phosphates on the DNA
Precipitation Part III: addition of ethanol to pull DNA out of solution	***Precipitation Part III***: addition of ethanol to pull DNA out of solution
Wash and resuspend: DNA is washed in ethanol, dried, and resuspended in H20 or TE buffer.	***Wash and resuspend:*** DNA is washed in ethanol, dried, and resuspended in H20 or TE buffer.

Outline

- Introduction
- DNA preparation protocol
- Quick methods

Overview of DNA Extraction



Introduction

- It is a routine procedure in the laboratory
- DNA isolation is a process of purification of DNA from sample using a combination of physical and chemical methods.
- The first isolation of DNA was done in 1869 by Friedrich Miescher.


Friedrich Miescher
1844 – 1895

Isolation/purification of total DNA

Sample preparation (mouse ear cutting)

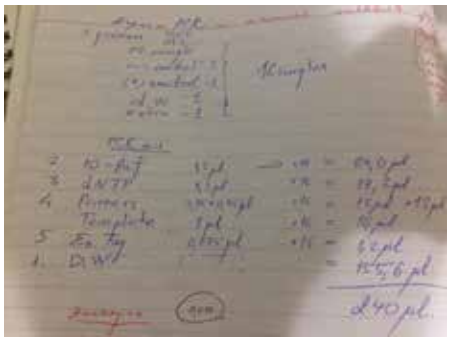
Next step of DNA preparation

- Now we need heating machine and centrifuge
- You need to heat heating machine until 90^0C
- Put samples to heating machine
  (at 90^0C, 10 min)
- Mix (vortex several seconds)
- Spin down 15000 rpm, 2 min
- The template is ready to PCR

Reagent

- Proteinase-K
  - Prepare mixture of lyses solution:
    lyses buffer (1,0 ml) + ProteinaseK (10ul), mix
  - For example, Today we have 12 ears samples.
  - We need 650 ul lyses
    12 x 50 ul + extra 50 ul = 650 ul
    650 ul lyses + 6.5ul ProK.
  - Add 6,5ul mixture to each tube with ear

The template is ready to PCR

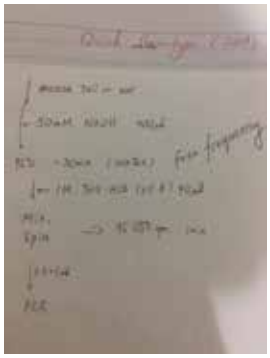


Incubation time

- Spin 15000 rpm several seconds
- Incubate at least 4 hours at 50^0C
  (or at maximum overnight at 50^0C)

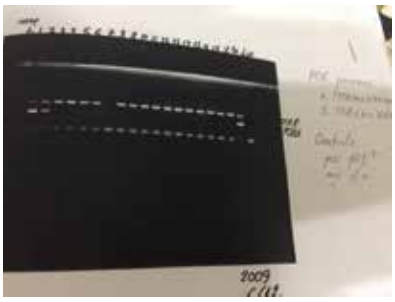
The end of the incubation time, add 450 ul DW to sample.

Quick Genotype (DNA) preparation



- Be careful when you use NaOH. Contact may severely irritate skin, eyes, and mucous membranes. Toxic by ingestion. Corrosive to metals and tissue.

Analyzing DNA samples in the lab



Thank you!

Jambaldorj Jamyansuren /MD, PhD/

Mongolian National University of Medical Sciences (MNUMS)

☎: (976)-89894417
✉: jambaldorj@mnums.edu.mn

Education:

PhD at Department of Neurology, Division of Molecular Biology June 2013
Institute of Health Biosciences, The University of Tokushima, Graduate School

Residency training in Laboratory Medicine, March 2005
Postgraduate Institute, Health Sciences University of Mongolia

Medical Doctor (Bachelor) School of Medicine, May 2003
Health Sciences University of Mongolia

Work Experience:

2016 - present
Lecturer at Department of Molecular Biology and Genetics School of BioMedicine
Lecturer of Cyber University, (Stem cell and Molecular biology)
Mongolian National University of Medical Sciences,

2015-2016
Mongolia Head of Department of Molecular Biology and Genetics,
School of Pharmacy and BioMedicine
Mongolian NationalUniversity of Medical Sciences, Mongolia

2013-2015
Lecturer Department of Molecular Biology and Genetics,
School of Pharmacy and BioMedicine
Mongolian National University of Medical Sciences, Mongolia

2010-2012
Associate Researcher at Advanced Molecular Epidemiology
Research Institute, Faculty of Medicine, GCOE project
The University of Yamagata, Japan

2003-2006
Research Assistant at Central Scientific Research Laboratory
The Institute of Medical Sciences Mongolia

Laboratory Experience:

- SNP genotyping using Illumina systems (HD Infinium and Golden gate assay)
- Karyotyping using Illumina systems (Human Sentrix-12)
- Polymerase chain reaction (nested, cloning, real time, PCR-RFLP and etc)

- TaqMan SNP genotyping
- Cloning (screening, amplification and confirmation)
- Animal experiments with rat and mouse
- Blotting (Southern and Western)
- Sanger sequencing and Gene scan
- General Laboratory Protocols
- Data mining and analyzing
- Cell culture (HeLa and B95)
- SEM and live cell imaging
- Gene expression and developmental experiment
- Recombinant molecule

Past Research Experience

- Research of Charcaot Marie Tooth disease.
- GWAS on QTL: body height in Mongolians.
- Genetics on Cranio cervical dystonia.
- TAF1 on XDP dystonia, developmental study
- Research on SMA.
- Yamagata University Genomic Cohort Consortium, Cohort study on Takahata population.
- GWAS on COPD and epilepsy.

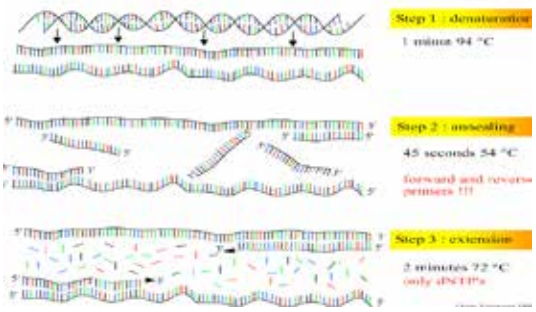
Ongoing Projects

- Genomic research on COPD
- Oligonucleotide synthesis
- Genomic research on dyslipidemy
- Genomic research on epilepsy
- Target gene searching on CMT, BHD and AMD.
- Animal project on mesenchymal stem cell

Essentials in qPCR

Jambaldorj J MD, Ph.D
Department of Molecular Biology and Genetics
Board member of Mongolian Neuroscience Society

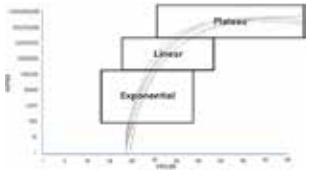
Principle of PCR



History

- 1983, Kary Mullis, PCR
- 1987, First PCR
- 1992, Higuchi R, realtime PCR device
  Higuchi R, Dollinger G, Walsh PS, Griffith R., Simultaneous amplification and detection of specific DNA sequences, Biotechnology (N Y). 1992 Apr;10(4):413-7.

Principle of PCR

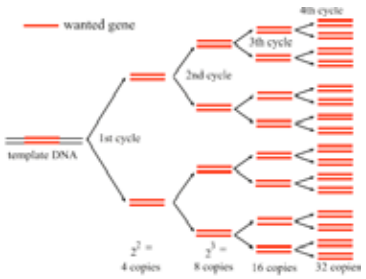


END POINT PCR

- **Exponential**
- **Linear**
- **Plateau**

Principle of PCR

- Amplify target sequence by 30-40 cycles
- After 36 cycles, target will be amplified 68 bln pieces.

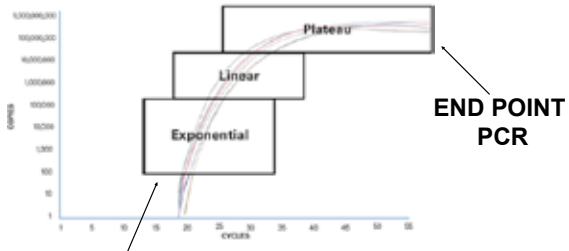


Difference PCR and qPCR

Disadvantage of PCR

- Less accurate
- Less sensitive
- Non automated
- Evaluation on DNA size
- Nonquantitative
- Post processing required

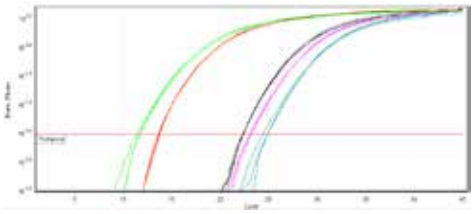
qPCR



- Real time PCR - qPCR

PCR vs qPCR

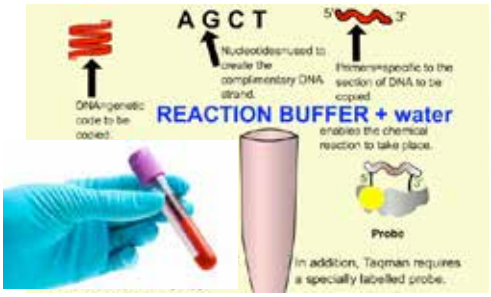
qPCR



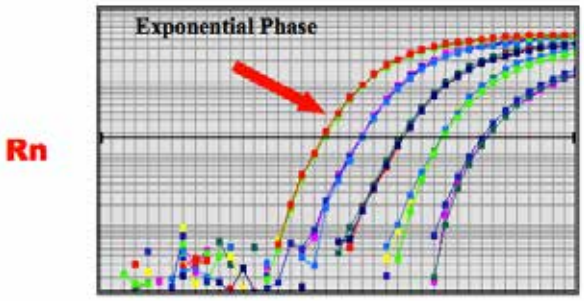
Threshold

- FAM, TAMRA, HEX, CY3, CY5 dye

Reaction mixture



qPCR

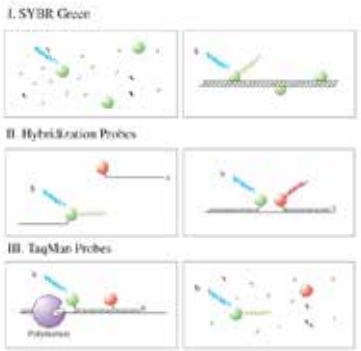


qPCR devices

Bio-Rad, Roche, ABI, DNA technology

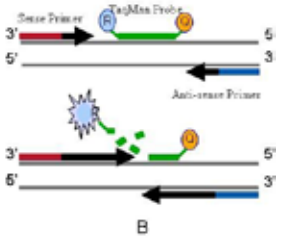
Detection methods

1. SYBR Green chemical
2. Hybridization probe usage
3. TaqMan probe usage



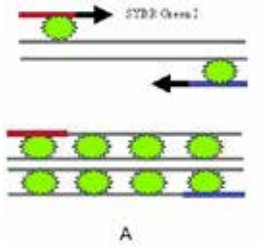
TaqMan method

- Probe contains Dye and Quencher
- Probe recognizes specific site and separates Dye and quencher leads to emission.

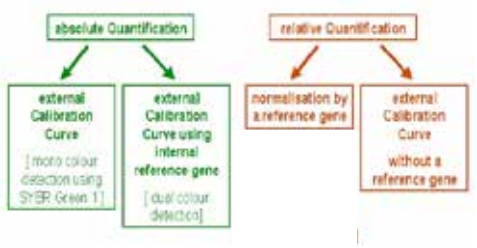


SYBR Green method

- SYBR Green binds between 2 DNA strands.
- Light will be emitted.

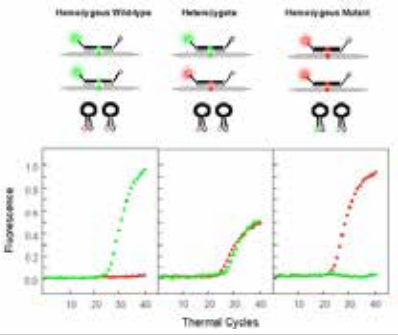


Absolute and relative quantification



Molecular beacon method

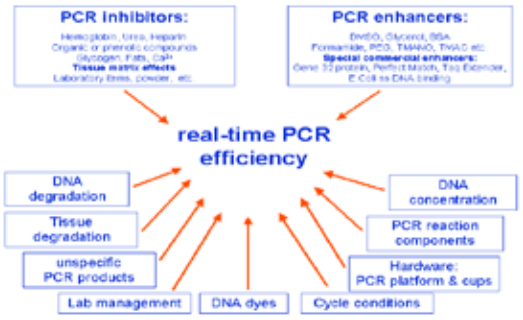
- Probe has specific hairpin structure.
- When dye and quencher close together there is no emission.
- Different probes detects mutant alleles and normal alleles.



Advantage of qPCR

- Accuracy and quantifcation
- No post processing (no preparation of gel, no staining)
- Less toxic due to no use of radioactive and toxic chemicals.

Possible effect of qPCR



Modern trends

- WiFi models and miniatures
- All cycles can be performed on 30 minutes.

https://www.chaibio.com/openqpcr

12 well
18-34min/30cycles

Applications

Diagnostic purpose

- Infectious disease
- Cancer
- Inherited disease
- Food safety
- Detection of GMO

Research purpose

- Gene expression profiles
- SNP determination
- Copy number variation
- Detection of mutation.

INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

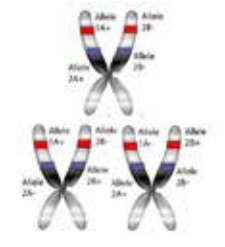
АШУУИС

Genotyping & Haplotyping

Sevjidmaa.B Ph.D

Linkage disequilibrium, LD

- Alleles are in **LD**, if they are inherited together more often than could be expected based on allele frequencies
- Two loci are inherited together, because recombination during meiosis separates them only seldom



I.Genotyping

- Analysis of DNA-sequence variation
- Human DNA sequence is 99.9% identical between individuals →3000000 varying nucleotides
- Polymorphism: normal variation between individuals (frequency> 1% of population)
- Genetic variation
  - May cause or predispose to inheritable diseases
  - Determines e.g. individual drug response
  - Used as markers to identify disease genes

Linkage Disequilibrium

$P_{AB} \neq P_A P_B$

$D_{AB}=P_{AB}-P_A P_B$ →

$P_{AB}=P_A P_B+D_{AB}$

$P_{Ab}=P_A(1-P_B)-D_{AB}$

$P_{aB}=(1-P_A)P_B-D_{AB}$

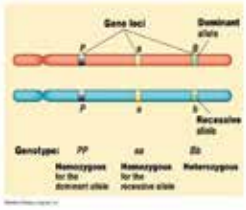
$P_{ab}=(1-P_A)(1-P_B)+D_{AB}$

Allele frequencies

	A1	A2	Total
B1	p1q1+D	p2q1-D	q1
B2	p1q2-D	p2q2+D	q2
Total	p1	p2	

Important terms

- Allele
  - Alternative form of a gene or DNA sequence at a specific chromosomal location (locus)
  - At each locus an individual possesses two alleles, one inherited from each parent
- Genotype
  - Genetic constitution of an individual, combination of alleles
- Genetic marker
  - Polymorphisms that are highly variable between individuals: **Microsatellites** and single nucleotide polymorphisms **(SNPs)**
  - Marker may be inherited together with the disease predisposing gene because of linkage disequilibrium (LD)



Microsatellite markers

Di-, tri-, tetranucleotide repeats

GAACGTACTCACACACACACACA**TTTGAC**
TTCGATGATAGATAGATAGATAGATA**CGT**

- the number of repeats varies (→ 30)
- highly polymorphic
- distributed evenly throughout the genome
- easy to detect by PCR

SNP markers

- **Single Nucleotide Polymorphisms (SNPs)**

GTGGACGTGCTT[G/C]TCGATTTACCTAG

- The most simple and common type of polymorphism
- Highly abundant; every 1000 bp along human genome
- Most SNPs do not affect on cell function
  - some SNPs could predispose people to disease or
  - influence the individual's response to a drug

Research strategies

- **Genome-wide scan**
  - ~400 microsatellite markers at 10 cM interval
  - Family-data
- **Fine mapping**
  - Candidate regions identified by a genome scan
  - Project specific microsatellite or SNP markers
- **SNP genotyping**
  - Candidate genes
  - Fine mapping
  - Sequenom: MassArray MALDI-TOF

SNP genotyping techniques

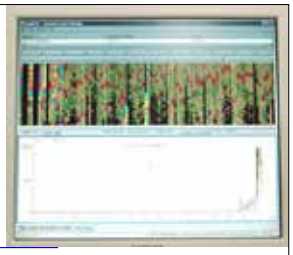
- Over **100 different** approaches
- Ideal SNP genotyping platform:
  - high-throughput capacity
  - simple assay design
  - robust
  - affordable price
  - automated genotype calling
  - accurate and reliable results

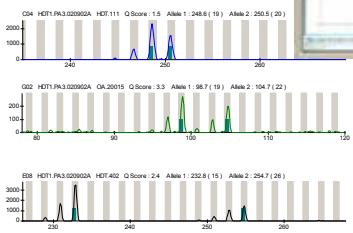
Setting up PCR-reactions

...SNP genotyping techniques

- PCR
- **discrimination between alleles:**
  - allele-specific hybridization
  - allele-specific primer extension
  - allele-specific oligonucleotide ligation
  - allele-specific enzymatic cleavage
- **detection of the allelic discrimination:**
  - light emitted by the products
  - mass
  - change in the electrical property

Electrophoresis run for microsatellites



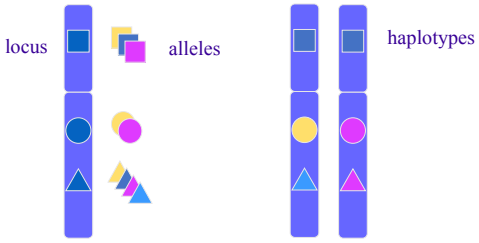


Microsatellite data

Marker	Well ID	SampleID	Allele1	Allele2	Size1	Size2
D7S513	H01	OA.11616	26	28	190.93	195.02
D7S517	C07	DYS.5020	26	26	262.19	262.19
D7S640	B02	DYS.3819	26	29	133.41	139.41
D7S640	G12	OA.152826	29	133.59	139.46	
D7S669	E05	OA.11615	26	29	190.37	196.61
D8S258	B06	DYS.5001	26	27	159.38	161.38
D8S260	C02	DYS.3931	26	26	215.57	215.57
D8S264	H01	OA.11616	26	26	158.86	158.86

II. Haplotype

- Multiple loci in the same chromosome that are inherited together
- Usually a string of SNPs that are linked



SNP data

ASSAY_ID	CHIP_ID	WELL_ID	SAMPLE_ID	GENOTYPE	DESCRIPTION
rs10563	1	A01	IDE.26738	AC	A.Conservative
rs10563	1	A02	IDE.35271	A	A.Conservative
rs3527	1	B05	IDE.68466	TG	A.Conservative
rs6779	2	A01	IDE.35357	G	B.Moderate
rs135627	2	B02	IDE.35328	C	A.Conservative
rs42778	3	C04	IDE.87378	AC	A.Conservative
rs755555	4	D12	IDE.83257	A	A.Conservative
rs45167	5	E10	IDE.54727	A	A.Conservative
rs47890	6	F01	IDE.25335	AC	A.Conservative

Haplotype construction

- No good molecular methods available to identify haplotypes

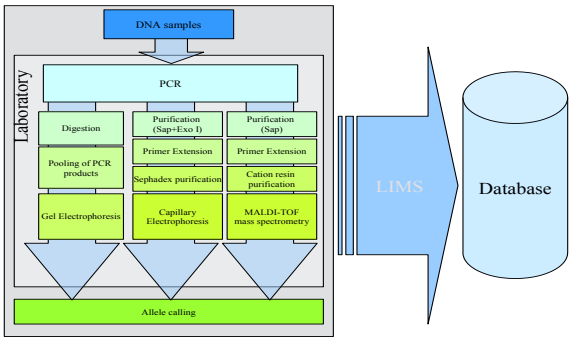
Genotypes → Haplotypes, two alternatives

SNP1 AT A T A T

SNP2 GC G C C G

→ Computational methods to create haplotypes from genotype data

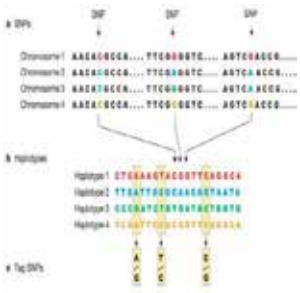
SNP genotyping workflow at FGC



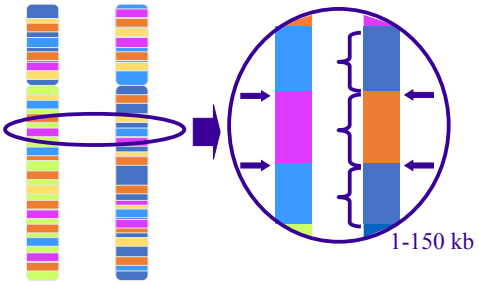
...Haplotype construction

- Family-based haplotype construction
  - Linkage analysis softwares: Simwalk, Merlin, Genehunter, Allegro...
- Population-based haplotype construction
  - Not as reliable as family-based
  - EM-algorithm (expectation maximization algorithm), described in http://www-gene.cimr.cam.ac.uk/clayton/software/
  - SnpHap
  - PHASE

Haplotype blocks



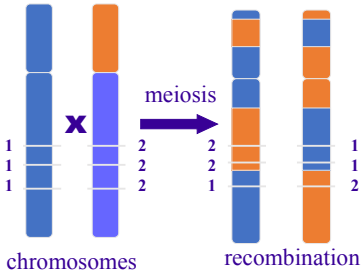
- Low recombination rate in the region
- Strong LD
- Low haplotype diversity
- Small number of SNPs in the block are enough to identify common haplotypes; tag SNPs



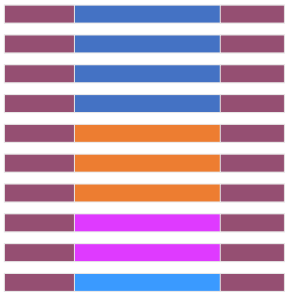
Average block size

- African populations: 11 kb
- Non-african populations: 22 kb
- 60%-80% of the genome is in the blocks of > 10 kb

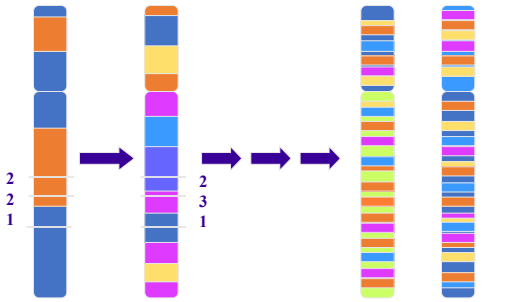
Formation of haplotype blocks



Block frequencies



- Typically, only 3-5 common haplotypes account for >90% of the observed haplotypes

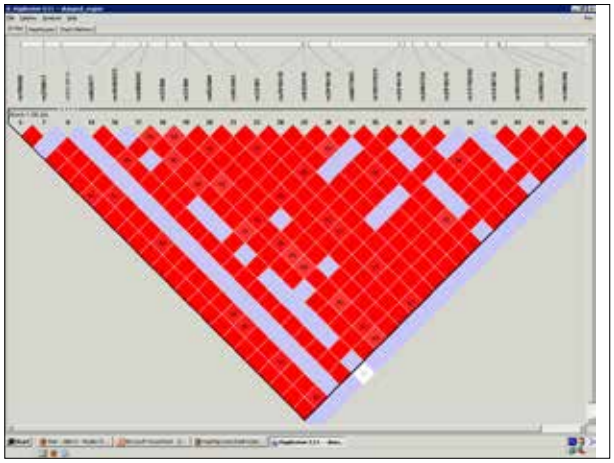


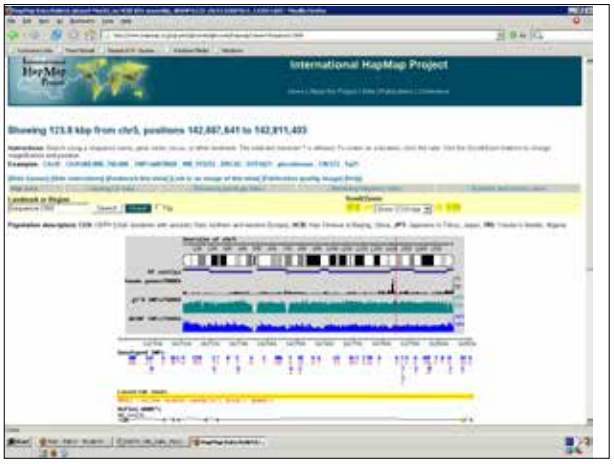
Benefits of haplotypes instead of individual SNPs

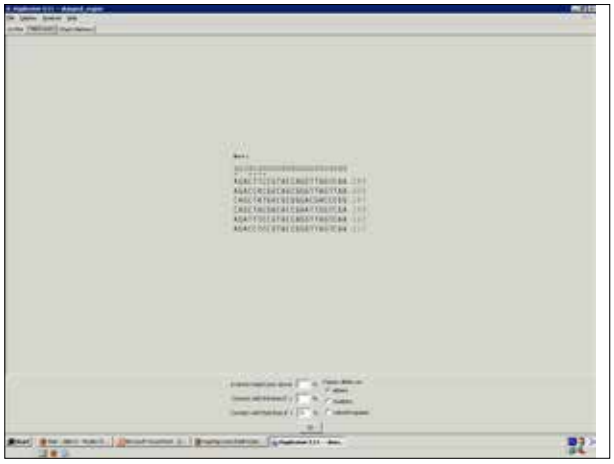
- Information content is higher
- Gene function may depend on more than one SNP
- Smaller number of required markers
  - The amount of wrong positive association is reduced
- Replacing of missing genotypes by computational methods
- Elimination of genotyping errors
- Challenges:
  - Haplotypes are difficult to define directly in the lab; computational methods
  - Defining of block boarders is ambiguous; several different algorithms

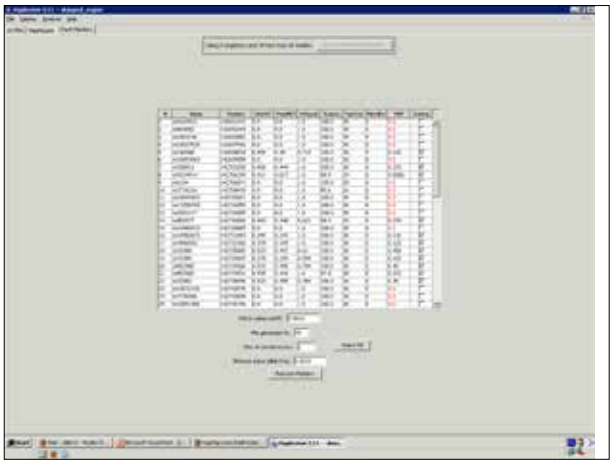
The HapMap project

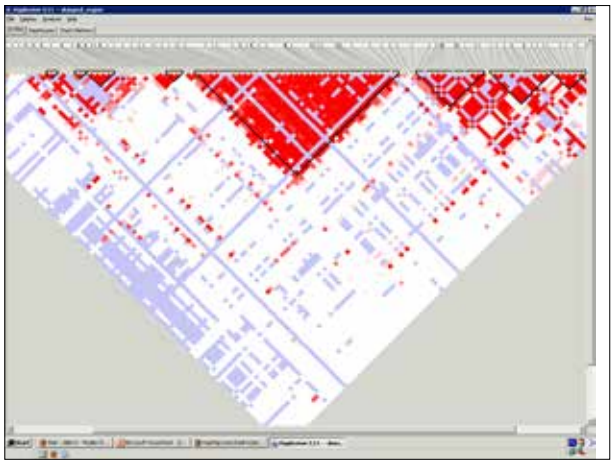
- International collaboration to create a map of human genetic variation
- The map is based on common haplotype patterns
- Includes information on
  - SNPs (location, frequency, sequence)
  - Haplotype block structure
  - Distribution of haplotypes in different populations

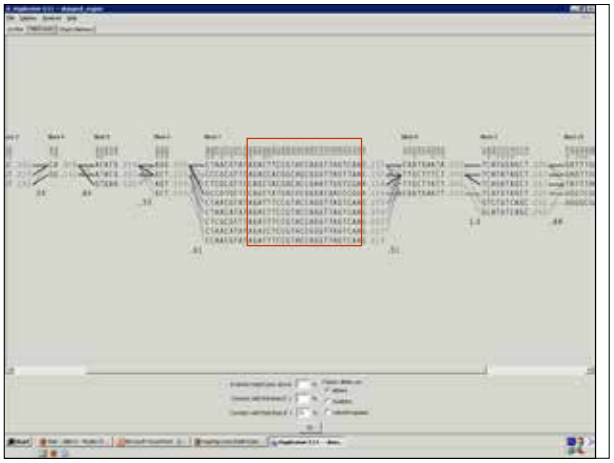






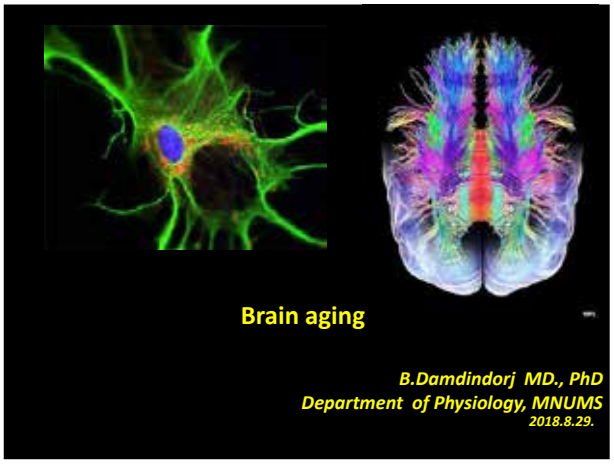




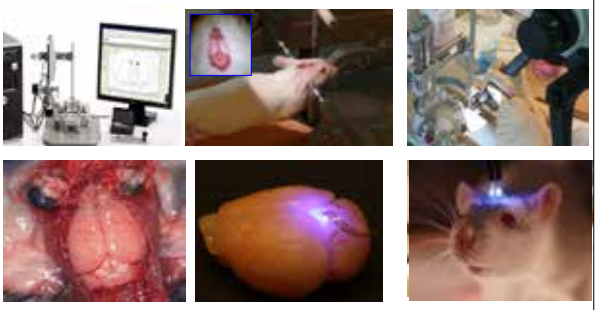



Thank you for your attention!

TL 5 – Lecturer: Damdindorj B, MNUMS



Stereotaxic surgery:



Нейромедиаторууд, пептид дааврааар үйлчлэлийг тархи, мэдрэлийн төв, мэдрэлийн эсүүдэд шууд тодохойлно.

Neuroscience ?

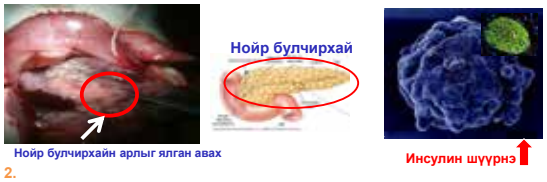
- Neurophysiology
- Neuroendocrinology
- Neurology
- Neurosurgery
- Molecular biology
- Mathematics
- Computer science
- Chemistry
- Cell physiology
- Endocrinology
- Psychology
- Cell signaling
- Neurobiology
- Physics
- Information technology
- Sociology…

"Neuroscience"-ийн судалгааны ололт, нээлтүүдийн үр шим

Тархины эдийн сөнөрөлт өвчнүүд Альцхеймер, Паркинсоны өвчин, Хавдар,Таргалалт, Чихрийн шижинг эмчлэхэд:

Нейрон, дотоод шүүрлийн эсүүдийн анхдагч өсгөвөр:

1.

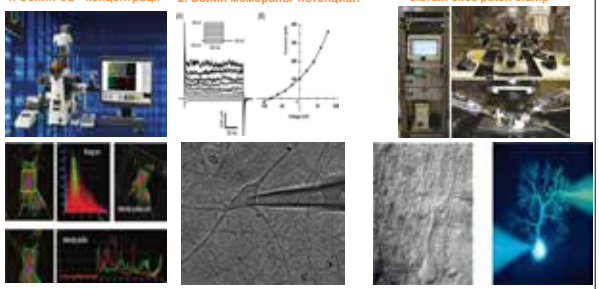


2.

Дотоод шүүрлийн эсүүдийн үйл ажиллагааны нарийн нийлмэл механизмуудыг тодорхойлж чадна

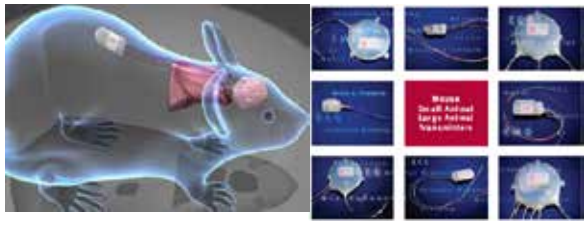
"Live cell imaging technology"

1. Эсийн Ca^{2+} концентраци
2. Эсийн мембраны потенциал
3.brain slice patch clamp



Амьд эс тухайлбал, мэдрэл, дотоод шүүрлийн эсүүдийн механизмуудыг молекулын өндөр нарийвчлалтайгаар эсийн бүтэц үйл ажиллагааг алдагдуулалгүй тодорхойлно

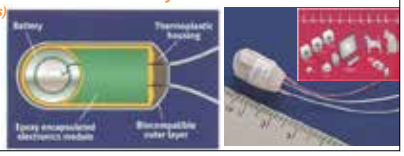
Telemetric system:

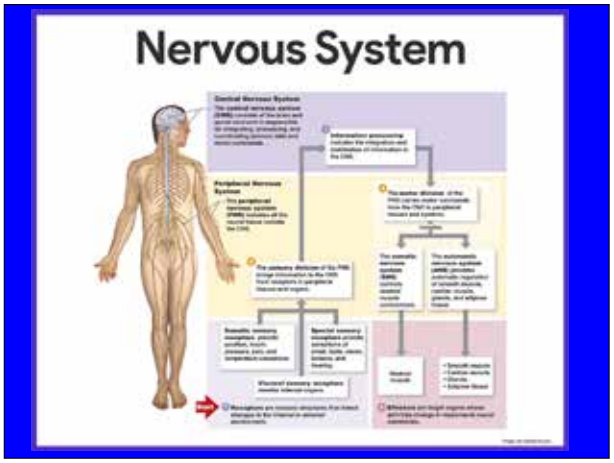


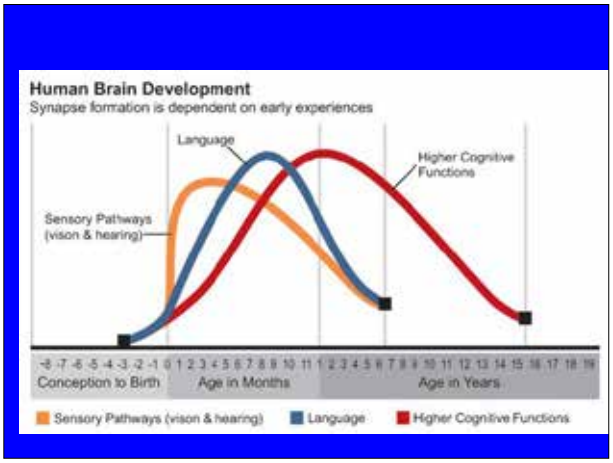
Parameters include:
(mice or other neonatal rodents)

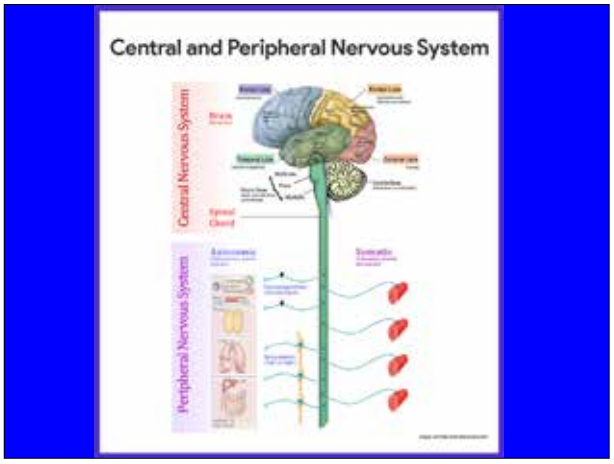
- Pressure and activity
- Temperature and activity
- Biopotentials and activity
- Combination of pressure, biopotential(s), temperature and activity

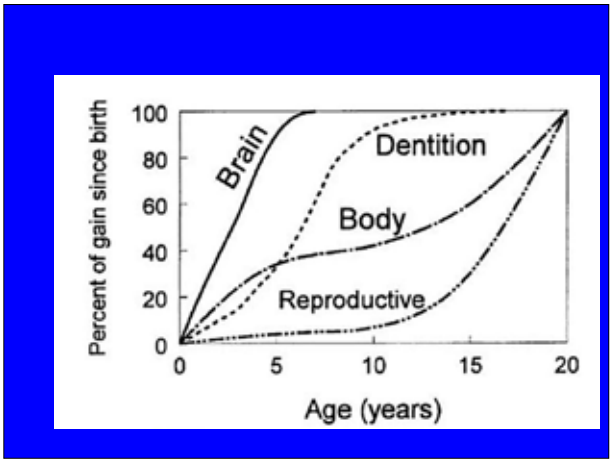
PhysioTel transmitters

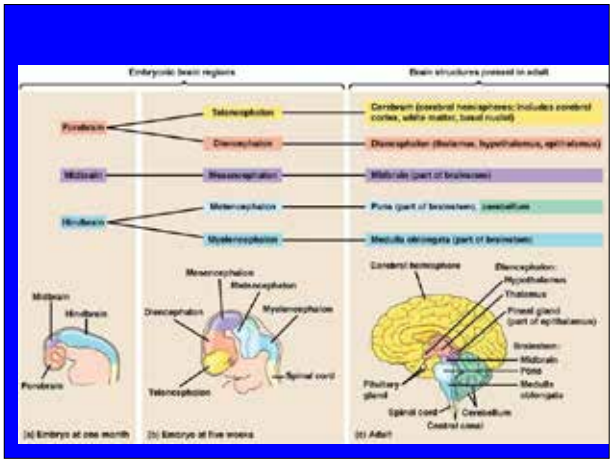


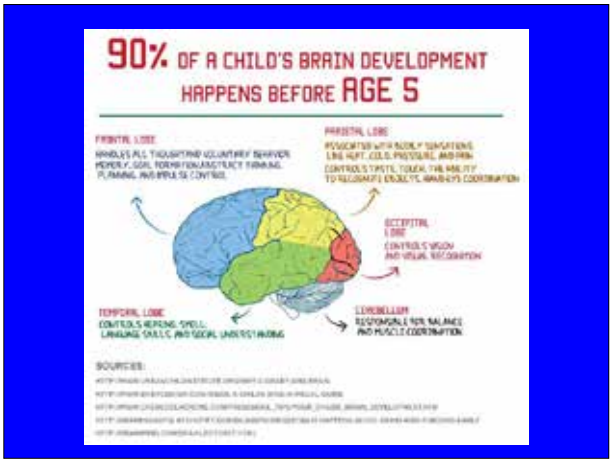

Nervous System

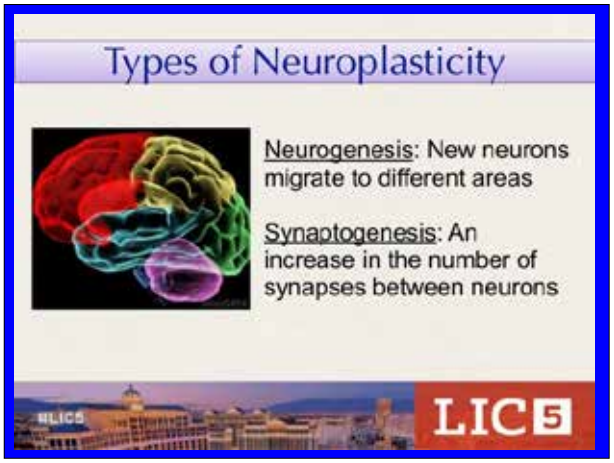

Human Brain Development
Synapse formation is dependent on early experiences
Language
Higher Cognitive Functions
Sensory Pathways (vison & hearing)
Conception to Birth
Age in Months
Age in Years
Sensory Pathways (vison & hearing)
Language
Higher Cognitive Functions

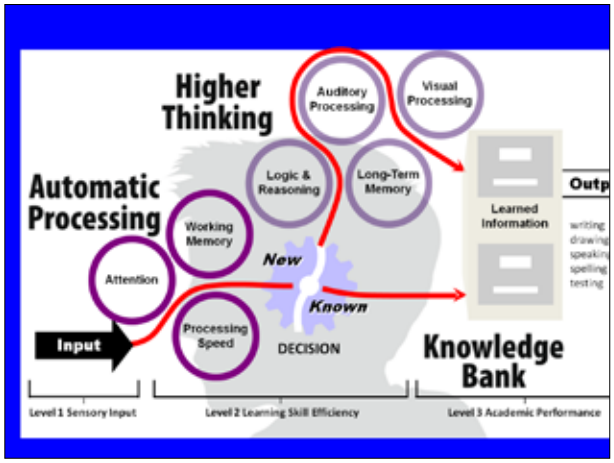

Central and Peripheral Nervous System
Central Nervous System
Peripheral Nervous System

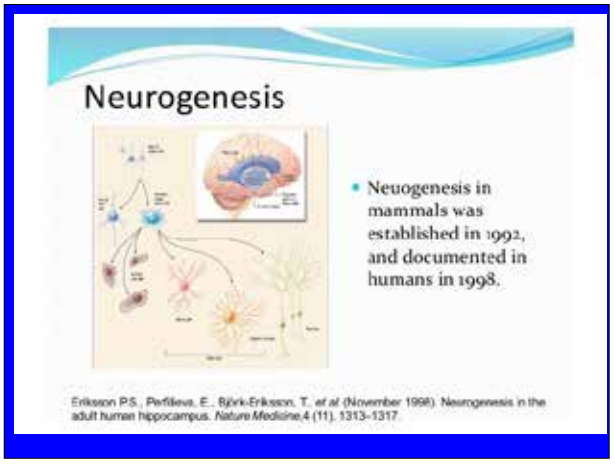

Percent of gain since birth
100
80
60
40
20
0
0
5
10
15
20
Age (years)
Brain
Dentition
Body
Reproductive

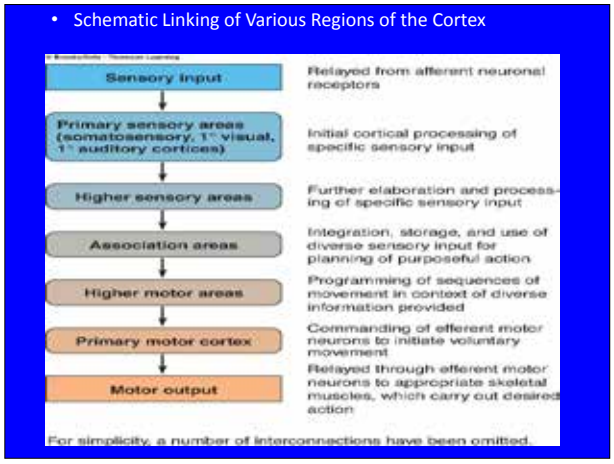

Embryonic brain regions
Brain structures present in adult
Telencephalon
Diencephalon
Mesencephalon
Metencephalon
Myelencephalon
Cerebrum (cerebral hemispheres; includes cerebral cortex, white matter, basal nuclei)
Midbrain
Pons (part of brainstem; cerebellum)
Medulla oblongata (part of brainstem)
Hindbrain
Forebrain
Midbrain
Hindbrain
Spinal cord
Cerebral hemisphere
Thalamus
Pineal gland (part of epithalamus)
Brainstem:
Midbrain
Pons
Medulla oblongata
Pituitary gland
Cerebellum
Central canal
(a) Embryo at one month
(b) Embryo at five weeks
(c) Adult

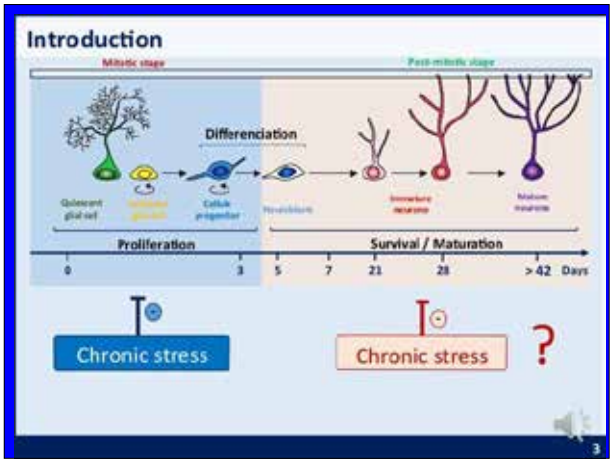

90% OF A CHILD'S BRAIN DEVELOPMENT HAPPENS BEFORE AGE 5
FRONTAL LOBE
PARIETAL LOBE
OCCIPITAL LOBE
CONTROLS VISION AND VISUAL RECOGNITION
TEMPORAL LOBE
CONTROLS HEARING, SPEECH, LANGUAGE, SKILLS, AND SOCIAL UNDERSTANDING
CEREBELLUM
SOURCES:

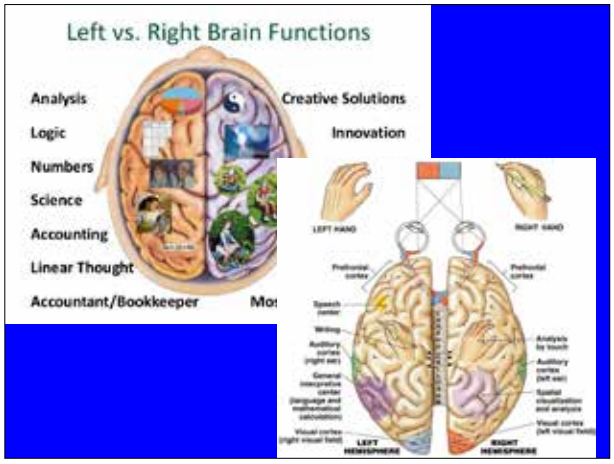

Types of Neuroplasticity
Neurogenesis: New neurons migrate to different areas
Synaptogenesis: An increase in the number of synapses between neurons
LIC5

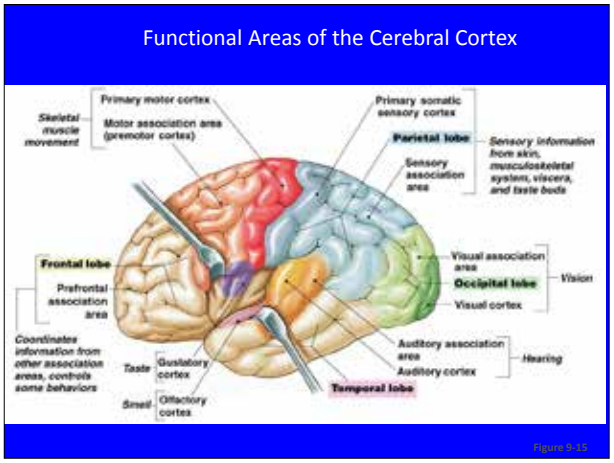

Higher Thinking
Auditory Processing
Visual Processing
Automatic Processing
Logic & Reasoning
Long-Term Memory
Working Memory
Attention
New
Known
Processing Speed
DECISION
Input
Learned Information
Knowledge Bank
Level 1 Sensory Input
Level 2 Learning Skill Efficiency
Level 3 Academic Performance

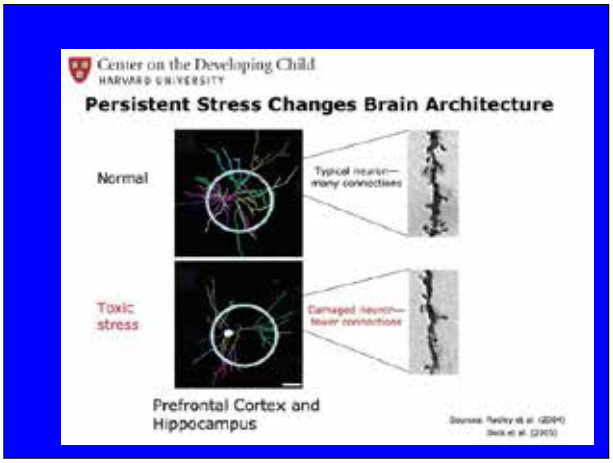

Neurogenesis
Neuogenesis in mammals was established in 1992, and documented in humans in 1998.
Eriksson P.S., Perfilieva, E., Björk-Eriksson, T. et al (November 1998). Neurogenesis in the adult human hippocampus. Nature Medicine,4 (11), 1313–1317.

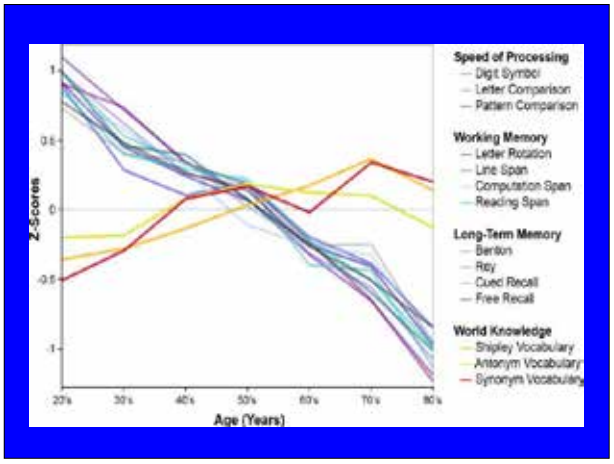

Schematic Linking of Various Regions of the Cortex
Sensory input
Relayed from afferent neuronal receptors
Primary sensory areas (somatosensory, 1° visual, 1° auditory cortices)
Initial cortical processing of specific sensory input
Higher sensory areas
Further elaboration and processing of specific sensory input
Association areas
Integration, storage, and use of diverse sensory input for planning of purposeful action
Higher motor areas
Programming of sequences of movement in context of diverse information provided
Primary motor cortex
Commanding of efferent motor neurons to initiate voluntary movement
Motor output
Relayed through efferent motor neurons to appropriate skeletal muscles, which carry out desired action
For simplicity, a number of interconnections have been omitted.

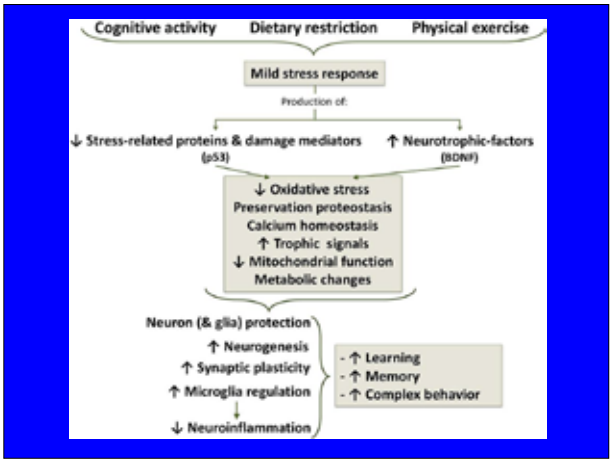

Introduction
Differenciation
Proliferation
Survival / Maturation
0
3
5
7
21
28
>42 Days
Chronic stress
Chronic stress
?


Left vs. Right Brain Functions
Analysis
Logic
Numbers
Science
Accounting
Linear Thought
Accountant/Bookkeeper
Creative Solutions
Innovation
LEFT HEMISPHERE
RIGHT HEMISPHERE

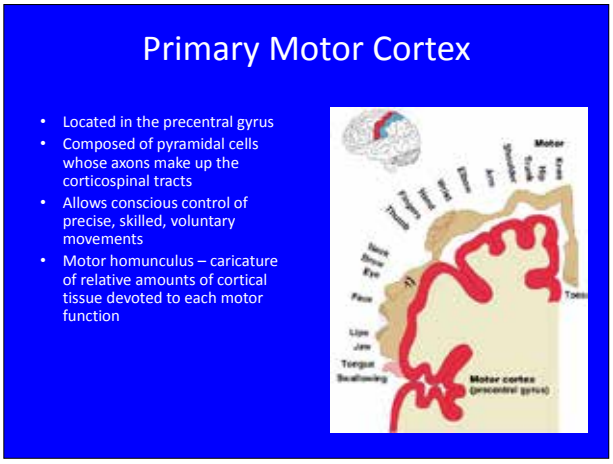

Functional Areas of the Cerebral Cortex
Primary motor cortex
Skeletal muscle movement
Motor association area (premotor cortex)
Primary somatic sensory cortex
Parietal lobe
Sensory information from skin, musculoskeletal system, viscera, and taste buds
Sensory association area
Frontal lobe
Prefrontal association area
Coordinates information from other association areas, controls some behaviors
Visual association area
Occipital lobe
Vision
Visual cortex
Auditory association area
Hearing
Auditory cortex
Taste
Gustatory cortex
Smell
Olfactory cortex
Temporal lobe


Center on the Developing Child
HARVARD UNIVERSITY
Persistent Stress Changes Brain Architecture
Normal
Typical neuron—many connections
Toxic stress
Damaged neuron—fewer connections
Prefrontal Cortex and Hippocampus


Speed of Processing
Digit Symbol
Letter Comparison
Pattern Comparison
Working Memory
Letter Rotation
Line Span
Computation Span
Reading Span
Long-Term Memory
Benton
Rey
Cued Recall
Free Recall
World Knowledge
Shipley Vocabulary
Antonym Vocabulary
Synonym Vocabulary
Z-scores
Age (Years)

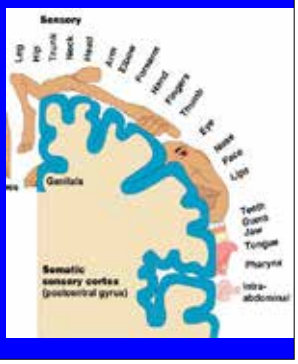

Cognitive activity
Dietary restriction
Physical exercise
Mild stress response
Production of:
↓ Stress-related proteins & damage mediators (p53)
↑ Neurotrophic-factors (BDNF)
↓ Oxidative stress
Preservation proteostasis
Calcium homeostasis
↑ Trophic signals
↓ Mitochondrial function
Metabolic changes
Neuron (& glia) protection
↑ Neurogenesis
↑ Synaptic plasticity
↑ Microglia regulation
↓ Neuroinflammation
- ↑ Learning
- ↑ Memory
- ↑ Complex behavior


Neurogenesis
Enhanced by:
• Exercise
• Complex Environments
• New Learning
• Nutrition
• Low Stress
Reduced by:
• Distress
• Inactivity
• Boredom
• Depression
• Poor Nutrition


Primary Motor Cortex
• Located in the precentral gyrus
• Composed of pyramidal cells whose axons make up the corticospinal tracts
• Allows conscious control of precise, skilled, voluntary movements
• Motor homunculus – caricature of relative amounts of cortical tissue devoted to each motor function
Motor
Motor cortex (precentral gyrus)

Primary Somatosensory Cortex

- Located in the postcentral gyrus, this area:
  - Receives information from the skin and skeletal muscles
  - Exhibits spatial discrimination
- Somatosensory homunculus – caricature of relative amounts of cortical tissue devoted to each sensory function



How Meditation Affected in to Brain Structure and Functions?

Limbic system

- Amygdaloid body
- Hippocampus ("seahorse")
- Cingulate gyus
- Parahippocampal gyrus
- Hypothalamus
- bodies
- ucleus of

Function of the Limbic System

Amygdala	Arousal; controls autonomic responses associated with fear, emotional responses, aggression, hormonal secretions
Hippocampus	Consolidation of new memories, formation of long-term memories, emotions, navigation, spatial orientation
Cingulate gyrus	Coordinates sensory input with emotions, emotional response to pain, regulates aggressive behavior, cognitive and attentional processing
Hypothalamus	Controls autonomic functions, emotions, endocrine functions, homeostasis
Mammilary body	Involved in the formation of memory
Nucleus accumens	Involved in reward, pleasure, addiction
Putamen (a part of basal ganglia)	Transposes information from the cortex, important in movement control

Blood Supply to the CNS

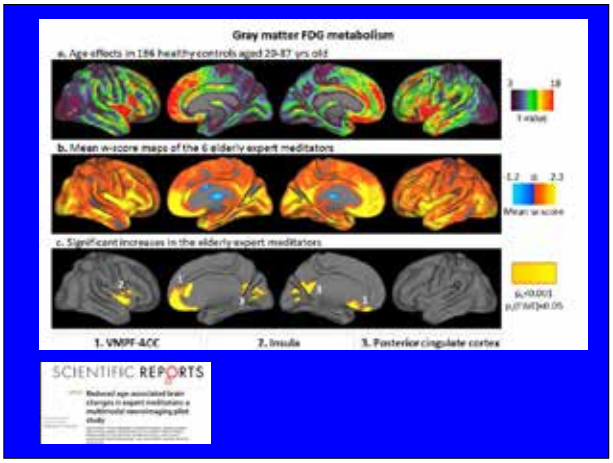
- CNS comprises 2% of body weight (3–4 pounds)
  - Receives 15% of blood supply
- High metabolic rate
  - Brain uses 20% of oxygen consumed by body at rest
  - Brain uses 50% of glucose consumed by body at rest
- Depends on blood flow for energy

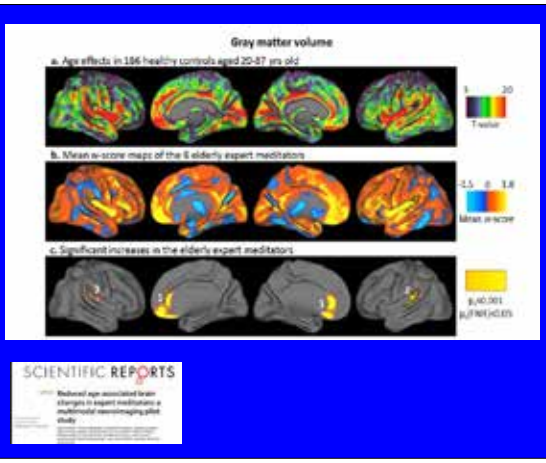
Functions of Limbic system

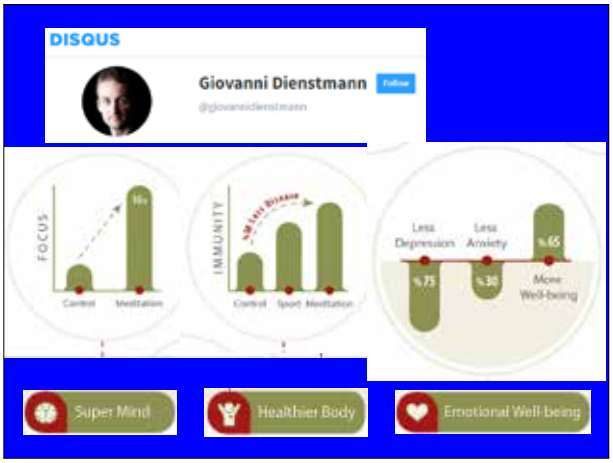
- "Emotional brain
  - Emotional and motivational aspects of behavior.
  - Provides emotional component to learning process: Especially the amygdala.
- Associated with memory
  - Especially the hippocampus.
- Associated with pain/pleasure, rage

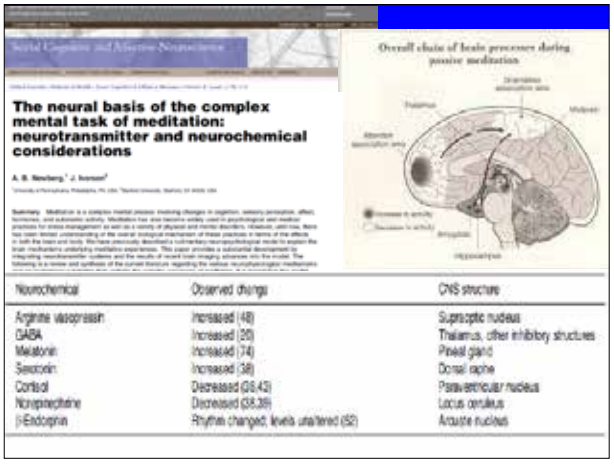
Meditation

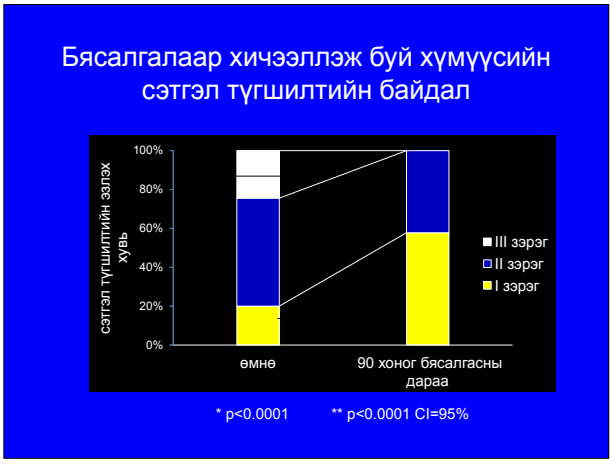
Review


Gray matter FDG metabolism
a. Age effects in 186 healthy controls aged 20-87 yrs old
b. Mean w-score maps of the 6 elderly expert meditators
c. Significant increases in the elderly expert meditators
1. VMPF-ACC
2. Insula
3. Posterior cingulate cortex
SCIENTIFIC REPORTS

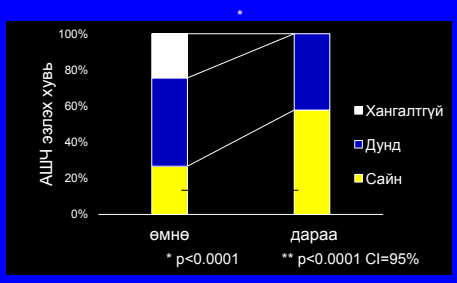

Gray matter volume
a. Age effects in 186 healthy controls aged 20-87 yrs old
b. Mean w-score maps of the 6 elderly expert meditators
c. Significant increases in the elderly expert meditators
SCIENTIFIC REPORTS


DISQUS
Giovanni Dienstmann
FOCUS
IMMUNITY
Less Depression
Less Anxiety
More Well-being
Super Mind
Healthier Body
Emotional Well-being

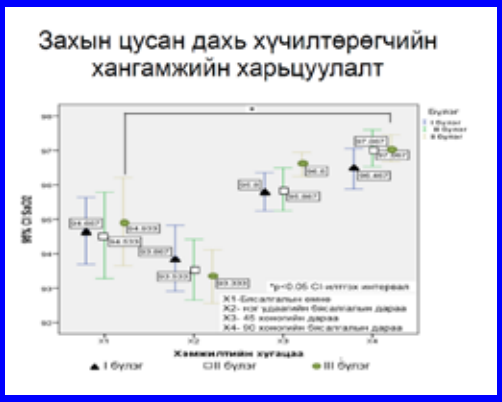

The neural basis of the complex mental task of meditation: neurotransmitter and neurochemical considerations
Overall chain of brain processes during passive meditation
Neurochemical
Observed change
CNS structure
Arginine vasopressin
GABA
Melatonin
Serotonin
Cortisol
Norepinephrine
β-Endorphin
Suprioptic nucleus
Thalamus, other inhibitory structures
Pineal gland
Dorsal raphe
Paraventricular nucleus
Locus ceruleus
Arcuate nucleus


Бясалгалаар хичээллэж буй хүмүүсийн сэтгэл түгшилтийн байдал
сэтгэл түгшилтийн эзлэх хувь
100%
80%
60%
40%
20%
0%
өмнө
90 хоног бясалгасны дараа
III зэрэг
II зэрэг
I зэрэг
* p<0.0001
** p<0.0001 CI=95%

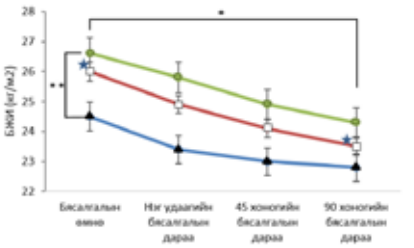
Анхаарал шилжүүлэх чадварт бясалгалын нөлөө



Захын цусан дахь хүчилтөрөгчийн хангамжийн харьцуулалт



Бясалгалаар хичээллэгчдийн БЖИ-ийн харьцуулалт

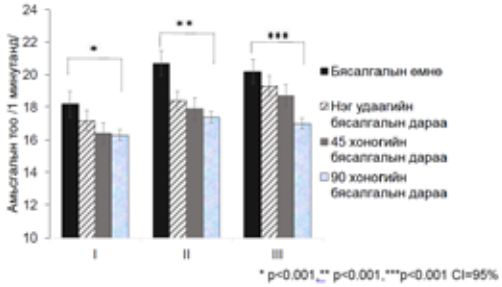


WHY DO WE EAT

- **SATIETY**

  If the quest for food is successful the brain signals the body to stop eating (hunger is suppressed).

Бясалгалаар хичээллэгчдийн амьсгалын тоо



- **Hunger**
  - **Physiological (internal) drive to eat**
  - **The feeling that prompts thought of food and motivates food consumption**
  - **Influenced by nutrients in the bloodstream, eating patterns, climate, etc**
  - **Controlled internally**
- **Appetite**
  - **Psychological (external) drive to eat**
  - **Often in the absence of hunger**
  - **Often of particular type of food**
  - **Combination of internal and external signals drive us to eat**
  - **Appetite is affected by a variety of external forces**
  - **Not a perfect system; desire to eat can be overwhelming**

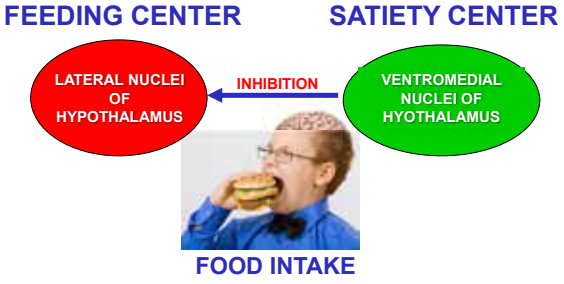
WHY DO WE EAT

- **Hunger**
  - Physiological (internal) drive to eat
  - The feeling that prompts thought of food and motivates food consumption
  - Influenced by nutrients in the bloodstream, eating patterns, climate, etc
  - Controlled internally

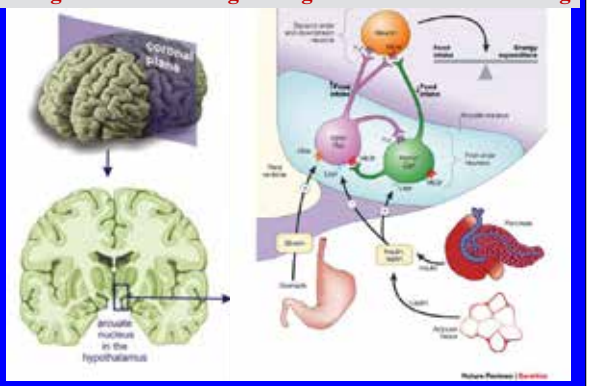
NEUROTRANSMITERS AND HORMONES

ANOREXIGENIC	OREXIGENIC
1. **α MSH (Melanocyte-stimulating hormone)**	1. **Neuropeptide Y (NPY)**
2. **Leptin**	2. Agouti-related protein (AgRP)
3. Serotonine	3. **MCH (Melanin-concentrating hormone)**
4. Nor-epinephrine	4. Orexins A and B
5. Corticotropin- realeasing hormone	5. Endorphins
6. **Insulin**	6. **Galanin (GAL)**
7. **CCK (Cholecystokinin)**	7. Amino acids (glutamate & GABA)
8. GLP (glucagon-like peptide)	8. Cortisol
9. CART (Cocain and amphetamine related transcript)	9. **Ghrelin**
10. Peptide YY	10. **Cannabinoids**

HUNGER AND SATIETY CENTRE

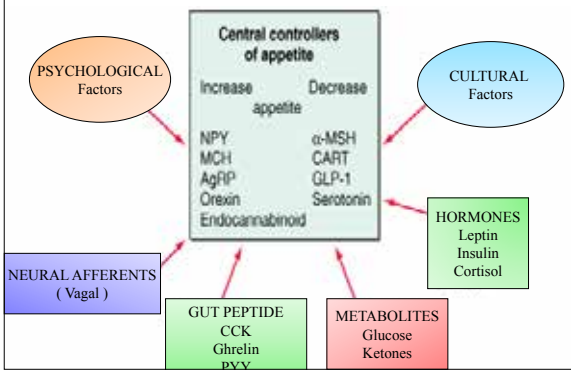


Orexigenic and anorexigenic signals are effects on feeding



Gregory S. Barsh & Michael W. Schwartz. *Nature Reviews Genetics* 3, 589-600

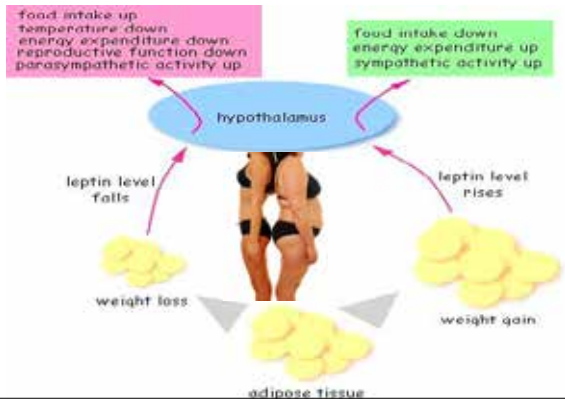
THE FACTORS THAT REGULATE APPETITE THROUGH EFFECTS ON CENTRAL NEURAL CIRCUIT



FEEDBACK SIGNALS FROM ADIPOSE TISSUE BY LEPTIN

- **Hypothalamus senses energy storage through the actions of LEPTIN**
- **LEPTIN: a peptide hormone released from adipose tissue**
- **adipose tissue increase to LEPTIN production**

FEED BACK SIGNALS FROM ADIPOSE TISSUE



Clock gene

- ***Circadian Locomotor Output Cycles Kaput***

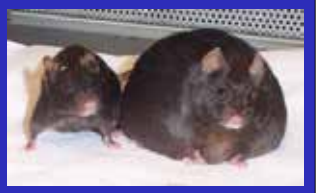
What is ob/ob mice?

In 1994, *ob* gene discovered by Friedman , is located in 7 chromosome.
Ob gene is produced new hormone leptin in adipose tissue. (Ob-obese)

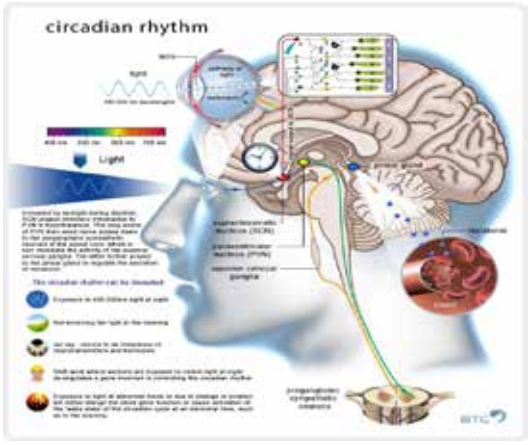
Leptin plays a key role in regulating energy intake and expenditure, including feeding, appetite and hunger, metabolism and feeding behavior.

Lep^{ob} or *ob/ob* is not produced leptin *db/db* is not expressed leptin receptor

ob/ob mice exhibit :
- obesity
- hyperphagia
- hyperglycemia
- glucose intolerance
- elevated plasma insulin
- subfertility
- hypometabolic
- hypothermic



Wild type mice ob/ob mice

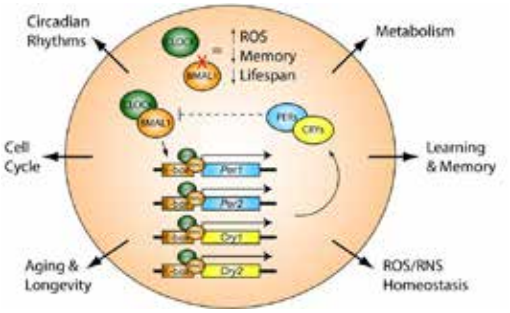


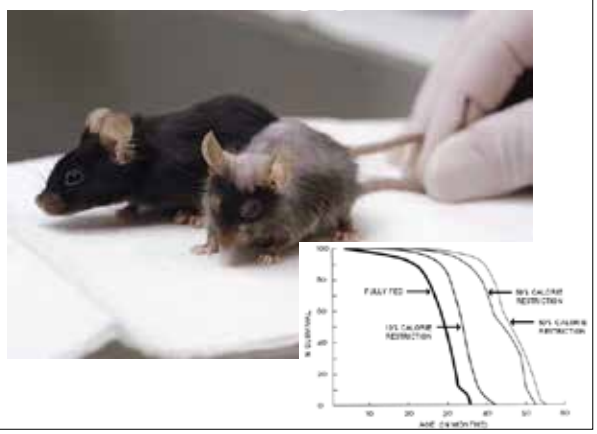
Clock gene

The core mammalian clock machinery consists of at least eight distinct proteins:

- ***BMAL1*, *CLOCK*, *PER1*,*PER2*, *PER3*, *CR Y1*, *CRY2*, and *REV-ERB*.**
- **CLOCK protein** has been found **to play** a central role as a transcription factor in the **circadian pacemaker.**
- Locates in chromosome 4.

Clock gene





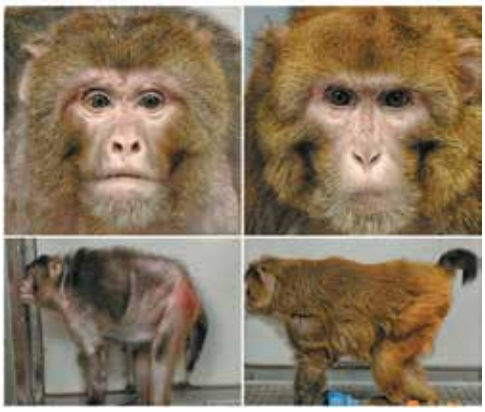
Clock gene

In mice, *Clock* has been implicated

- **in sleep disorders**
- **metabolism,**
- **pregnancy**
- **mood disorders**

***Clock* mutant mice sleep less than normal mice each day.**

The Curious Case Of Caloric Restriction



TL 6 – Lecturer: Javkhlan B, MNUMS

АШУУИС

Cell viability assay method

Javkhlan.b mnums (core laboratory)

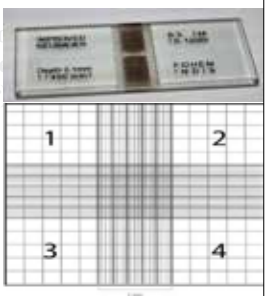
- Manual:
  - Hemocytometer (Double Neubauer ruled metalized counting chamber)
- Automated:
  - Spectrophotometry
  - Coulter Counter
  - Flow Cytometry
  - Image based
- Advantages and Disadvantages

Introduction

- Cell counting:
  - Why?
  - How?
  - Recent advances
- Cell viability assays
  - Why?
  - How?
  - Recent advances

Hemocytometer (Double Neubauer Ruled Metalized Counting Chamber)

- Each counting chamber has a mirrored surface with a 3 Ч 3 mm grid of 9 counting squares
- The chambers have raised sides that can hold a cover slip exactly 0.1 mm above the chamber floor
- Each of the 9 counting squares holds a volume of 0.0001 mL (1 mm x 1 mm x 0.1 mm)
- The average count in the squares marked 1 to 4 in the figure, multiplied by 10000 gives the cell count/mL.

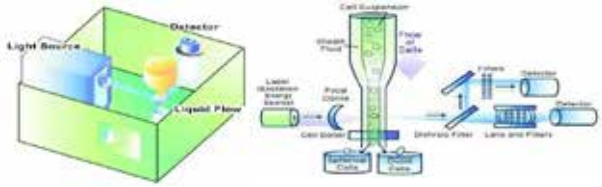


- For maintaining cell cultures
  - Splitting cells or preparing for the next passage (usually cells are diluted into a new culture flask with fresh media for optimal growth)
- For preparing cells for transfection experiments
- For preparing cells for downstream experiments that require accurate and consistent numbers of input cells, including qPCR

Cell Counting Using Hemocytometer:

- Remove bleached media from the T-flasks and rinse the cell monolayer with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium.
- Remove DPBS and add trypsin-EDTA solution (1 mL for T-25 flasks and 3 mL for T-75 flasks) to the flasks followed by incubation at 37°C, to dissociate the cells from the adhering surface
- When the cells appeared to be detached, add complete growth media to neutralize the trypsin in volumes that were double that of the trypsin-EDTA used for dissociation
- >95% of the cells should be single cells
- After mixing the cell suspension to ensure uniform distribution of cells, load 10 µL of the cell suspension into the counting chamber.
- Place Neubauer chamber was placed under an inverted microscope and view the cells at 100 x magnification
- Count the cells in quadrants labeled 1, 2, 3 and 4 and multiply the average value by 10000 to obtain the number of cells per mL.

Automated Cell Counting – Flow Cytometry



- If suitable dyes are used, viability also can be assessed
- Acridine Orange (AO)- Cell membrane permeable → Stains nucleus
- Propidium Iodide (PI)- Impermeable

Cell Viability Assays

- Non-fluorescence based
  - Trypan Blue
  - Erythrosin B
  - MTT
  - XTT
- Fluorescence based
- Chemiluminescence based

Automated Cell Counting – Spectrophotometry

- Not very reliable
- More cells → More turbidity → High OD
- Relative count
- Absolute count: When you have a sample with known cell number
- Not suitable if media is turbid

Trypan Blue Exclusion Test

- Live cells possess intact cell membranes that exclude certain dyes such as trypan blue, whereas dead cells do not
- Add 10 µL of 0.4% trypan blue to 10 µL of the cell suspension
- After proper mixing of the dye and the cell suspension, load 10 µL of the mixture into the counting chamber
- Viable cells are characterized by a clear cytoplasm whereas nonviable cells possess a blue cytoplasm
- Count the viable cells in quadrants 1 to 4 of the Neubauer chamber within 5 minutes and multiply the average of this value by 10000 x dilution factor (2, in this case)
- This gives the number of viable cells per mL of the cell suspension
- This can also be expressed as a percentage of the total number of cells.

Cell Viability Assays:

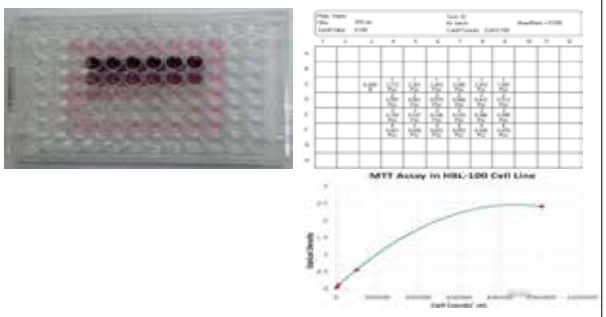
- For maintaining cell cultures
  - Splitting cells or preparing for the next passage (usually cells are diluted into a new culture flask with fresh media for optimal growth)
- For preparing cells for transfection experiments
- For preparing cells for downstream experiments that require accurate and consistent numbers of input cells, including qPCR
- To assess toxic effect of drugs/ chemicals
- After cryopreservation

- **Trypan Blue**
  - Incubation: 2-5 minutes
  - Binds to serum proteins
  - Less clear background
  - Potential carcinogen
- **Erythrosin B**
  - No incubation
  - No binding
  - Clear background
  - Less toxic

MTT assay

- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
- Reduction of yellow MTT by mitochondrial succinate dehydrogenase yields insoluble dark purple formazan

- The cells are then solubilised with an organic solvent (e.g. DMSO) and the released, solubilised formazan reagent is measured spectrophotometrically at 570 nm using a micro-titer platereader



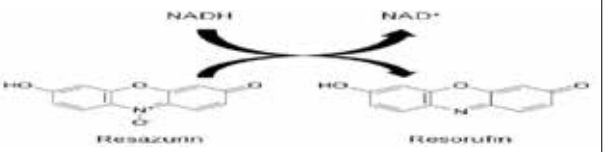
MTT assay

- Prepare the cell suspension and count the cells
- Plate the cells onto a 96-well tissue culture plate, with seeding densities of $1x10^6$, $1x10^5$, $1x10^4$, and $1x10^3$ cells/mL
- Make dilutions so as to maintain 200µL of complete media/well and seed the wells in hexaplicate
- Only the inner rows and columns of the plate are to be used so as to minimize cell growth variations due to different medium evaporation rates at the periphery
- One well should be maintained as blank, in which only media is added

- Incubate the tissue culture plate in a CO_2 incubator at 37°C overnight.
- Check the cell growth on the next day

Fluorescence based Detection:

- Live cells contain esterases
- Non-fluorescent substrates → Fluorescent molecules
- Intact intracellular membrane retains the cleaved fluorescent products inside the cell
- Dead cells, are deficient in esterase activity and their compromised membranes lead to substrate leaks from cells



MTT assay

- Remove the bleached media from each well and add 100 µL of MTT (1 mg/mL) diluted in DPBS to each well
- Incubate at 37°C for 4 hours, remove the supernatant and add 100µL of DMSO to each well
- Incubate the plate in the dark for 60 minutes and measure the absorbance using a micro-titer plate reader at 570nm
- Calculate the mean absorbance and plot it against the number of cells/mL.

- For maintaining cell cultures
  - Splitting cells or preparing for the next passage (usually cells are diluted into a new culture flask with fresh media for optimal growth)
- For preparing cells for transfection experiments
- For preparing cells for downstream experiments that require accurate and consistent numbers of input cells, including qPCR

Cell Counting Methods:

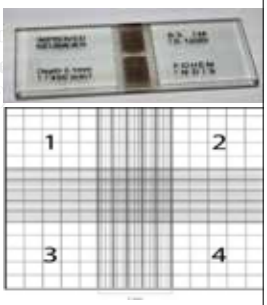
- Manual:
  - Hemocytometer (Double Neubauer ruled metalized counting chamber)
- Automated:
  - Spectrophotometry
  - Coulter Counter
  - Flow Cytometry
  - Image based
- Advantages and Disadvantages

Automated Cell Counting - Coulter Counter

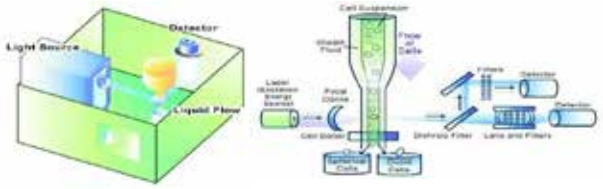
- Only cell count
- Cannot measure viability

Hemocytometer (Double Neubauer Ruled Metalized Counting Chamber)

- Each counting chamber has a mirrored surface with a 3 Ч 3 mm grid of 9 counting squares
- The chambers have raised sides that can hold a cover slip exactly 0.1 mm above the chamber floor
- Each of the 9 counting squares holds a volume of 0.0001 mL (1 mm x 1 mm x 0.1 mm)
- The average count in the squares marked 1 to 4 in the figure, multiplied by 10000 gives the cell count/mL.



Automated Cell Counting – Flow Cytometry



- If suitable dyes are used, viability also can be assessed
- Acridine Orange (AO)- Cell membrane permeable → Stains nucleus
- Propidium Iodide (PI)- Impermeable

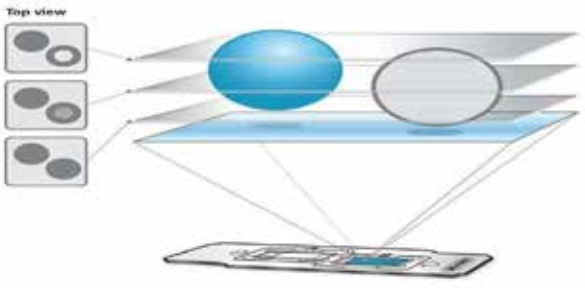
Cell Counting Using Hemocytometer:

- Remove bleached media from the T-flasks and rinse the cell monolayer with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium.
- Remove DPBS and add trypsin-EDTA solution (1 mL for T-25 flasks and 3 mL for T-75 flasks) to the flasks followed by incubation at 37°C, to dissociate the cells from the adhering surface
- When the cells appeared to be detached, add complete growth media to neutralize the trypsin in volumes that were double that of the trypsin-EDTA used for dissociation
- >95% of the cells should be single cells
- After mixing the cell suspension to ensure uniform distribution of cells, load 10 µL of the cell suspension into the counting chamber.
- Place Neubauer chamber was placed under an inverted microscope and view the cells at 100 x magnification
- Count the cells in quadrants labeled 1, 2, 3 and 4 and multiply the average value by 10000 to obtain the number of cells per mL.

Automated Cell Counting – Spectrophotometry

- Not very reliable
- More cells → More turbidity → High OD
- Relative count
- Absolute count: When you have a sample with known cell number
- Not suitable if media is turbid

Automated Cell Counting – Image Based



- These reductions take place only when reductase enzymes are active, and therefore conversion is often used as a measure of viable (living) cells.
- However, it is important to keep in mind that other viability tests (such as the CASY cell counting technology) sometimes give completely different results, as many different conditions can increase or decrease metabolic activity.
- Changes in metabolic activity can give large changes in MTT or MTS results while the number of viable cells is constant.
- When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death, or changing metabolism of cells, can be deduced through the production of a dose-response curve.

Cell Viability Assays:

- For maintaining cell cultures
  - Splitting cells or preparing for the next passage (usually cells are diluted into a new culture flask with fresh media for optimal growth)
- For preparing cells for transfection experiments
- For preparing cells for downstream experiments that require accurate and consistent numbers of input cells, including qPCR
- To assess toxic effect of drugs/ chemicals
- After cryopreservation

- The MTT Cell Proliferation and Viability Assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation or, when metabolic events lead to apoptosis or necrosis, reduction in cell viability.
- Cells are cultured in flat-bottomed, 96-well tissue culture plates. The cells are treated as per experimental design and incubation times are optimized for each cell type and system.
- The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals by mitochondrial enzymes associated with metabolic activity. The reduction of MTT is primarily due to glycolytic activity within the cell and is dependent upon the presence of NADH and NADPH

MTT assay

The **MTT assay** and the **MTS assay** are colorimetric assays for measuring the activity of enzymes that reduce MTT or close dyes (XTT, MTS, WSTs) to **formazan** dyes, giving a purple color

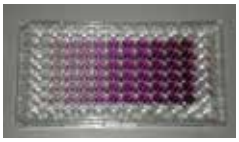
The main application allows to assess the viability (cell counting) and te proliferation of cells (cell culture assays)

It can also be used to determine cytotoxicity of potential medicinal agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth

- Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. Samples are read directly in the wells.
- The optimal wavelength for absorbance is 570 nm, but any filter that absorbs between 550 and 600 nm may be used.
- The data is analyzed by plotting cell number versus absorbance, allowing quantitation of changes in cell proliferation.
- The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

Reagents and storage conditions

Reagent	Storage
MTT Reagent	2 - 8° C
Detergent Reagent	18 - 24° C



A microtiter plate after an MTT assay. Increasing amounts of cells resulted in increased purple colouring

Reaction

Applications:

-Cell proliferation assays
-Cytotoxicity analysis
-Apoptosis screening

Features:

- ✓ Convenient →Stabilized formulation is stored in your refrigerator and does not require thawing before use.
- ✓ Non-isotopic →Assay for cell proliferation, cytotoxicity, and viability does not require isotopic reagents
- ✓ Fast →High throughput microplate format
- ✓ Flexible →The reaction product can be visualized directly by microscopy to evaluate cell to cell reactivity, or solubilized and evaluated by microplate reading.
- ✓ Safe →Reaction product is solubilized using a non-organic solvent
- ✓ Common methods for determining cell viability depend upon membrane integrity (e.g. trypan blue exclusion), or incorporation of nucleotides during cell proliferation (e.g. BrdU or 3H-thymidine)

Experimental Protocol

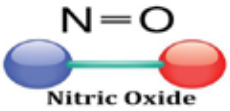
- Plate cells into 96-well tissue culture plates. In general, cells should be seeded at densities between 5000 and 10,000 cells per well since they will reach optimal population densities within 48 to 72 hours.
- Carry out your experiment by adding chemicals or biological agents into appropriate well. The final volume of tissue culture medium in each well should be 0.1mL, and the medium may contain up to 10% Fetal Bovine Serum.
- Thaw one vial of MTT solution for each 96-well plate assay.
- ❖ Note: If sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained. Add 10µL MTT solution to each well. Mix by tapping gently on the side of the tray or shake briefly on an orbital shaker.

- ✓ These methods are limited by the impracticality of processing large numbers of samples, or by the requirement for handling hazardous materials.
- ✓ The MTT Assay, in contrast, provides a rapid and versatile method for assessing cell viability.
- ✓ The assay is used to measure changes in cell proliferation.
- ✓ In actively proliferating cells, an increase in MTT conversion is spectrophotometrically quantified.
- ✓ Comparison of this value to an untreated control provides a relative increase in cellular proliferative activity. Conversely, in cells that are undergoing apoptosis, MTT reduction decreases, reflecting the loss of cell viability.

- Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours
- Add 200µL DMSO into each well to dissolve the formazan by pipetting up and down several times
- Measure the absorbance on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal (OD570-OD630).

TL 7 – Lecturer: Baasansuren E, MNUMS

Nitric oxide and Nervous system

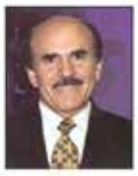


Baasansuren E
Core Laboratory, MNUMS

Nobel Prize Awarded to Scientists for Nitric Oxide Discoveries

They discoveries concerning "***Nitric Oxide as a signaling molecule in the cardiovascular system***"

Robert F Furchgott, born 1916, Dept. of Pharmacology, SUNY Health Science Center New York



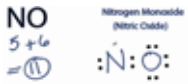
Louis J Ignarro, born 1941, Dept. of Molecular and Medical Pharmacology UCLA School of Medicine Los Angeles

Ferid Murad, born 1936 Dept. of Integrative Biology Pharmacology and Physiology University of Texas Medical School, Houston

What is Nitric Oxide?

- ✓ A chemical compound with formula NO is a free radical gas.
- ✓It is produced in mammals by the enzyme-catalesyed.
- ✓At high concentration, fight against infectious organism and cancer cell.
- ✓ At lower concentration helps in regulating the central nervous system.
- ✓ Ca++ clamudulin complex is necessary for nitric oxide

The structure and nature of nitric oxide



- Nitric oxide is a di atomic free radical consisting of one atom of nitrogen and one of oxygen.
- Lipid soluble and very small for easy passage between cell membranes.
- Short lived, usauallu degrade or reacted within a few seconds.
- The natural form is a gas.

History of Nitric oxide

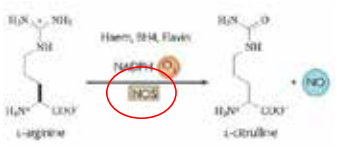
- *Endothelium-derived relaxing factor* (EDRF) was discovered in 1980 by Furchgott and Zawadzki.
- In 1987, it was determined that EDRF was actually nitric oxide (NO).
- Further clarification found that NO was the primary EDRF but other molecules play a role as well.
- NO is an inorganic molecule that naturally occurs in mammalian biology and has been widely studied for health effects in the body.

Function of Nitric oxide

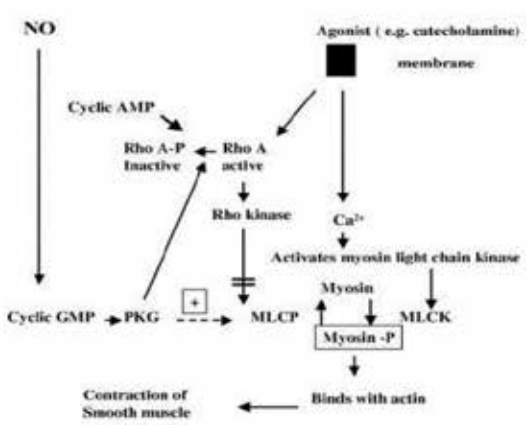
- The endothelium-derived realxing factor, which caudses vasodilation by relaxing vascular smooth muscle.
- Acts as a neurotransmitter.
- Prevents platelet aggregation.
- An essential role in macrophage function
- Vasodilator
- NO and peroxy nitrite induce apoptosis in different types of neural cells.

Synthesis of Nitric Oxide

- Nitric oxide is synthesized from L-arganine.
- This reaction is catalyzed by nitrix oxide synthase, a 1,2,9,4 amino acid enzyme.

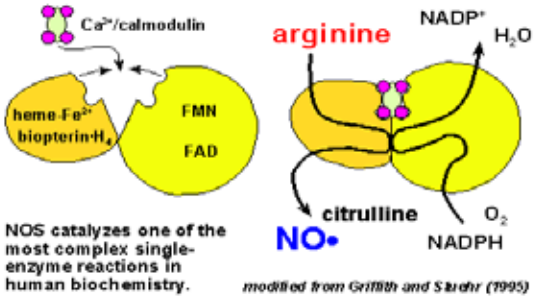


NOS is a homodimeric protein of 125-to 160-kD subunits, each subunit contains one FMN, one, FAD, one tetrahydrobiopterin and one Fe(III)-heme. These cofactors facilitate the five-electron oxidation of arginine to produce NO.



Intracellular mechanism

- When nitric oxide forms in large parts because superoxide anion has height affinity for NO.
- Superoxide anion reduces NO bioavailability.
- NO also binds to the heme mioety of hemoglobin and heme mioety of enzyme gunayl cyclase8 which is found in smooth muscle cell and most other cells of body.
- When NO formed by vascular endothelium it rapidly diffuses into the blood where it binds to hemoglobin and subsequently broken down.



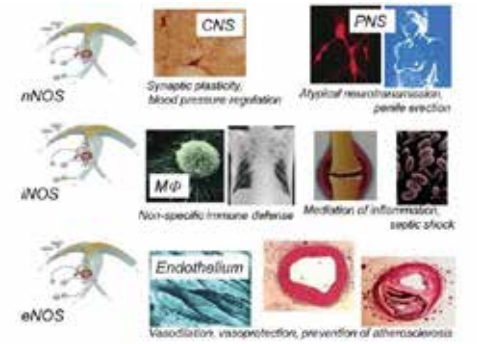
Http://www.kumc.edu/research/medicine/biochemistry/bioc800/sig02-06.htm

- It also diffuses into vascular soomth muscle cells adjacent to the endothelium where it binds to and activate gunyl cyclase. This enzyme catalyse the dephosprylation of CTP to cGMP which serve as a second messenger for many importmant cellular function, particular for signaling smooth muscle contraction.
- cGMP induces cells relaxation by multiple mechanism including.
- Increased intracellular cGMP which inhibit Ca++ entry into the cell and decrease intracellular Ca++ concentration,
- Activates K+ channel which leads to hyper polarization and relaxation.
- Stimulates a cGMP dependent protein kinase that actives mysosin light chain phosphate (MLCK) the enzyme that dephosphorylate myosin light chain leads cells.

Nitric Oxide Synthase (NOS) isoforms

NOS Isoforms	*Gene*	*Type*	*location*	*Cytosolic/ membrane assocaited?*
Endothelial NOS (eNOS) Type II	Chromosome 7	Constitutive Ca2+ calmodulin dependent	Endothelial tissue of blood vessels	Membrane associated
Neuronal NOS (nNOS) Type I	Chromosome 12	Constitutive Ca2+ calmodulin dependent	Central and peripheral neurons	Cytosolic
Inducible NOS (iNOS) Type III	Chromosome 17	Inducible Ca2+ calmodulin independent	Immune cells, astrocytes and microglial cells	Cytosolic
Mitochondrail NOS (mtNOS)	=?	=?	Mitochondrail inner membrane	Membrane associated

Function of Nitric Oxide Synthase



Nitric Oxide in the Nervous System

Nitric oxide as a neurotransmitter

- NO is a signaling molecule, but not necessarily a neurotransmitter
- NO signals inhibition of smooth muscle contraction, adaptive relaxation, and localized vasodilation

Nitric oxide believed to play a role in long term memory

- Memory mechanism proposed is a retrograde messenger that facilitates long term potentiation of neurons (memory)
- Synthesis mechanism involving Ca/Calmodulin activates NOS-I
- NO travels from postsynaptic neuron back to presynaptic neuron which activates guanylyl cyclase, the enzyme that catalyzes cGMP production
- This starts a cycle of nerve action potentials driven by NO

Role of Nitrix Oxide

Nitric oxide in the human body has many role.

I. No in the nervous system
II. NO in the circulatory system
III. NO in the muscular system
IV. NO in the immune system
V. NO in the digestive system
VI. NO in the reproductive system

NO in the gene toxicity

NO in the apoptosis

Is NO a neurotransmitter?

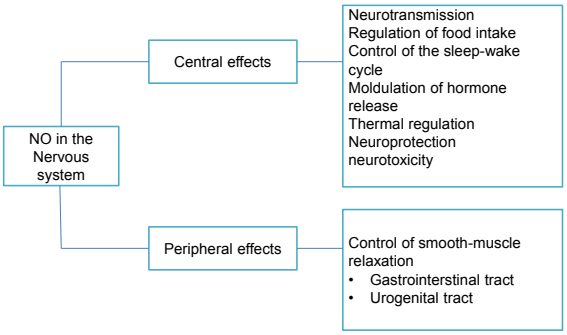
NO serves in the body as a neurotransmitter, but there are definite differences between other neurotransmitters used commonly in the body.

- NO synthasizd on demand and constant synthesis.
- NO diffuses out of the cells making it vs. storage in vesicles and release by exocytosis.
- NO does not bind to surface receptors, but instead exits cytoplasm, enters the target cell, and binds with intracellular guanil cyclase.

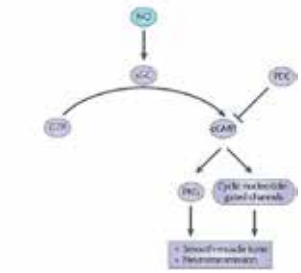
Similarities to normal NTs

- present in presynaptic terminal
- Natural removel from synaptic junction

NO in the nervous system

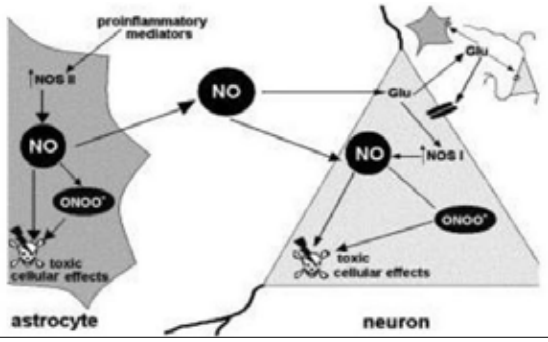


Nitric oxide signaling in the NS

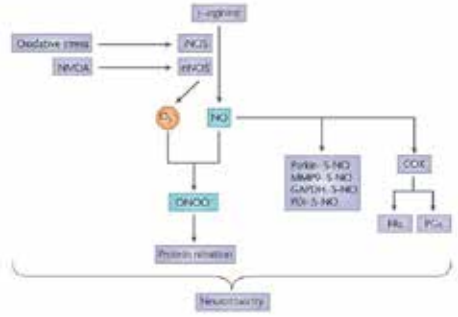


NO-mediated signalling indicated that this gas interacts with soluble guanylyl cyclase (sGC) and stimulates its activity. The consequent increase in intracellular levels of cyclic GMP can influence synaptic plasticity, smooth muscle relaxation, neurosecretion and neurotransmission.

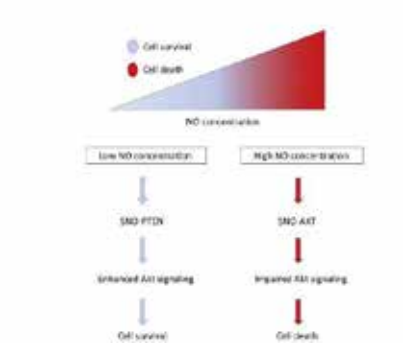
NO mediated effects mediated by astrocytes and neurons.



Neurotoxic effects of nitric oxide.



Actions of NO in neuronal signaling depend on its concentration



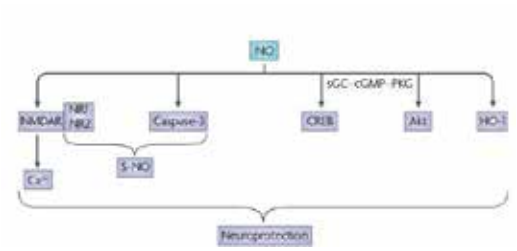
Role in Neurodegenerative disease

Implicated in - Alzheimer disease

- Parkinson disease
- Huntington disease
- Amyotrophic leteral sclerosis

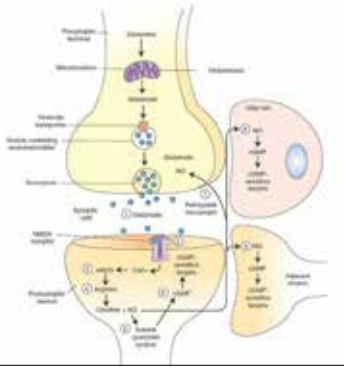
All are related to the excessive release of NO & glutamate both. But in Parkinson's disease Glial cells produce excessive levels of nitric oxide, which may be neurotoxic for a sub population of dopaminergic neurons, especially those not expressing NADPH- diaphorase activity. The presence of glial cells expressing nitric oxide synthase in the substantia nigra of patients with Parkinson's disease represents a consequence of dopaminergic neuronal loss.

Neuroprotective effects of nitric oxide.



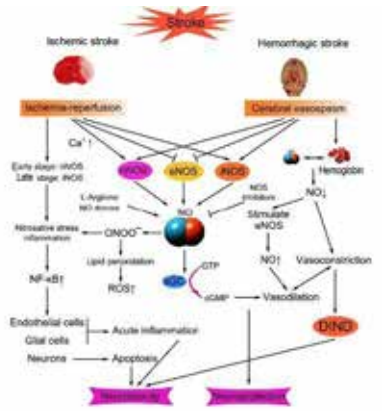
NO confers a neuroprotective effect through multiple mechanisms.

Neurotransmitter



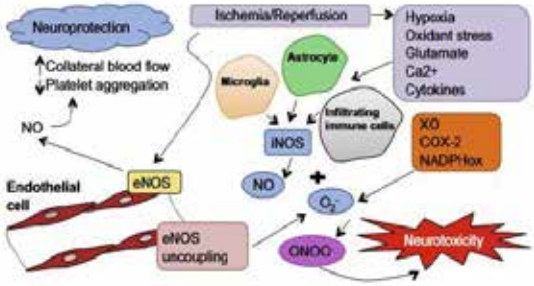
NO and Stroke

- Stroke can be either occlusive or haemorrhagic, and NO appears to play a role in the both types.
- It is proposed that ischemia cause release of NO from the vascular endothelium in an attempt to limit the degree of damage by increasing local blood flow. As ischemia develops and the infarct evolves, NO may have a more deleterious effect.



- Glutamate excitotoxicity has been implicated in stroke: isvhemia markedly increases release due to depolarisation of the pre-synapticneuron. In addition to NMDAR-mediated Ca2+ entry and stimulation of NOS I, NOS II expression also occurs in astrocytes.
- The release of other inflammatory mediators including NF-kβ, TNFαand IRF1 also potentiates NOS II-mediated generation of NO, which peaks between 12 and 24h after ischemia develops.

Roles of NO and peroxynitrite in the pathophysiology of acute brain injury



- Haemorrhagic stroke results in blood spilling into the brain tissue. In addition to focal ischemia “downstream” of the bleed, as haemoglobin is a powerful scavenger of NO, when blood comes into contact with the outside of a blood vessel, this can lead to acute vasospasm.
- The presence of blood out with the vasculature will mediate inflammation, again resulting in activation of the inflammatory cascade including NOS II.

Darambazar Gantulga /MD, PhD/

Mongolian National University of Medical Sciences (MNUMS)

☎: (976)-99193543
✉: darambazar@mnums.edu.mn

Education

2009-2013 Ph.D. Dept. of Physiology, Graduate School of Medicine, Jichi Medical University, Japan. Thesis titled "Nucleobindin-2/nesfatin-1 in the hypothalamic paraventricular nucleus: regulation by metabolic factors and role in energy homeostasis". (Mentor: Toshihiko Yada)

2003 – 2005 M.S. Dept. of Medical Biology, School of Biomedicine, Health Sciences University, Mongolia. Thesis titled "Seroepidemiological study on Taenasis in Mongolian adult population". (Mentor: Temuulen Dorjsuren)

1997 – 2003 B.S. Medical Doctor and Bachelor degree, School of Medicine, Health Sciences University of Mongolia

Research Experience

1. Research Assistant on research project titled "Hypothalamic nesfatin system in maintaining homeostasis" which supported by Strategic Research Program for Brain Sciences (BrainPro), MEXT at Dept. of Physiology, Jichi Medical University, Japan, 2009-2013.
2. Participant of the Lab Course titled "Molecular biology and Confocal microscopy" at Department of Biomedical Sciences, University of Copenhagen, Denmark, in September, 2008
3. Yonsei Scholarship Program 2005, I completed six month training course in Molecular Biology techniques in the Dept. of Parasitology, Yonsei University, Korea.

Work Experience

2005 -2009 Biology and Parasitology lecture Teaching Assistant, Department of Medical Biology, School of Biomedicine, Health Sciences University, Mongolia

2013 -2014 Biology instructor, Department of Biology, School of Biomedicine, School of Biomedicine, National University of Medical Sciences, Ulaanbaatar, Mongolia

2014 - Principal, University High School, National University of Medical Sciences, Ulaanbaatar, Mongolia

Scholarships and awards

1. Research Encouragement Award 2011, this grant is given by Jichi Medical University for graduate student with excellent research projects.
2. Research Grant for Young Scientists 2007, this grant is given by Ministry of Education, Culture and Science of Mongolia on competitive basis.

Laboratory experience

Neuroscience: Single neuron preparation, calcium imaging in single neuron, cannulations and microinjections in rodent brain, brain fixation, sectioning and immunohistochemistry

Physiology: Lab animal handling, breading, GTT, ITT, calorimetry measurements.

Molecular biology: DNA/RNA extraction, PCR amplification, gel electrophoresis, Western Blotting, ELISA

Publications

1. Ando A, Gantulga D, Nakata M, Maekawa F, Dezaki K, Ishibashi S, Yada T. Weaning stage hyperglycemia induces glucose-insensitivity in arcuate POMC neurons and hyperphagia in type 2 diabetic GK rats. Neuropeptides. 2018 Apr;68:49-56.
2. Nakata M, Gantulga D, Santoso P, Zhang B, Masuda C, Mori M, Okada T, Yada T. Paraventricular NUCB2/Nesfatin-1 Supports Oxytocin and Vasopressin Neurons to Control Feeding Behavior and Fluid Balance in Male Mice. Endocrinology. 157(6):2322-32. 2016
3. Kurita H, Xu KY, Maejima Y, Nakata M, Dezaki K, Santoso P, Yang Y, Arai T, Gantulga D, Muroya S, Lefor AK, Kakei M, Watanabe E, Yada T. Arcuate Na+,K+-ATPase senses systemic energy states and regulates feeding behavior through glucose-inhibited neurons. Am J Physiol Endocrinol Metab. 309(4):E320-33, 2015
4. Maejima Y, Rita RS, Santoso P, Aoyama M, Hiraoka Y, Nishimori K, Gantulga D, Shimomura K, Yada T. Nasal oxytocin administration reduces food intake without affecting locomotor activity and glycemia with c-Fos induction in limited brain areas. Neuroendocrinology. 101(1):35-44, 2015
5. Darambazar G, Nakata M, Okada T, Wang L, Li E, Shinozaki A, Motoshima M, Mori M, Yada T. Paraventricular NUCB2/nesfatin-1 is directly targeted by leptin and mediates its anorexigenic effect. Biochem Biophys Res Commun. 456(4):913-8, 2015
6. Aoki H, Nakata M, Dezaki K, Lu M, Gantulga D, Yamamoto K, Shimada K, Kario K, Yada T. Ghrelin counteracts salt-induced hypertension via promoting diuresis and renal nitric oxide production in Dahl rats. Endocrine Journal. (Epub ahead of print http://dx.doi.org/10.1507/endocrj.EJ12-0371)
7. Gantulga D, Maejima Y, Nakata M, Yada T. Glucose and insulin induce Ca2+ signaling in nesfatin-1 neurons in the hypothalamic paraventricular nucleus. Biochem Biophys Res Commun. 420(4), 811-815, 2012
8. Kohno D, Sone H, Tanaka S, Kurita H, Gantulga D, Yada T. AMP-activated protein kinase activates neuropeptide Y neurons in the hypothalamic arcuate nucleus to increase food intake in rats. Neurosci Lett. 499(3):194-8, 2011
9. Yoshida N, Maejima Y, Sedbazar U, Ando A, Kurita H, Damdindorj B, Takano E, Gantulga D, Iwasaki Y, Kurashina T, Onaka T, Dezaki K, Nakata M, Mori M, Yada T. Stressor responsive central nesfatin-1 activates corticotropin-releasing hormone, noradrenaline and serotonin neurons and evokes hypothalamic pituitary adrenal axis. Aging 2(10): 775-784, 2010
10. Lee H, Jeong KY, Shin KH, Yi MH, Gantulga D, Hong CS, Yong TS. Reactivity of German cockroach allergen, Bla g 2, peptide fragments to IgE antibodies in patients' sera. Korean J Parasitol. 46(4):243-6, 2008
11. Lee JW, Gantulga D, Yong TS. PCR identification of Anopheles species using species-specific primers designed from ITS2. Mongolian Journal of Health Science, 2: 64, 2006
12. Gantulga D, Temuulen.D, Gurbadam.A. Indirect ELISA test for diagnosis on taeniasis and cysticercosis. J Mongolian Medicine 3: 48, 10, 2004
13. Gantulga D, Dulamsuren.D and Temuulen.D. A Seroepidemiological study on taeniasis in Mongolian Adult population. J Mongolian Medicine 2: 132, 14, 2005

Primary neuronal cell culture

Darambazar Gantulga MD., PhD.

Dissection

- Regardless of the tissue source, the general method for producing primary neuronal cultures can be summarized in a few major steps: Dissection, dissociation, plating, and maintenance.
- In preparing for the dissection and culturing steps, sterility is critical to prevent cultures from becoming contaminated. Tools must be sterilized with alcohol, and a laminar flow hood is often used to remove airborne contaminants.
- For culturing rodent neurons, tissue should be removed from freshly euthanized animals into a cold, buffered salt solution. Using a dissecting microscope, smaller parts of the tissue – such as the hippocampi – can be carefully isolated for further processing.

Overviev

- The complexity of the brain often requires neuroscientists to use a simpler system for experimental manipulations and observations.
- One powerful approach is to generate a primary culture by dissecting nervous system tissue, dissociating it into single cells, and growing those cells *in vitro*.
- Primary cultures make neurons and glia easily accessible to the experimental tools required for techniques like genetic manipulation and time-lapse imaging.
- Furthermore, these cultures represent a highly controllable environment in which to study complex phenomena such as cell-cell interactions.

Dissociation

- It's necessary to break the sample down into its cellular components in a process known as dissociation.
- The dissected tissue is first minced using a scalpel or scissors. The resulting tissue pieces are then transferred to a new container.
- A proteolytic enzyme such as trypsin or papain is then added to digest the extracellular matrix proteins that bind the cells together. Following a short incubation in a warm incubator or water bath, the tissue pieces are gently washed with buffer to remove the enzymes.
- The softened tissue pieces can now be dissociated by trituration, which involves passing the tissue through a pipet multiple times so that the cells are freed into a single cell suspension. At this point, the cells can be counted for concentration and checked for viability.

Tissue Sources for Preparing Primary Neuronal Cultures

- Many of the animal models important to neuroscience research can be used to prepare primary neuronal cultures. With mice and rats – two of the most commonly used mammals in biomedical research – neuronal tissue from embryos, newborn pups, and adults can be dissected for culturing.
- Embryos and perinatal pups are typically preferred since the brain cells are immature and less susceptible to damage.
- Various nervous system tissues can be isolated and used to produce different types of primary neuronal cultures. For example, specific parts of the brain can be dissected, such as the cerebellum, cortex and hypothalamus.
- The spinal cord or components of the peripheral nervous system, such as the dorsal root ganglia, can also be dissected and cultured to grow specific types of neurons.

Plating and Maintenance

- The composition of the culture media that is used to grow the neurons is very important. Special supplements that support neuronal survival and growth are usually added to the media. Other additives can include drugs that inhibit the division of non-neuronal cells such as glial cells.
- After the neuronal cell suspension is mixed with the media, the cells are ready to be plated in the desired containers. These can include culture dishes and plates or glass coverslips placed inside these containers. Since neurons cannot grow directly on glass, the coverslips are treated beforehand to create better surfaces for cell attachment and growth. These treatments can include coating with synthetic extracellular matrix proteins; for example, poly-lysine and laminin; or acid etching to roughen the surface.
- Once plated, the neurons are grown inside a warm, humidified incubator.

Applications

- One basic method commonly applied to neuronal cultures is genetic manipulation by transfection or viral transduction. Genetic material encoding, for example, a mutant protein, can be introduced into the cultured neurons to alter neuronal function. Alternatively, transfection with double stranded RNA is an effective technique for blocking protein expression. The availability of multiple methods and reagents for genetic manipulation make this a particularly valuable application for a wide variety of research questions.
- Primary neuronal cultures are also very useful for electrophysiology studies, in which the electrical activity of single cells can be measured using microelectrodes. The single layer of neurons in a culture means that individual cells are easy to target, and manipulations such as pharmacological treatments are quickly and evenly applied. For example, the effect of a test compound on a single neuron's activity can be measured with the patch clamp technique in cultured neurons.
- Calcium imaging

Summary

- Primary neuronal cultures can be made from a wide variety of tissue sources, typically using a method that involves dissection, dissociation into a cell suspension, and growing in culture media in incubators.

1 Preparation for Cell Culture

Aliquoting of penicillin-streptomycin mixture (1-1)

RPMI and DMEM medium of cell culture required antibiotics that 100 U penicillin and 0.1 mg/mL streptomycin. This solution contains 10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin. Therefore, its convenient for further experiments to divide 5ml of antibiotic mix from the 500 ml stock.

Materials:

Reagents:

1. Penicillin-Streptomycin 100 mL (-200C)

Consumable items:

1. 10 mL pipette
2. 5 mL tube x 20 ps

Equipment:

1. Water bath
2. Clean bench
3. -20° C freezer

Preparation:

1. Penicillin- Streptomycin solution is thawed at 37° C in a water bath.
2. Prepare 20 tubes of 5 ml tubes.

Procedure:

1. Aliquote the antibiotic mix with the volume of 5 ml.
2. Clog and mark the tube and store at -200C.

Heat inactivation of fetal bovine serum (FBS) and aliquoting (1-2)

Purpose:

FBS contains nutritious substances but at the time. It contains proteins and immunoglobulins. So it has to be inactivated.

Materials:

Reagents :

1. FBS (500 mL, -20°C)

Consumable items:

1. 50 ml tube x 12 ps
2. 50 ml pipette x 1 ps
3. Cell Strainer (70 µm Nylon)

Equipment:

1. Water bath
2. -200C freezer
3. Clean bench

Preparation:

1. Thaw frozen FBS in the 37° C water bath.
2. Prepare 12 tubes of 50 ml.

Procedure:

1. Adjust the temperature of water bath at 57oC.
2. Aliquot the FBS into 50 ml tubes with the volume of 43-45 ml.
3. Store the bottled FBS at the -20° C.

DNA Extraction Protocol (1-3)

Materials:

Reagents:

1. Ethanol (96–100%)
   QIAamp DNA Blood Maxi Kit:
2. Spin Columns
3. Collection Tubes (50 ml)
4. Buffer AL
5. Buffer AW1 (concentrate)
6. Buffer AW2 (concentrate)
7. Buffer AE
8. Protease

Equipment:

1. Centrifuge
2. Vortex
3. Water bath or incubator set at 70°C
4. 50 ml centrifuge tubes
5. Eppendorf pipettes and tubes

Preparation:

1. Equilibrate samples to room temperature (15–25°C) before starting.
2. Prepare a 70°C water bath or incubator.
3. Pipet 5.5 ml distilled water into a vial of lyophilized QIAGEN Protease.
4. Mix Buffer AL thoroughly by shaking before use. If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C
5. Buffer AW1 and AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on each bottle.

Procedure:

1. Dispense 3–5 ml or 5–10 ml blood into centrifuge tubes.
2. Add 500 µl Protease to samples.
3. Mix Briefly.
4. Add 6 ml or 12 ml Buffer AL.
5. Mix thoroughly.
6. Incubate at 70°C for 30 min.
7. Add 5 ml or 10 ml ethanol (96–100%) to the samples.
8. Mix thoroughly.

9. Transfer half of the solution from step 8 onto the QIAamp Maxi column placed in a 50 ml centrifuge tube.
10. Close the cap and centrifuge at 3000 rpm for 3 min.
11. Remove the column, discard the filtrate, and place the column into new 50 ml centrifuge tube. Load the remainder half of the solution from step 8 onto the column. Close the cap and centrifuge again at 3000 rpm for 3 min.
12. Remove the column, discard the filtrate, and place the column back into the 50 ml centrifuge tube.
13. Add 5 ml Buffer AW1 to the column. Close the cap and centrifuge at 5000 rpm for 1 min.
14. Add 5 ml Buffer AW2 to the column. Close the cap and centrifuge at 5000 rpm for 25 min.
15. Place the column in a clean 50 ml centrifuge tube, and discard the collection tube containing the filtrate.
16. Incubate at 70°C for 10 min.
17. Add 600 µl or 1 ml Buffer AE directly onto the column membrane and close the cap. Incubate at RT for 5 min, and centrifuge at 5000 rpm for 5 min.
18. Apply DNA solution to a tube.

2 Culturing Cell Lines

Refreshing cell line (2-1)

Purpose:

To use frozen cells in the further experiments.

Materials:

Reagents:

1. DMEM (Dulbecco's Modified Eagle Medium)
2. FCS (Fetal Calf Serum)
3. END-D and RAW 265.7 cells

Consumable items:

1. 15 ml tube
2. 50 ml tube
3. 10 ml pipette
4. Autoclave bags

Equipment:

1. Clean bench
2. Centrifuge
3. Water bath
4. Incubator (CO2 5%, 370C)
5. Vortex

Preparation:

1. Prepare autoclave bag.
2. First warm the culture medium in 37°C water bath for at least 30 min.
3. Remove the cryopreserved cells.

Procedure:

1. Note the 15 ml tubes and add 5 ml of DMEM medium to each tube.
2. Remove the cryovial containing the frozen cells from liquid nitrogen storage and immediately place it into a 37°C water bath.
3. Transfer the cell into 15 ml tube and mix well.
4. Centrifuge the cell suspension at approximately 1800 rpm for 5 minutes. The actual

centrifugation speed and duration varies depending on the cell type. (Washing step 1)

5. Leave the all pellet and discard the supernatant. Wash again with cell culture medium (1800 rpm, room to, 5 min) (Washing step 2)
6. Discards the supernatant and add 5 to 7 ml DMEM medium (20% FBS-DMEM).
7. Close the cup of the 15 ml tube loosely and shake in the vortex.
8. Put cell suspension in 25 cm2 flask.
9. After 3-4 days, if the cell number increased or the medium color altered, add 1-2 ml of growth medium (20% FBS)
10. Depending on the cell growth rate and colony formation, change the growth medium, or subculture the cell cells into 6 well plate and 25 cm2 flask. (every 3-4 day growth medium should be changed with the concentration 10% FBS)

Precautions:

1. When removing samples from -80o C or liquid nitrogen tank use ethanol induced cotton to look the cap in order to release gases. Then re-tight cap and put into water bath.

Cell Counting Protocol (2-2)

Materials:

Reagents:

1. Cell staining dye (Trypan blue, Turk)
2. Cell medium

Equipment:

1. Hemocytometer with cover slip
2. Cell counter
3. Microscope
4. Eppendorf pipettes and tubes

Preparation:

1. To prepare the hemocytometer, the mirror-like polished surface is carefully cleaned with lens paper and ethanol.
2. Take the well sample for counting into the 96 well plate.

Procedure:

1. Dilution depends on the cell number. For example, using appropriate Eppendorf pipettes, combine 90 µl of 0.1% trypan blue and 10 µl of suspended cells in a for 10x dilution. Mix well.
2. Inject 10 µl of trypan blue/cell mixture into each side of hemocytometer.
3. Count cells in the 4 large squires which consist of 16 small squires. If a cell is stained blue, then it is dead and should not be counted.

- **Cell count per 1 ml = 4 large squires (16 small squires) / 4 x dilution factor x 104**

  Total cell number = cells per 1 ml x the original volume of fluid

Nucleic acid Quantification (DNA-Gel electrophoresis) (2-3)

Purpose

Separating DNA by size (e.g., length in base pairs) for visualization and purification.

Equipment

- Casting tray
- Well combs
- Voltage source
- Gel box
- UV light source
- Microwave

Reagent

- TAE
- Agarose
- Ethidum bromide
- Size marker

Procedure

Standard 1% Agarose Gel

1. Measure 1 g of agarose
2. Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel.
3. Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.
4. Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2-3 µl of lab stock solution per 100 mL gel).
5. Pour the agarose into a gel tray with the well comb in place.
6. Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

Loading Samples and Running an Agarose Gel:

1. Add loading buffer to each of your DNA samples
2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill gel box with 1xTAE (or TBE) until the gel is covered.
4. Load a molecular weight ladder into the first lane of the gel.
5. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage

6. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box
7. Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.
8. Using the DNA ladder in the first lane as a guide (the manufacturer’s instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes.

3 Experiments Based on the Cell Culture

Changing medium and passaging cell lines (3-1)

Purpose:

It is necessary to replace the medium in order to further enhance the incubation of cells. Color of the medium change, or the cells starting to die, the cell medium is considered to be inadequate. Following conditions the cell culturing medium will be replaced alteration in color of the medium. Cell death (causes cell debris turn into black and float on the surface). If the confluence of the flask reached the peak, passaging of the cell culture will be needed.

Materials:

Reagents:

1. FBS (stored at 40C)
2. DMEM medium (stored at 4°C)

Consumable items:

1. 50 ml tube
2. 25 см2 flask
3. 10 ml pipette
4. 50 ml pipette

Equipment:

1. Water bath
2. Centrifuge
3. Incubator
4. Clean bench

Preparation:

1. Put DMEM medium and FBS in 370C water bath.
2. Prepare tubes for washing the cells (select tubes depending on the cell and its number).
3. Prepare the flask (choose the size depending on the number of cells).
4. Prepare the autoclave bag.

Procedure:

1. Transfer the cells to an appropriate tube.
2. Centrifuge 1600 rpm, at ambient temperature, for 5 minutes. Leave the pellet remove the liquid on the cell to the waste container.

3. Re-suspend the cell pellet in a complete growth medium (10% FBS). Predictability of cellular preparation is anticipated. Adjust the volume according to the site of the cell culture flask.
4. Incubate the plate at 370 C, in presence of 5% CO2.
5. Media should be changed every 3-4 days until the cells attain 80-90% confluence.

Preparation of a Nitrite Standard Reference Curve (3-2)

A Nitrite Standard reference curve must be prepared for each assay for accurate quantitation of NO2– levels in experimental samples.

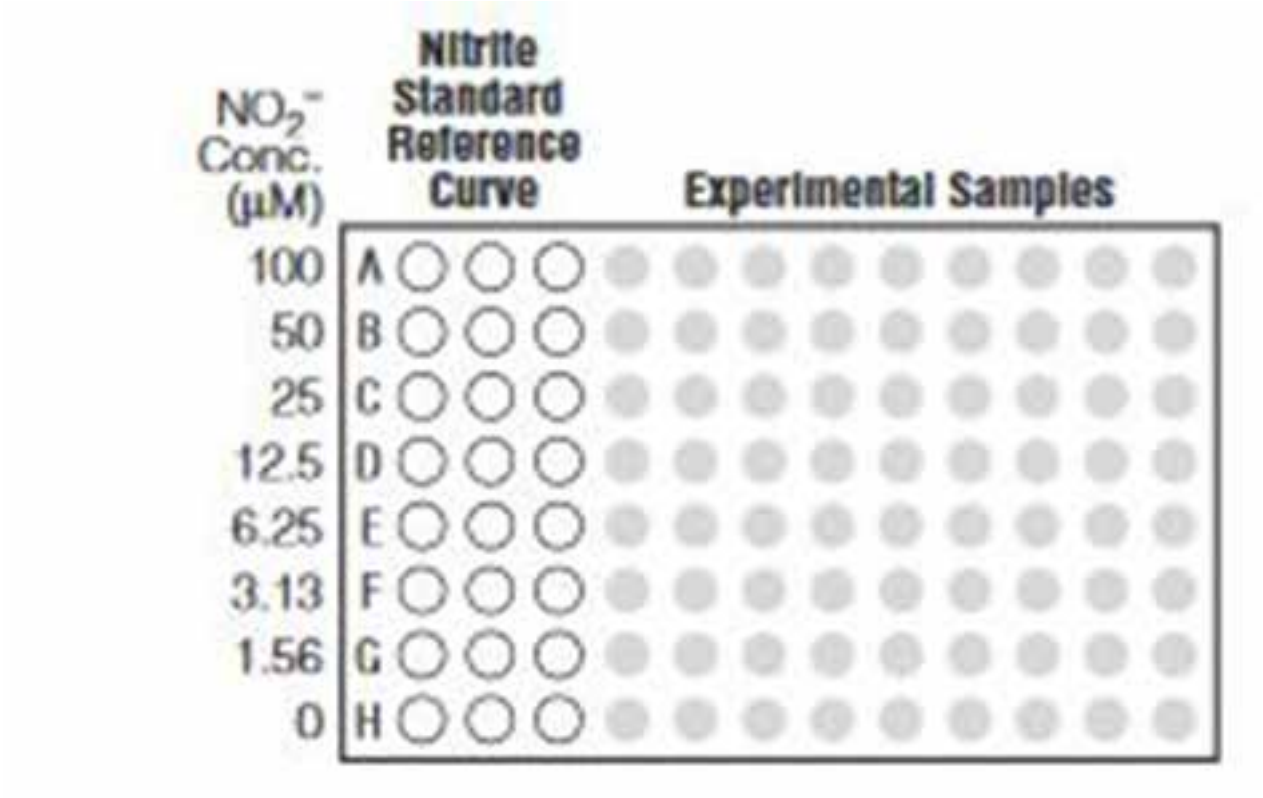


Figure: Suggested plate format for the Nitrite Standard reference curve

Procedure:

1. Prepare 1ml of a 100µM nitrite solution by diluting the provided 0.1M Nitrite Standard 1:1,000 in the DMEM used for the experimental samples.
2. Designate 24 wells in the 96-well plate.
3. Dispense 50 µl of the appropriate DMEM the wells in rows B–H.
4. Add 100 µl of the 100 µM nitrite solution to the remaining 3 wells in row A.
5. Immediately perform 6 serial twofold dilutions (50 µl/well) in triplicate down the plate to generate the Nitrite Standard reference curve (100, 50, 25,12.5, 6.25, 3.13 and 1.56 µM), discarding 50 µl from the 1.56 µM set of wells.Do not add any nitrite solution to the last set of wells (0 µM).
6. Add 25µl of the Sulfanilamide Solution.
7. Incubate 5–10 minutes at room temperature, protected from light
8. Dispense 25 µl of the NED Solution.
9. Incubate at room temperature for 5–10 minutes, protected from light.
10. Measure absorbance within 30 minutes in a plate reader with a filter 492 nm.

Determination to Nitric oxide (3-3)

Introduction of Theory

Nitric oxide (NO) is an important physiological messenger and effector molecule in many biological systems, including immunological, neuronal and cardiovascular tissues. One means to investigate nitric oxide formation is to measure nitrite (NO2–),which is one of two primary, stable and nonvolatile breakdown products of NO. This assay relies on a diazotization reaction that was originally described by Griess reagent. The Griess Reagent System is contain sulfanilamide and N-1-napthylethylenediamine dihydrochloride (NED).

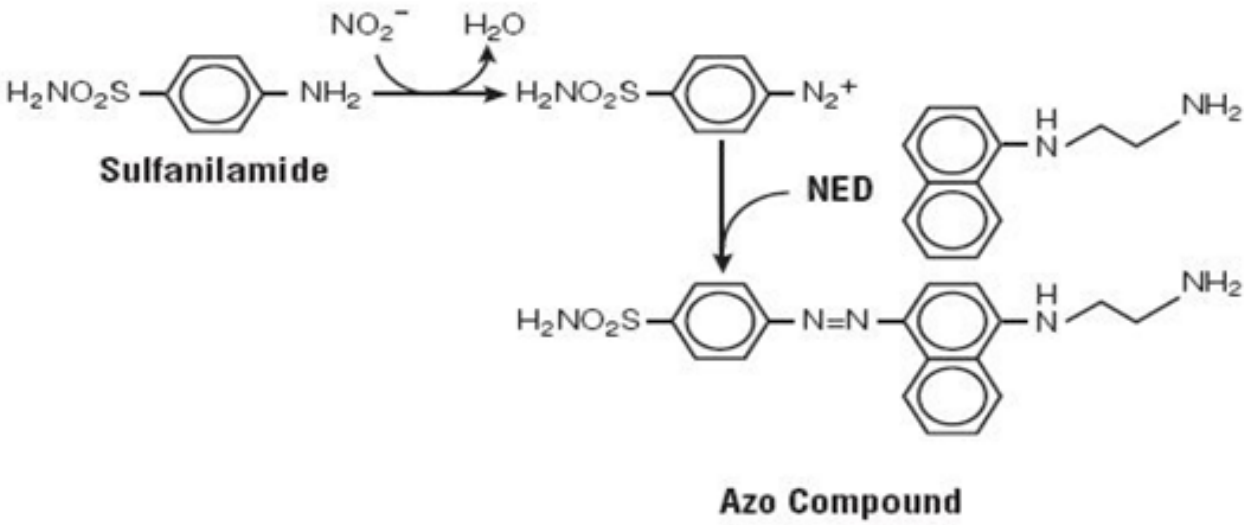


Figure: Chemical reactions involved in the measurement of NO2–using the Griess

Materials :

- Griess Reagent System
- DMEM
- pipettor
- 96-well flat-bottom
- ELISA reader etc,

Procedure:

1. Allow the Sulfanilamide Solution and NED Solution to equilibrate to room temperature (15–30 minutes).
2. Add 50 µl of each experimental sample to wells in duplicate or triplicate.
3. Add 25µl of the Sulfanilamide Solution.
4. Incubate 5–10 minutes at room temperature, protected from light.
5. Dispense 25 µl of the NED Solution.
6. Incubate at room temperature for 5–10 minutes, protected from light.
7. Measure absorbance within 30 minutes in a plate reader with a filter 492 nm.

IBRO-APRC Associate School 2018 Participants List

	First Name	Last Name	M/F	Profession	Research Title/Interest	Contact Information
1	Ariunzaya	Bat-Erdene	F	Biochemistry	Synergistic targeting of Sp1, a critical transcription factor for myeloma cell growth and survival, by panobinostat and proteasome inhibitors	myzoeis@yahoo.com
2	Anir	Khurelbaatar	M	Medical Doctor	Paraffin embedded tissue section preparation Chronic implant for rat brain	99196656 anirkh05@gmail.com
3	Anudari	Tseveendorj	F	Psychology	Psychology	99089223 anudari6891@gmail.com
4	Baigalmaa	Byambatseren	F	Biology-Biochemistry	Lactoferrin purification from bovine colostrum	99381257 baigal.b@mnums.edu.mn
5	Guljanat	Yerlan	F	Psychiatrist	Some results of study on determining emotional and behavioral problems among adolescents	88064236 guljanat_ye@yahoo.com
6	Enkhee	Ochirjav	F	Pathologist	WT1 expression in astrocytic brain tumors and its relationship with patients' prognosis	99122852 enhee002@gmail.com
7	Erdenezaya	Odkhuu	F	Medical Doctor	Regulation of NOS and COX cross-talk in HEI-OC1 auditory hairy cell by bacterial lipopolysaccharide	99169798 erdenezaya@mnums.edu.mn
8	Gantulga	Javzandulam	M	Psychiatrist	Addiction Medicine, Child psychiatry	89740615 gantulga9997@gmail.com
9	Javkhlan	Bold	M	Medical Doctor	Cell viability assay method	99212626 javkhlan.b@mnums.edu.mn
10	Mandakh	Mishig	F	Psychiatrist psycho-therapist	Association study of NRG1, DISC1, 5HTR2A and TPH2 genes with Schizophrenia	98170602 99058979 mandakhmishig17@gmail.com
11	Misheel	Davaadorj	F	Psychiatrist	Lead in solvent-based paints for home use in Mongolia	99995079 misheeld13@gmail.com
12	Munkhzaya	Borbandi	F	Psychologist	Study on some psychological features of children with internet gaming disorder	99741063 munkhzayaborbandi@gmail.com

13	Naranchimeg	Baatar	F	Medical Doctor	Abnormal brain development and delayed neurodevelopmental phenotypes resulting from c12orf57 mutation	94061337 b.naranchimeg@gmail.com
14	Orkhontuul	Shirmen	F	Neurologist Neurosurgeon	Influence of aneurysm location on the development of chronic hydrocephalus following SAH	99078871 orkhontuul.sh@gmail.com
15	Oyungerel	Dogsom	F	Medical Doctor / Lecturer	Bacterial culturing, All kind of ELISA, Immunodiffusion, B cell and T cell isolation from patient blood, clinical biochemical tests	99123401 oyungerel.d@mnums.edu.mn
16	Saraa	Unurkhaan	F	Neurologist Neurosurgeon	Clinical subtypes of stroke records at Shastin third general hospital of Mongolia – a single National Center Observati	80191537 saraan_he@yahoo.com
17	Sayamaa	Lkhagvadorj	F	Pathologist	Klotho Expression in Clear Cell Renal Cell Carcinoma and Its Correlation with Prognosis	89038022 sayamaa@mnums.edu.mn
18	Tsetsgee	Damdin	F	Oriental Medical Doctor	Traditional Chinese Medicine/Acupuncture studies	88052538 tsetsgeedamdin77@gmail.com
19	Tsevelmaa	Nanjidsuren	F	Medical Doctor /Biotechnology	Genetic engineering, animal reproduction, research of biological active compounds	88310330 tsevelmaa.n@mnums.edu.mn
20	Munkhtsetseg	Galdan	F	Linguist	Psycholinguistics Research of Language Acquisition Acts of Mongolian Children – Based on Language Facts of Children of Ages 6-12	80506506 moogie52004@yahoo.com
Additional Participants						
21	Otgon-Ujin	Munkhbaatar	F	Nano-technology	Mechanizm of Fiber Formation	88121110 otgonujin@gmail.com
22	Purevsuren	Munkhuu	F	Child psychologist	Standardizing the ‘ways of coping’ questionnaire	88952298 96686861 purevsuren. munkhuu.apdc@gmail.com
23	Janbolat	Ashim	M	Biotechno-logiest	Co-IP, RNA-FISH, Antibody-antigen interaction. Recombinant DNA methodology.	janbolatdna@gmail.com
24	Chibaatar	Enkhmurun	F	Neuroscience	Astroglial GLT-1 modulates synaptic plasticity in hippocampus and depressive behaviors in PSD rats	murun0824@gmail.com

MNUMS
Mongolian National University of Medical Sciences
1942

CORE LABORATORY

New idea

Best solution

Research University

THE CORE LABORATORY OF SCIENCE TECHNOLOGY CENTER

Mongolian National University of Medical Sciences (MNUMS) is opening a new laboratory called the "Core Laboratory" in scope of its goal to ensure integrity of medical education, health care services and scientific research with a funding of 5.3 billion MNT (over 4 million USD) from the Government of Mongolia.

This laboratory is facilitated with modern technological equipment from the US, Japan, Germany, PRC, India and South Korea, and offers a strong and cohesive research environment for researchers with its aim to support world-class research work at the university level.

Core laboratory will play a key role for MNUMS to attain international level research quality in six major disciplines, including cell culture, molecular biology, protein and chemical analysis, pathology and histology, live cell imaging technology, and experimental animal facilities.

LABORATORY TECHNOLOGIES:

- Cell culture
- Molecular biology
- Nucleic acid/genomic analysis
- Protein/chemical analysis
- Pathology/immunohisto-chemistry
- Live cell imaging
- Experimental animal laboratory

CORE EQUIPMENT

- Cell culture laboratory system
- PCR, RT-PCR, Gene sequencer system
- Gel electrophoresis system
- Mass spectrometry
- High performance liguid chromatography
- Immunohistochemistry and Immunoflourescence research
- Corr Sight TM electron/ Confocal microscopy complex

CELL CULTURE LABORATORY

Cell culture laboratory is focused on biomedical research that is built on primary and linear cell culture studies using experimental animals on various normal and pathologic *in vivo* research models as well as utilization of isolated primary and linear cell cultures in *in vitro* to investigate target cell differentiation, secreting cytokines and biological active substrates from the cells, and molecular integration within cellular level. The cell culture laboratory embraces PC 2 level of biosafety.

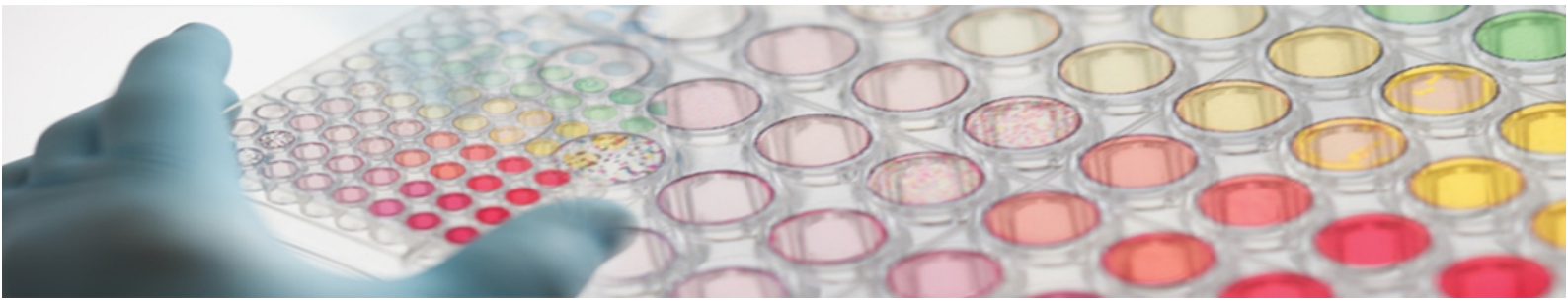
With the advancement of cryopreservation technology, the laboratory provides the major resource of cryopreserved linear cells for experimental studies, to freeze down samples and cells at 4^0C, -20^0C, -80^0C using refrigeration and at -200^0C with liquid nitrogen.

Biohazard Safety Clean Bench,
VS-1400LSN, Vision Scientific, Korea

Benchtop Refrigerated centrifuge,
Combi-514R, Hanil Science Industrial, Korea

- 80^0C ultra-low Deep freezer,
DW-86W420, Haier Laboratory and Medical Products, Chin

Liquid Nitrogen Tank,
Direct-Q-3, Fisher Scientific Korea



MOLECULAR BIOLOGY LABORATORY

The Molecular biology laboratory is housed with a range of most up-to-date molecular biology equipment to isolate, amplify, and sequence a variety of genetic materials, particularly nucleic acid from biological samples.

In order to determine a unique sequence of nucleic acids, the laboratory allows researchers to identify polymorphic type, genetic mutations and compare those with pathologic changes.

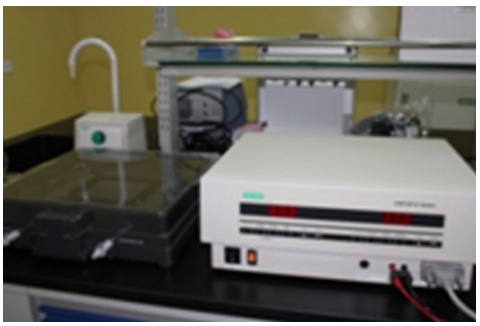
It conducts the following studies:

- Population genetic study
- Genetic study of Quantitative Trait Loci
- Study of gene and genomic region associated with diseases

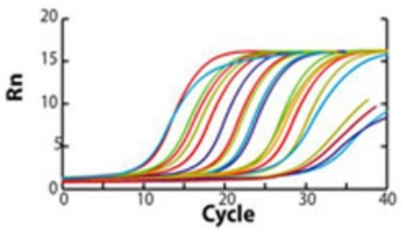
DNA or RNA automatic purification system ExiPrep and ExiCycler 96, Bioneer, Korea

Real Time-PCR, CFX96, Bio-Rad, USA

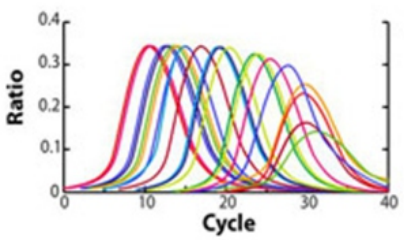
Personal Genome Machine, PGM ION TORRENT, Life Technologies, USA



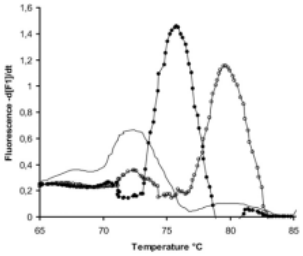
Pulse Field Gel Electrophoresis, Chef-Dr III system, Bio-Rad, USA

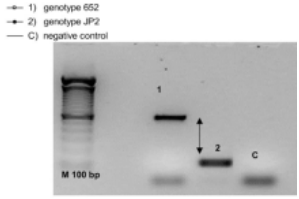


real-time PCR curve



*max*Ratio curve





PROTEIN AND CHEMICAL ANALYSIS

Protein and chemical analysis laboratory generates important insights into protein, vitamin, biological active substances, and drug molecule isolation with quantitative and qualitative rapid assays using high performance liquid chromatography, and mass spectrometry.

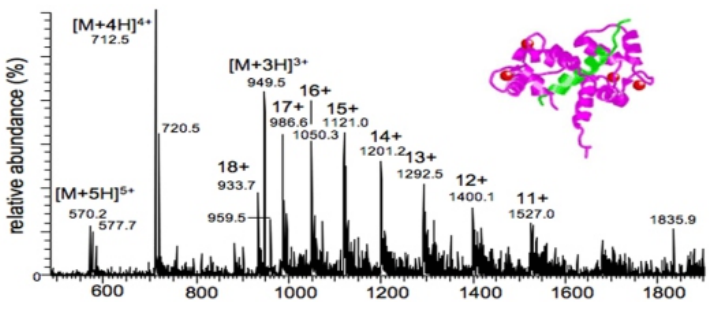
The laboratory has the capacity to determine biological active molecules from widespread plants, herbs, animals, and minerals in Mongolia, and generate quantitative and qualitative data. Isolating pure components from logical active molecules of bio-materials from widespread natural raw materials and determining molecular mechanisms, and developing medicinal drugs have a crucial impact to develop university brand research priorities.

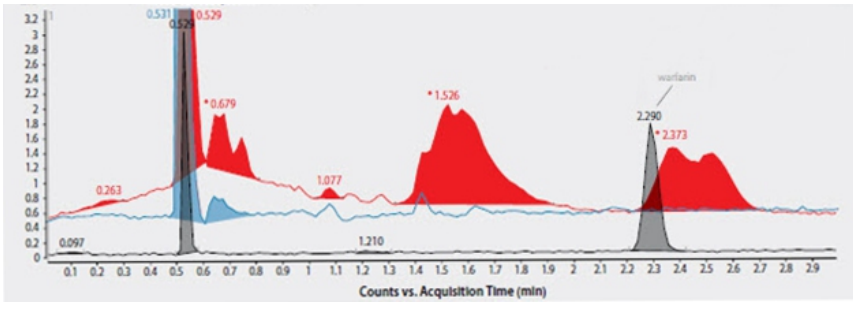
HPLC,
NEXERA X2, Shimadzu, Japan

Mass Spectrometer, LCMS-8040,
Shimadzu, Japan

Semi dry electrophoretic transfer cell, Trans-Blot SD cell 221 BR,
Bio-Rad, USA

ChemiDoc MP system,
Universal Hood III, Bio-Rad, USA

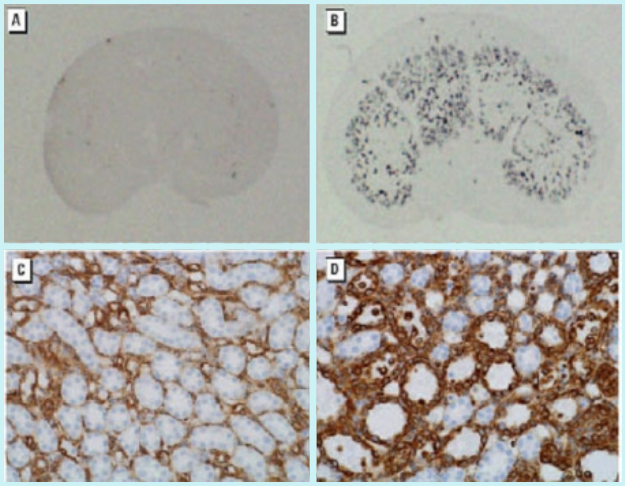




PATHOLOGY LABORATORY

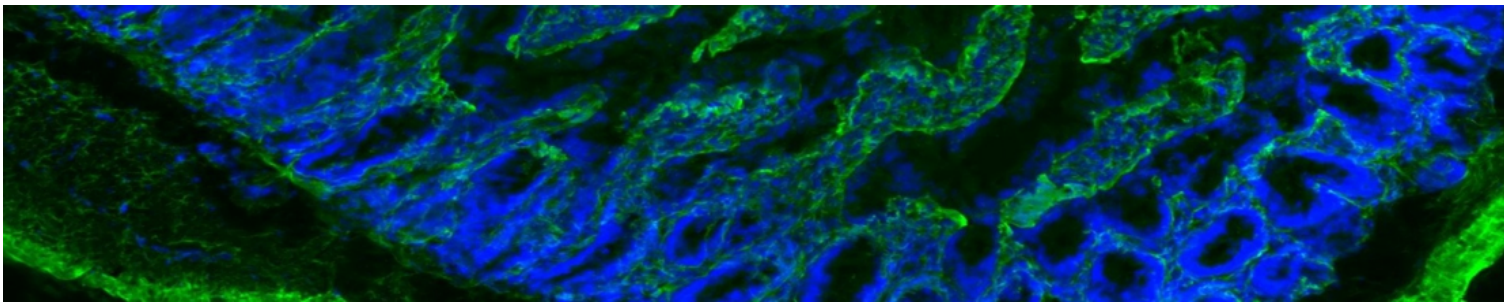
Pathology laboratory works toward isolating cell and tissue from biologic materials for fixation and is facilitated with cryo-sectioning microtome, immunofluorescence equipment to prepare tissue slides.

Immunohistochemistry and immunofluorescence assays are critical to demonstrate the results of pathology, cell culture, molecular biology, protein, and gene expression.



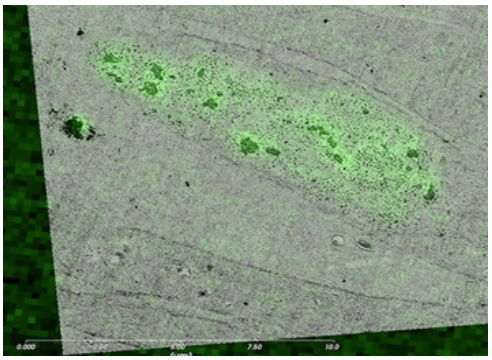
Fluorescence inverted microscopy, XYL 403, USA

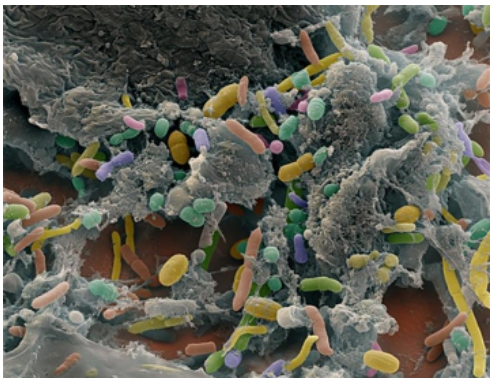
Microtome with freezer, Kryostat MEV, SLEE Medical, Germany

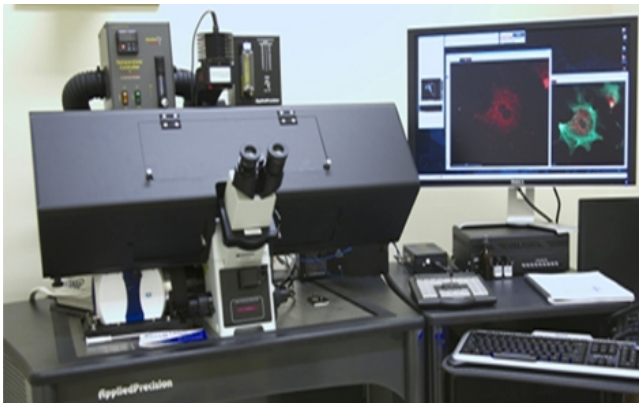


LIVE CELL IMAGING

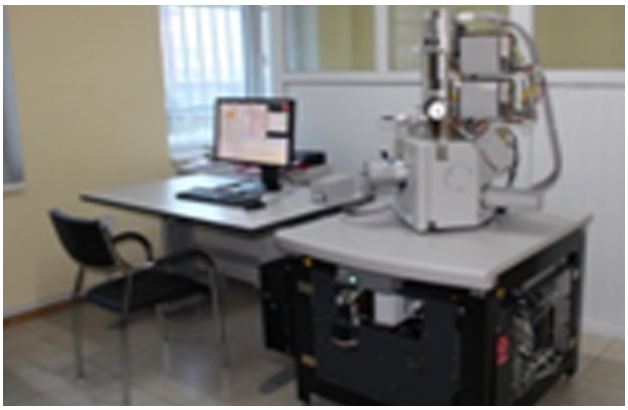
Live cell imaging laboratory is equipped with cutting-edge electron and confocal laser microscopy system that allows to study structure and functional interactions of live cells with the highest precision. With this system,researchers will have the opportunity to explain pathogenesis of diseases, develop prevention and therapeutic new strategy for diseases,as well as a new drug. Moreover, live cell imaging technology allows tostudy cell signaling, particularly the neural and neuro-endocrinology cell signaling mechanism.



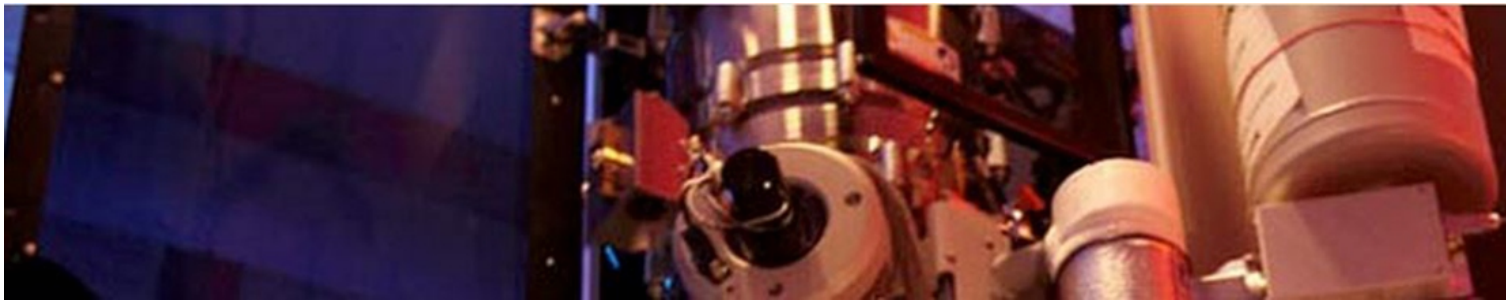




Digital LM and Confocal Laser microscopy sestem, CorrSight, FEI, USA



Electron microscopy system, QUANTA 205 FEG, FEI, USA



EXPERIMENTAL ANIMAL LABORATOTY

Experimental animal laboratory has the following cutting-edge equipment:

- Stereotaxic microsurgery equipment system for experimental mouse brain ventricles
- Movement, body temperature, cardiac pulse, blood pressure monitoring system, and telemetric system to obtain data from *in-situ* implanted transmitter.
- Automatic system to ventilate experimental animals with appropriate ratio of O_2/CO_2 gases, laboratory rodent feeding system for experimental animal breeding

Experimental animal breeding, acclimatizing and experimenting in accordance with biomedical research ethics breeding, acclimatizing and experimenting in accordance with biomedical research ethics

Stereotaxic surgery kit, CLEA, Japan

Telemetric system, CLEA, Japan

Animal cage rack with ventilation system, CLEA, Japan