

Molecular biology at the University of Gdansk: from basic research towards practical use of the results

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Gdansk, Poland



UNIwersytet Gdański



Department of Molecular Biology

One of 16 units of the Faculty of Biology
(12 Departments, 2 independent Laboratories,
and 2 field Stations)



Faculty of Biology

One of 11 Faculties of the
University of Gdansk



University of Gdansk

The largest higher education institution in
Northern Poland
~ 33,000 students,
~ 1,700 academic teachers and
researchers (professors, lecturers, post-
doctoral fellows)



UNIWERSYTET GDAŃSKI





Head of Department
Prof. Grzegorz Wegrzyn

Laboratory of
Molecular Biology
of Bacteria

Laboratory of
Molecular
Genetics

Laboratory of
Human Genetics
and Genomics

Laboratory of
Intracellular
Signalling

Prof. Agnieszka
Szalewska-
Palasz

Prof.
Grzegorz
Wegrzyn

Prof. Joanna
Jakobkiewicz-
Banecka

Prof. Anna
Herman-
Antosiewicz

Laboratory of Molecular Biology of Institute of Biochemistry and Biophysics of
Polish Academy of Sciences, affiliated with University of Gdansk

Prof. Magdalena Gabig-Ciminska



Grzegorz Wegrzyn

(born: 27 June, 1963; Gdansk, Poland)

Education (*degrees, dates, universities*):

MSc, 1987, University of Gdansk (Poland)

PhD, 1991, University of Gdansk (Poland)

Habilitation, 1995, University of Gdansk (Poland)

Professor (title), 1998

Career/Employment (*employers, positions and dates*):

University of Gdansk (Poland), Research Assistant, 1987-1991

University of Nottingham Medical School (UK), Research Fellow, 1991

University of California at San Diego (USA), Post-doctoral Fellow, 1992

University of Gdansk (Poland), Lecturer, 1992-1996

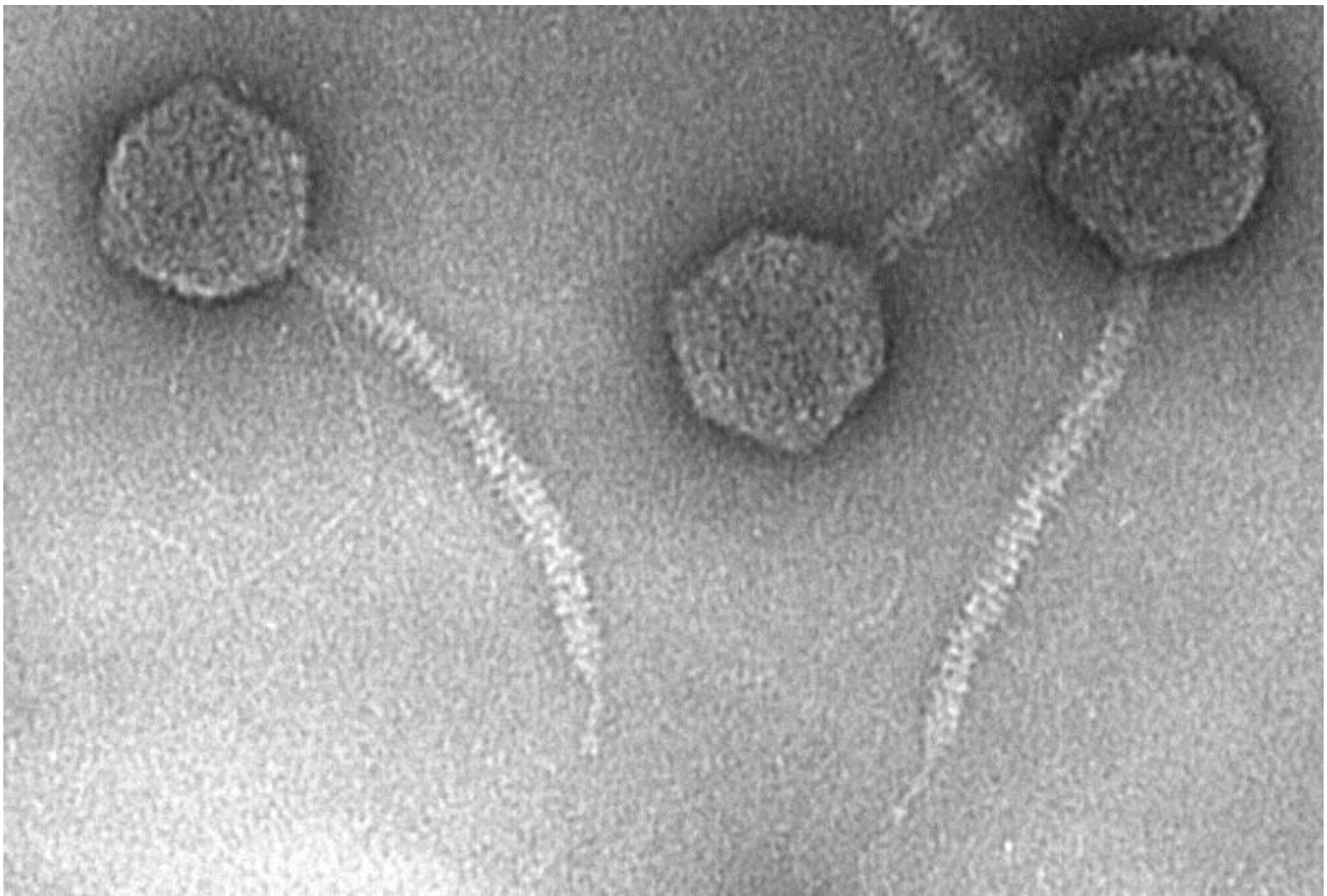
University of Gdansk (Poland), Assistant Professor, 1996-1998

University of Gdansk (Poland), Associate Professor, 1998-1999

University of Gdansk (Poland), Professor, 1999 – present

Research interest: Molecular genetics (gene expression regulation, control of DNA replication, extrachromosomal genetic elements, genetic diseases)

Publications: Over 300 articles in peer-reviewed international journals (including: *Proc. Natl. Acad. Sci. USA*, *EMBO J.*, *EMBO Rep.*, *Nature Commun.*, *Nucleic Acids Res.*, *J. Biol. Chem.*, *J. Mol. Biol.*, *Mol. Microbiol.*, *FEBS J*, *PLoS ONE*, *J. Bacteriol.*, and others)



Bacteriophage λ

Model virus in molecular biology,
used by us for many years

Shiga toxin-producing *Escherichia coli* (STEC)



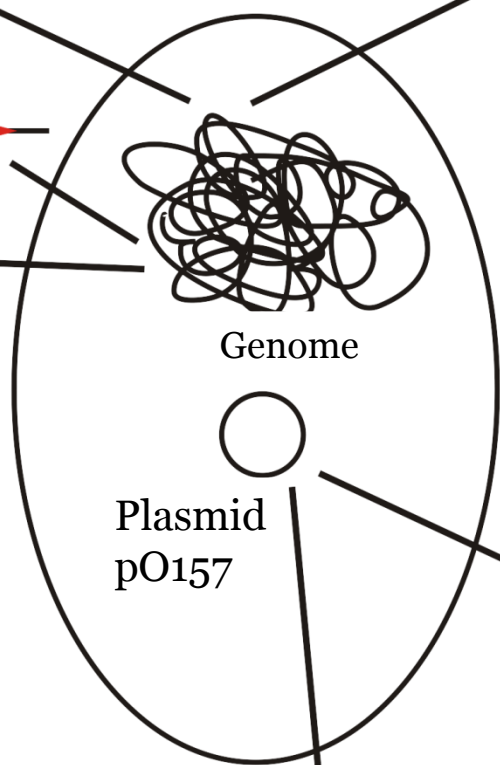
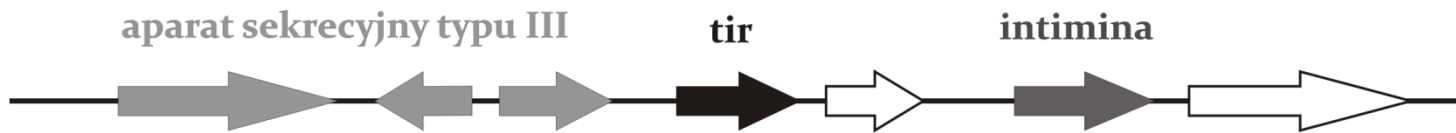
© 2004 Dennis Kunkel Microscopy, Inc.

- Identified in 1982 in USA
- EHEC (entero-haemorrhagic *Escherichia coli*), a subset of STEC strains, causes bloody diarrhea and colitis. It may also cause a haemorrhagic-uremic syndrome (HUS)
- HUS develops in about 10% of patients infected with EHEC. Among them, 2-10% dies due to renal failure
- The infection dose is very low, even less than 100 bacterial cells
- EHEC is relatively resistant to various environmental factors.

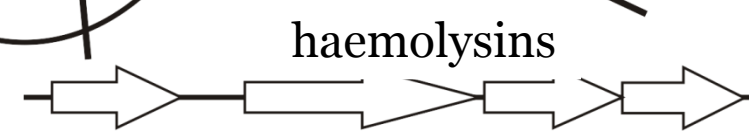
Epidemics caused by EHEC

Year	Country	No. of infected persons	Source of EHEC
1989	USA	243	Beef
1996	Japan	5727	Radish
1996	UK	496	Beef
2000	Canada	>2000	Drinking water
2011	Germany	>4000 (53 died)	Vegetables

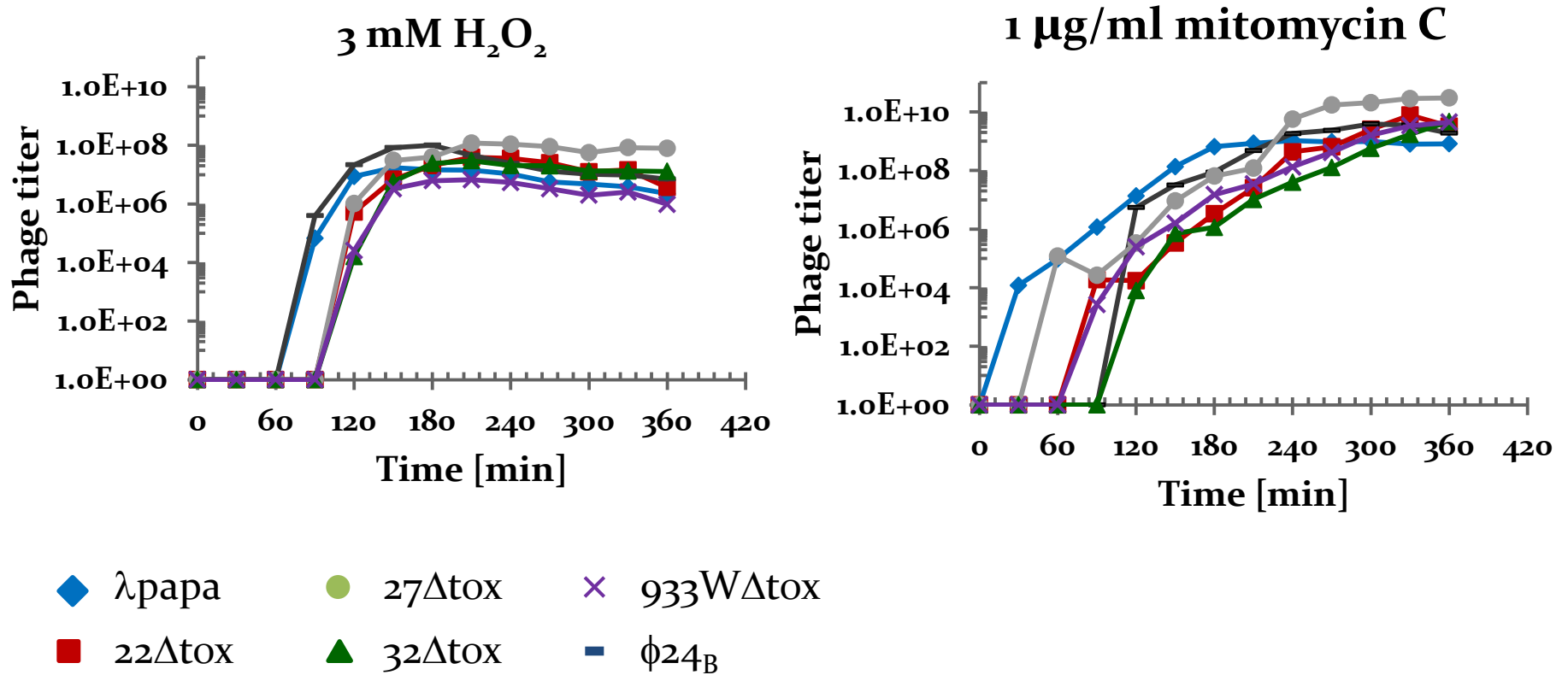
The estimated cost of the last outbreak in 2011 is over 4,000,000,000 Euro



Virulence factors



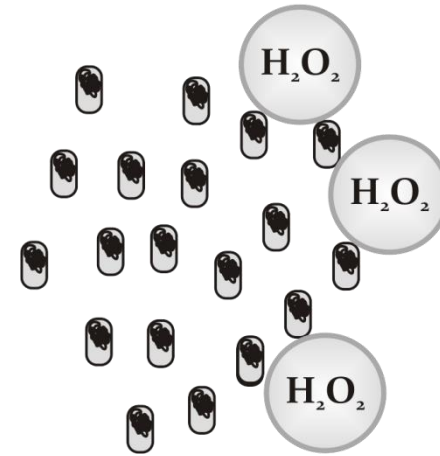
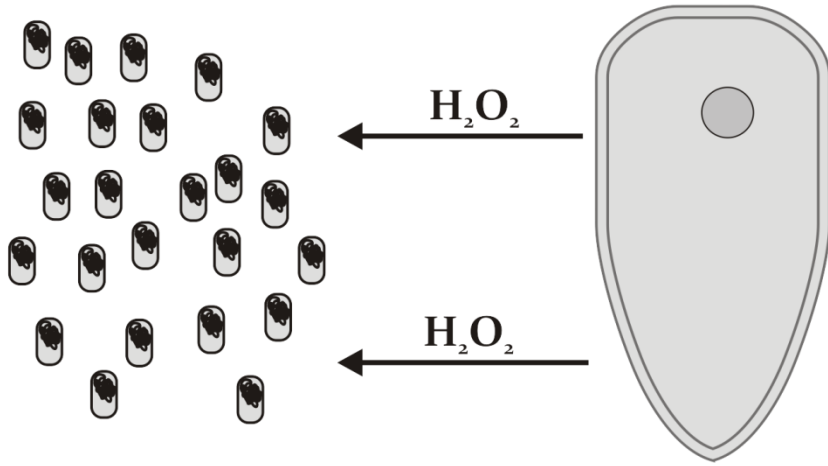
Oxidative stress causes induction of Shiga toxin-converting prophages



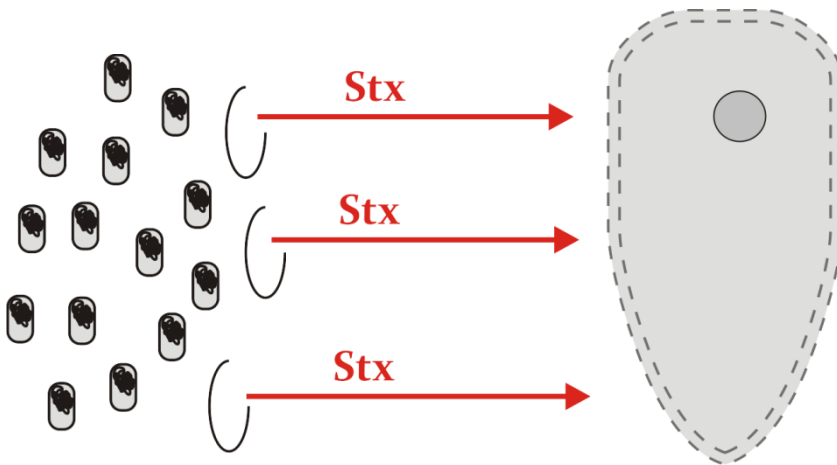
Escherichia coli O157:H7

Tetrahymena sp.

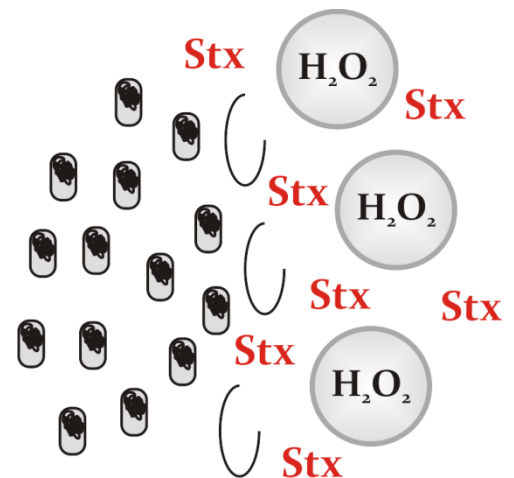
Neutrophils



Wagner et al. 2001, *J. Bacteriol.*, **183**, 2081

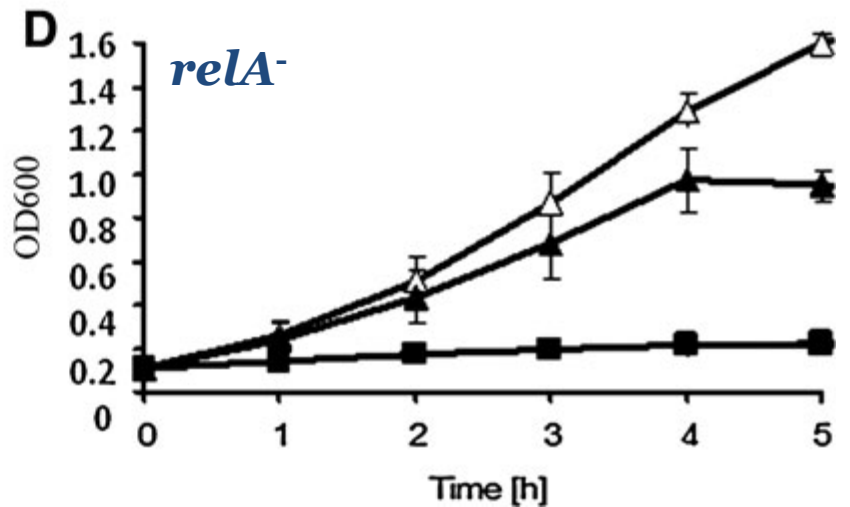
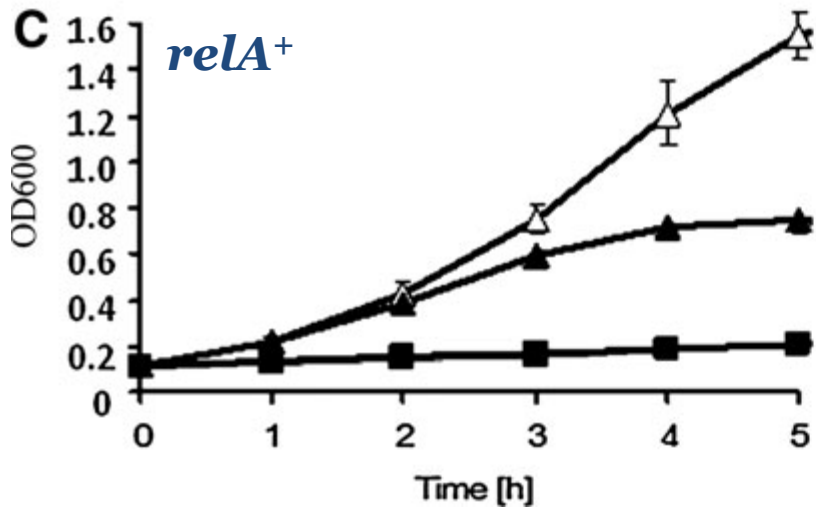
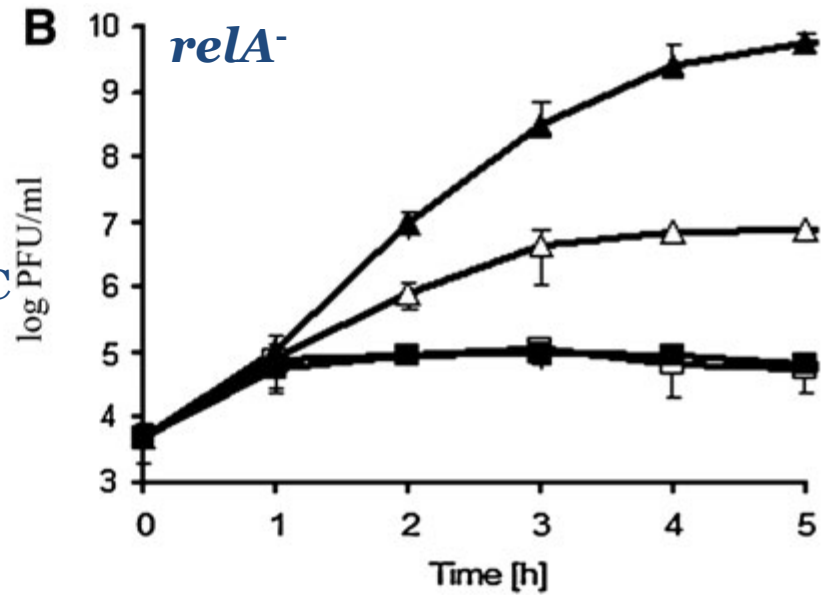
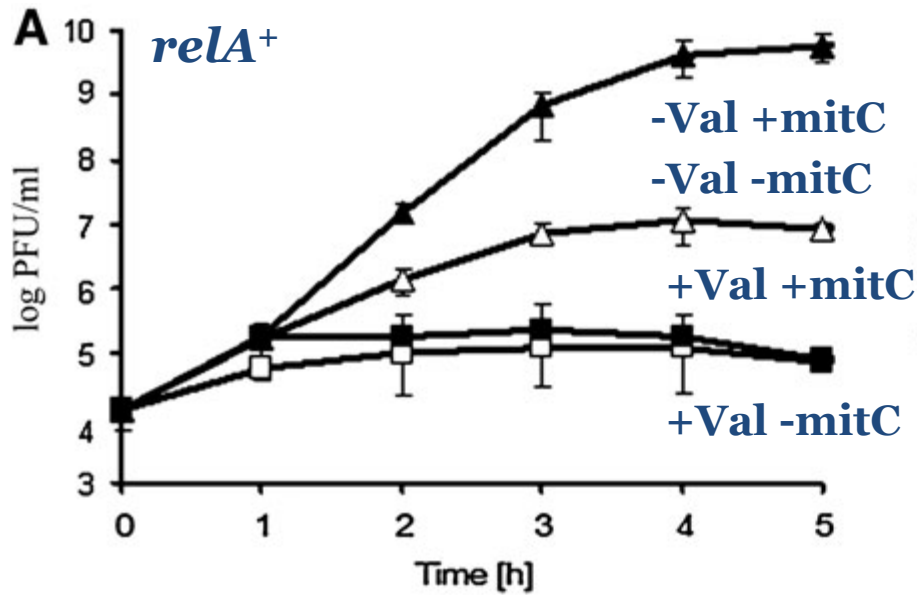


Lainhart et al. 2009, *J. Bacteriol.* **191**, 5116

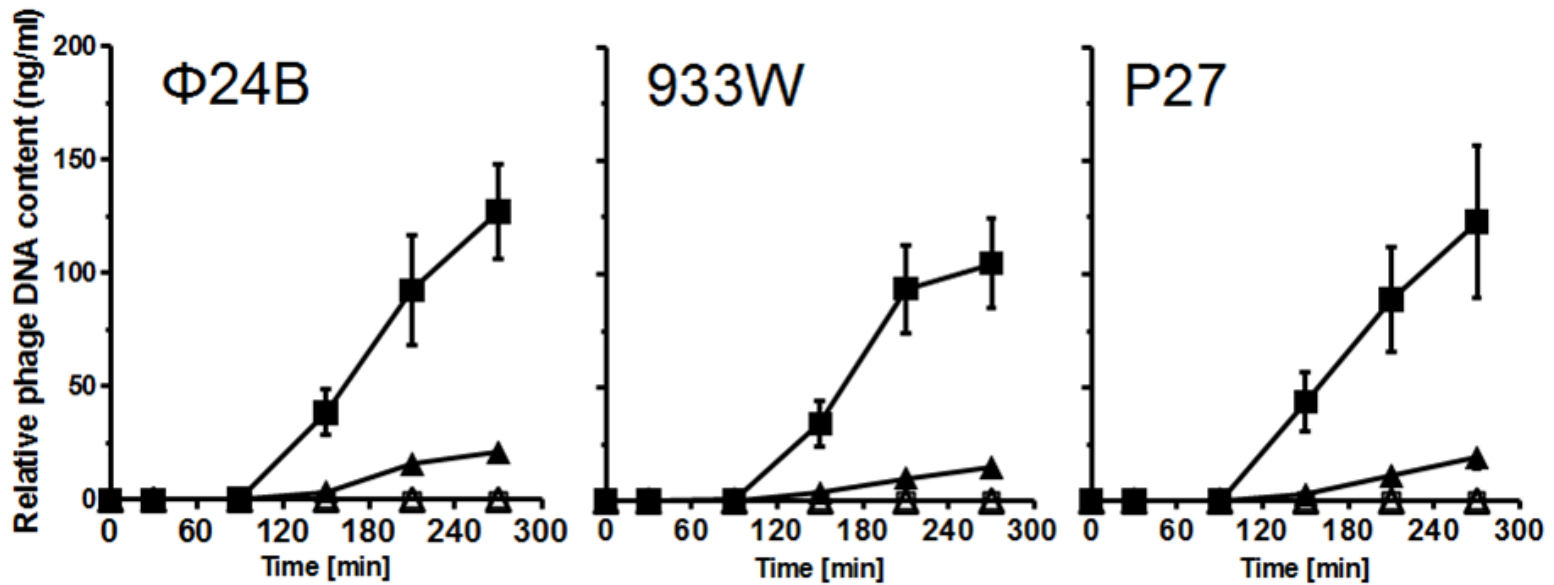


Los et al. (2013) *Frontiers in Cellular and Infection Microbiology*, **2**, 166

Development of bacteriophage 933W Δ tox



Phage DNA replication after prophage induction with mitomycin C



wild-type

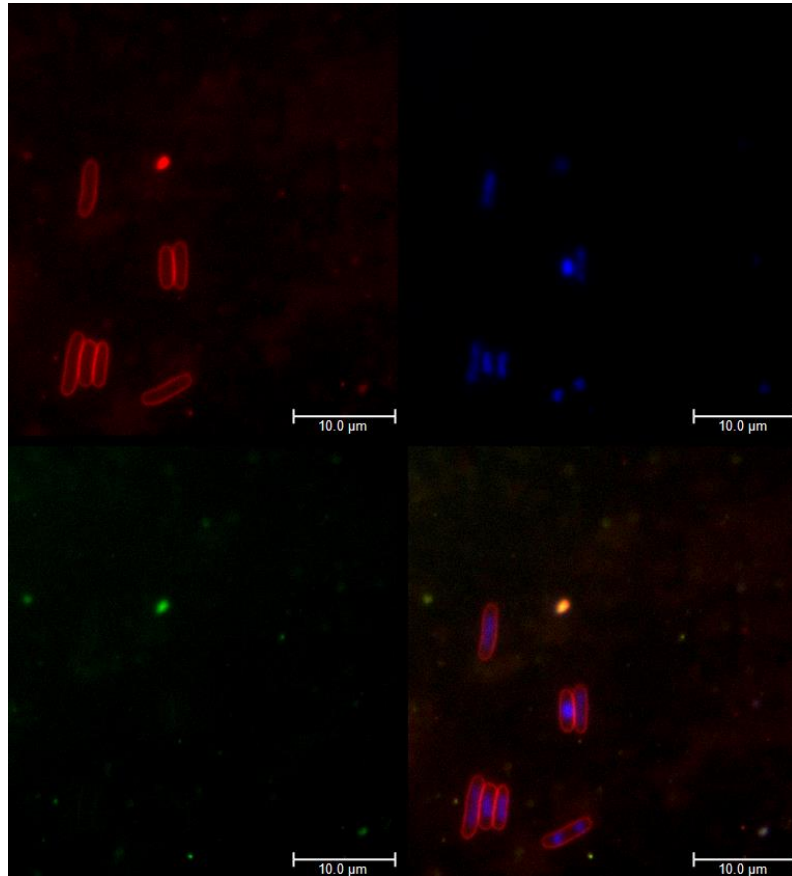


$ppGpp^0$

Wild-type host

FM4-64
(membranes)

DAPI
(DNA)



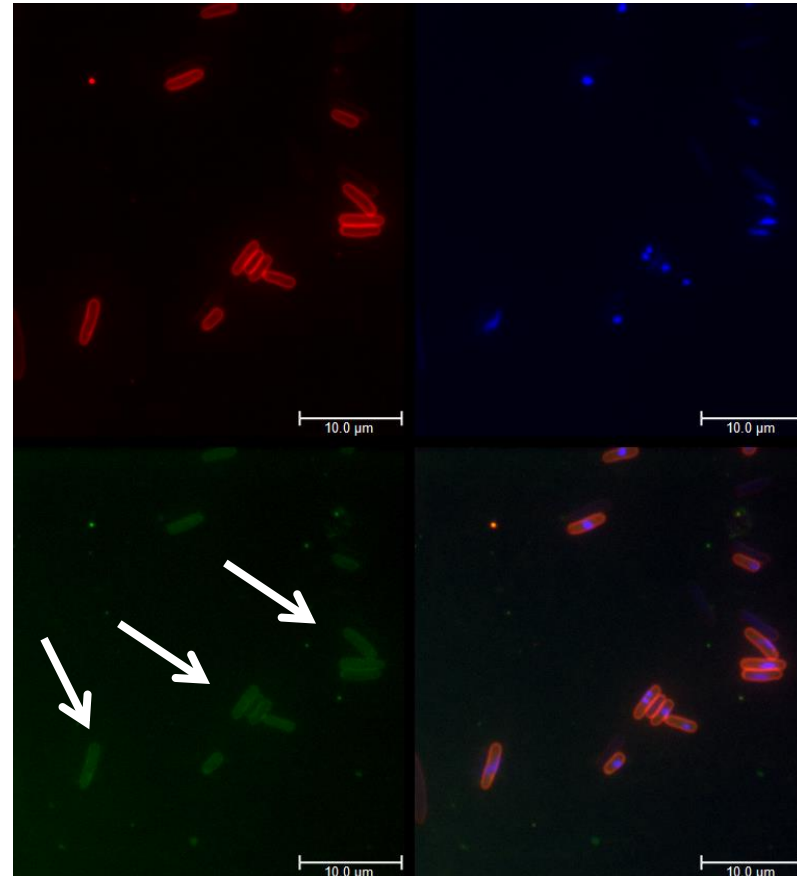
GFP
(Shiga toxin)

MERGE

ppGpp⁰ host

FM4-64
(membranes)

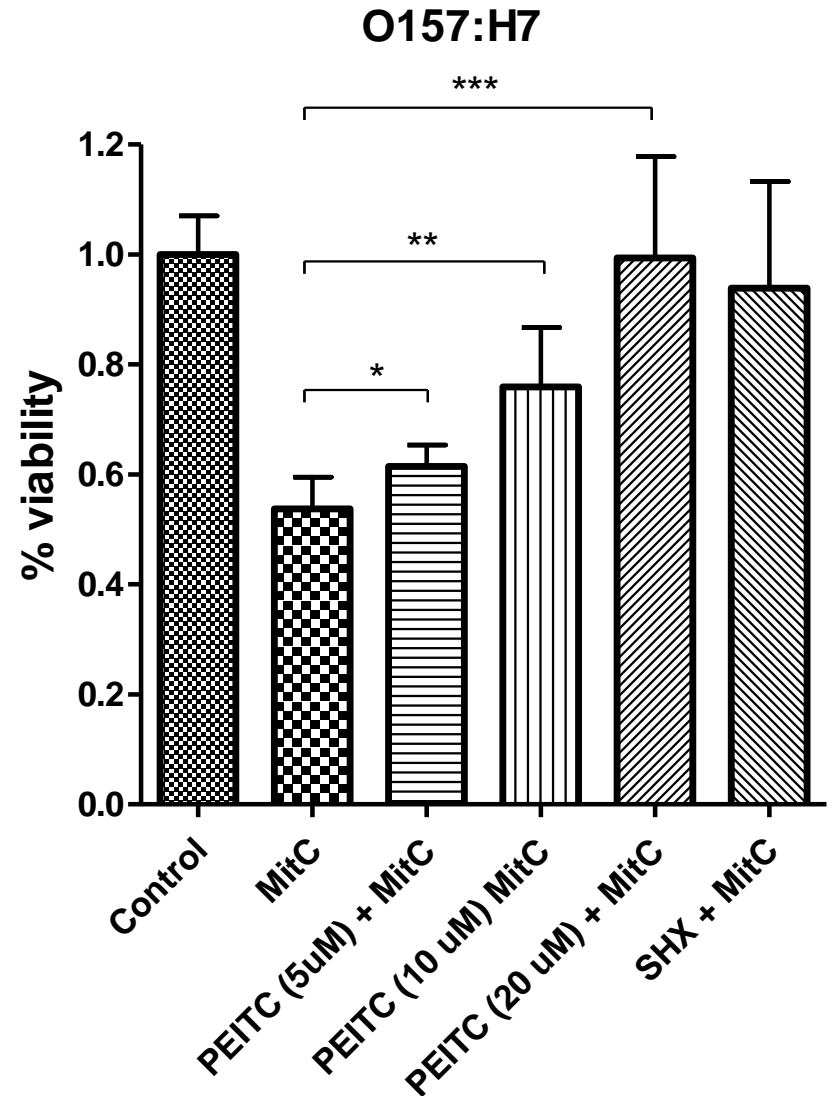
DAPI
(DNA)



GFP
(Shiga toxin)

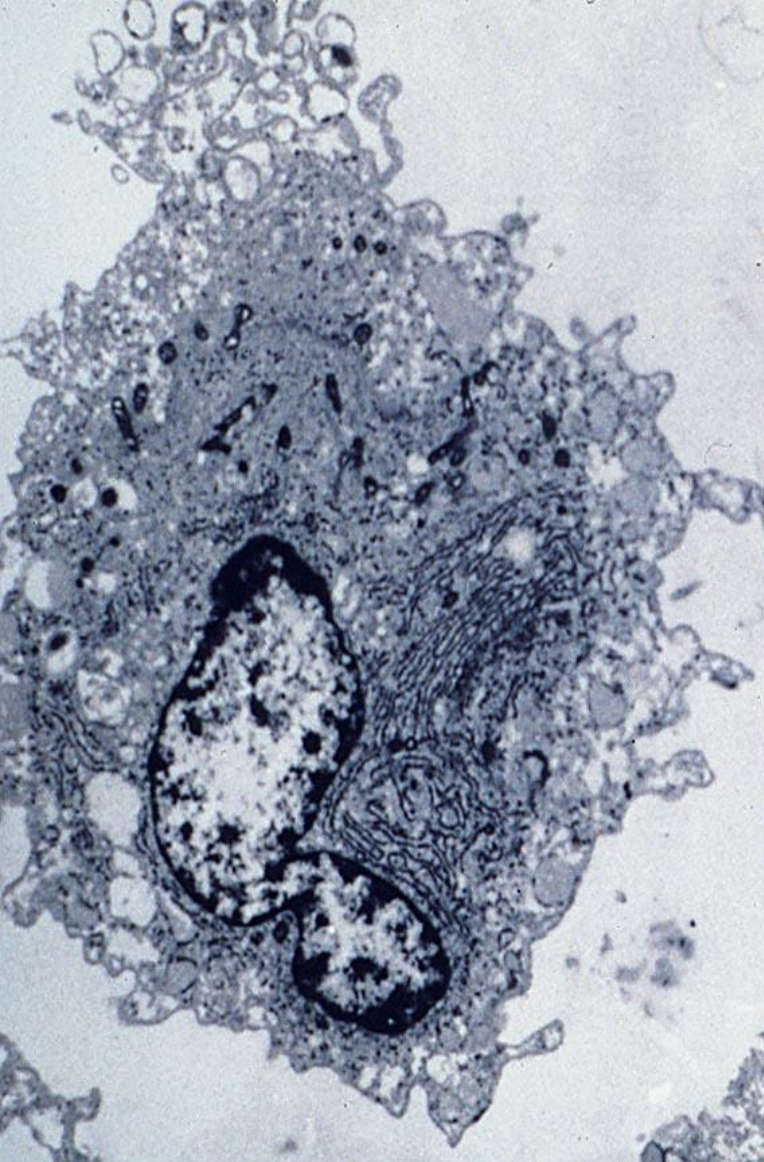
MERGE

PEITC prevents killing of human cells by *E. coli* O157:H7 extracts prepared after induction of the Stx prophage



Lysosomal storage diseases (LSD)

- A group of over 50 diseases
- Each disease is caused by a deficiency in:
 - (i) a specific lysosomal hydrolase (leading to an inability to degrade particular macromolecules, for example: sphingolipids, glycoproteins, glycosaminoglycans)
 - (ii) a protein involved in transport of particular compounds through lysosomal membranes
 - (iii) an enzyme that modifies lysosomal proteins, ensuring their proper localization and function
 - (iv) an activator of particular lysosomal enzyme



Normal fibroblast



lysosomes

nucleus

LSD fibroblast

The deposition of undegraded substrates in tissues throughout the body leads to a multisystemic disease



Growth deficiency
(dwarfism)

Central nervous system
and sensory organs affected



Bones and joints problems
(*Disostosis multiplex*)

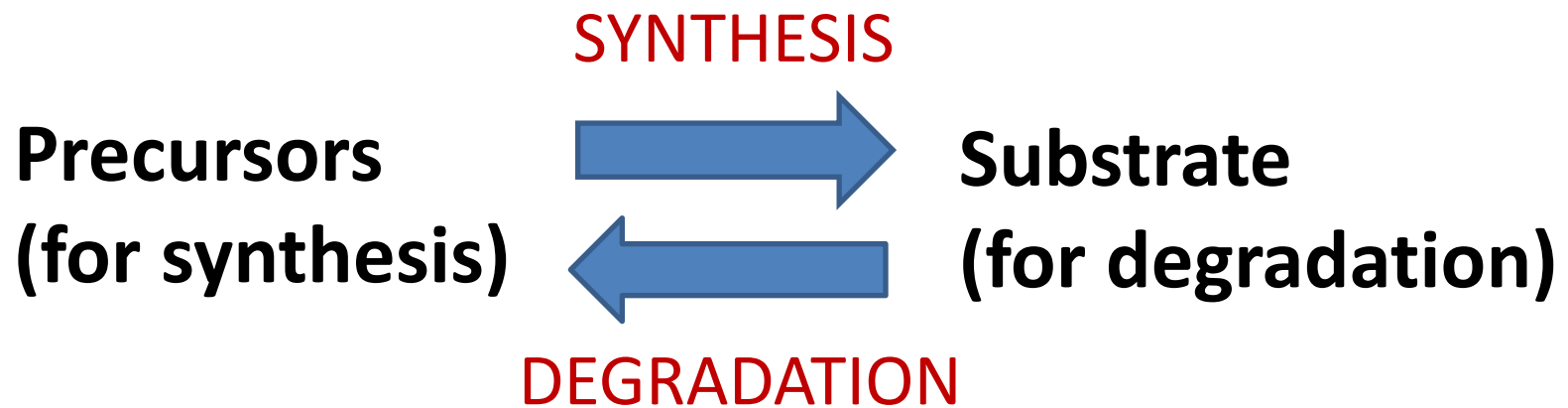


Visceral organs' dysfunctions

Potential therapies for LSD:

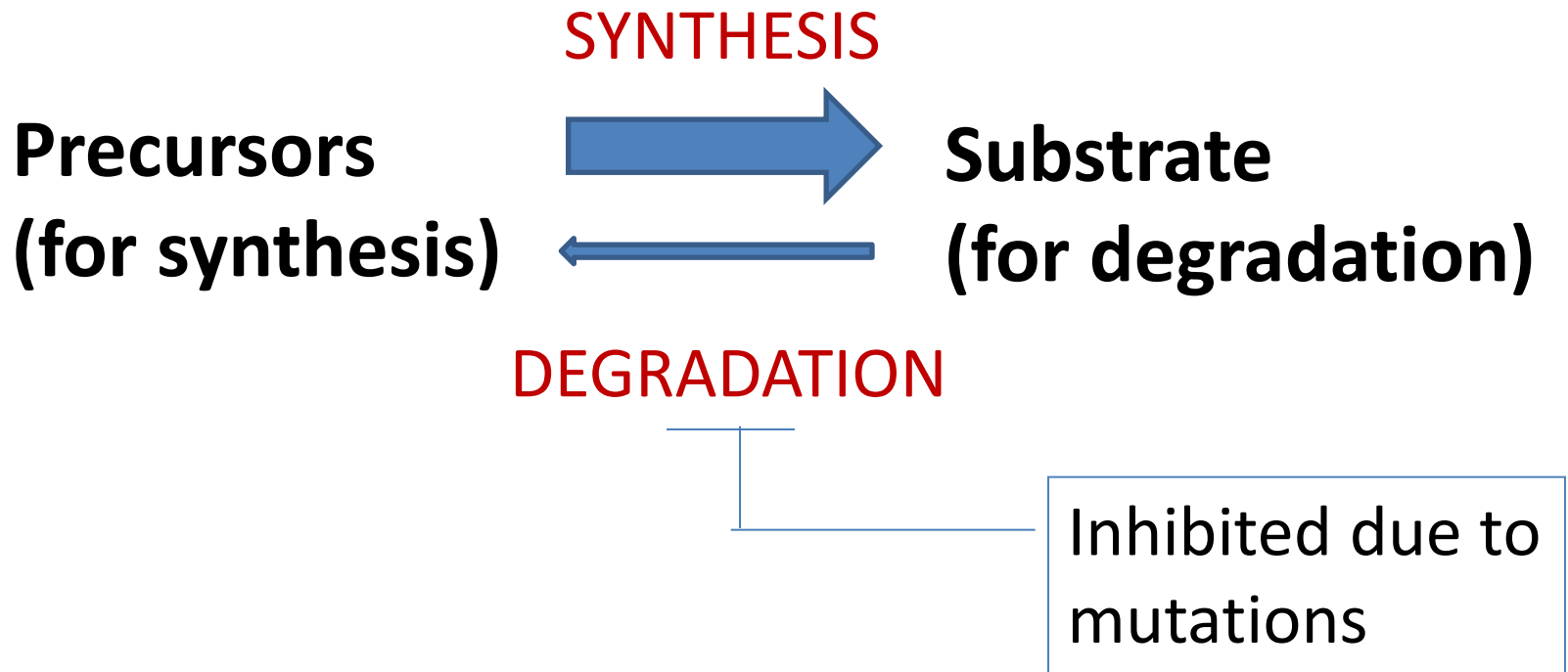
- Enzyme replacement therapy (ERT)
- Bone marrow (or stem cell) transplantation
- Gene therapy
- Stop codon read-through
- Small chaperones
- Substrate optimization therapy
- Substrate reduction therapy

The idea of substrate reduction therapy



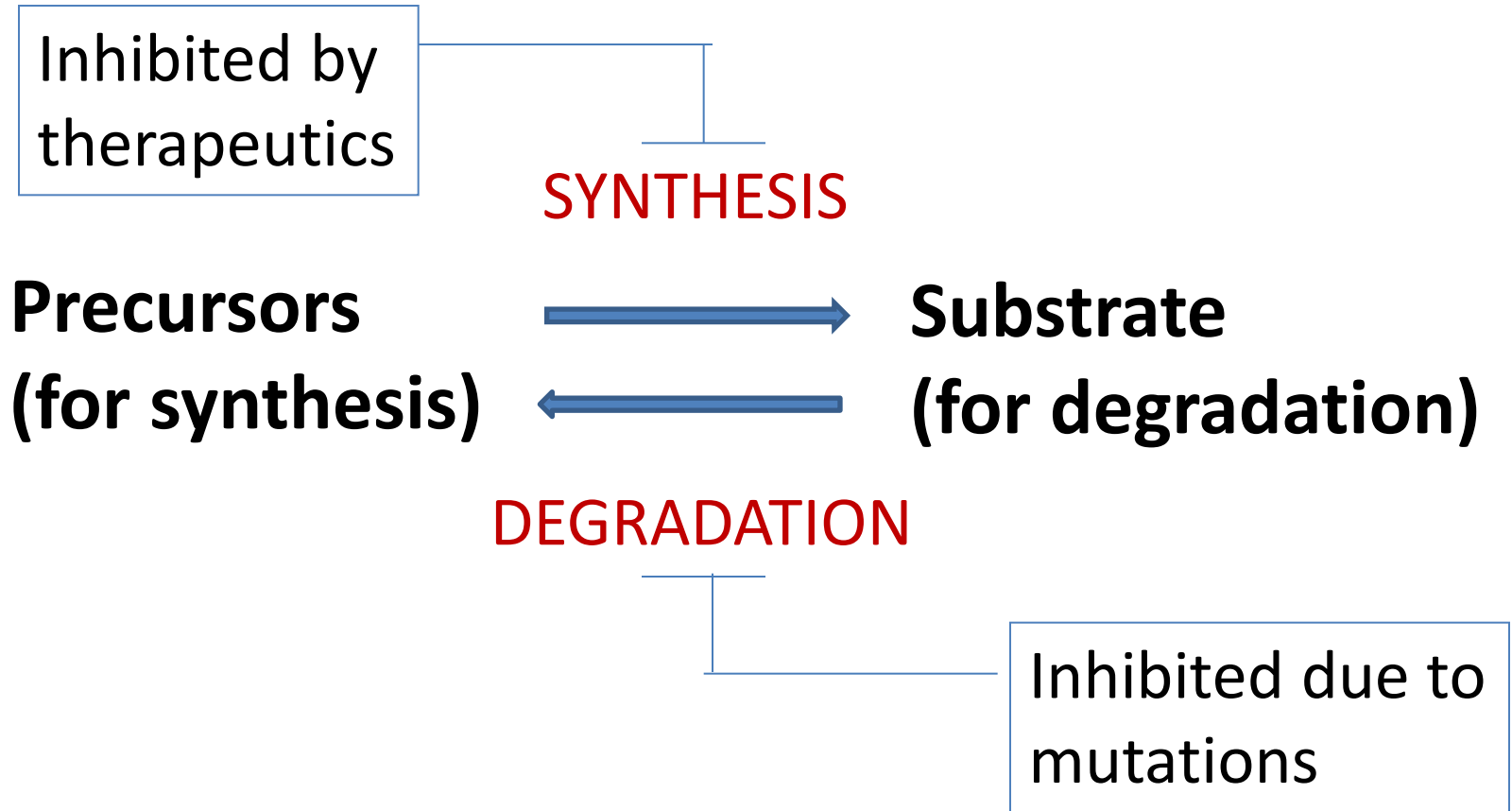
NORMAL (Synthesis = Degradation)

The idea of substrate reduction therapy

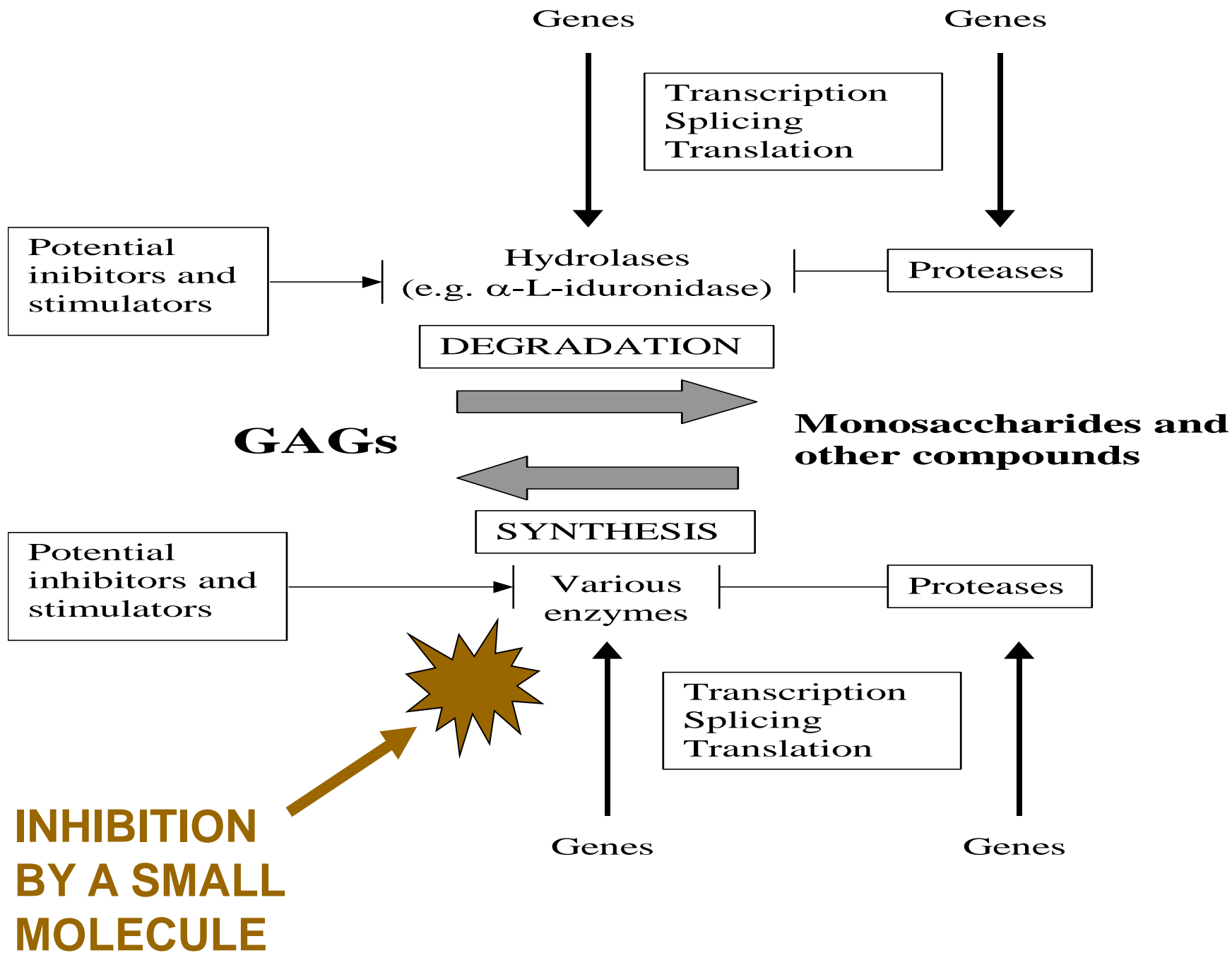


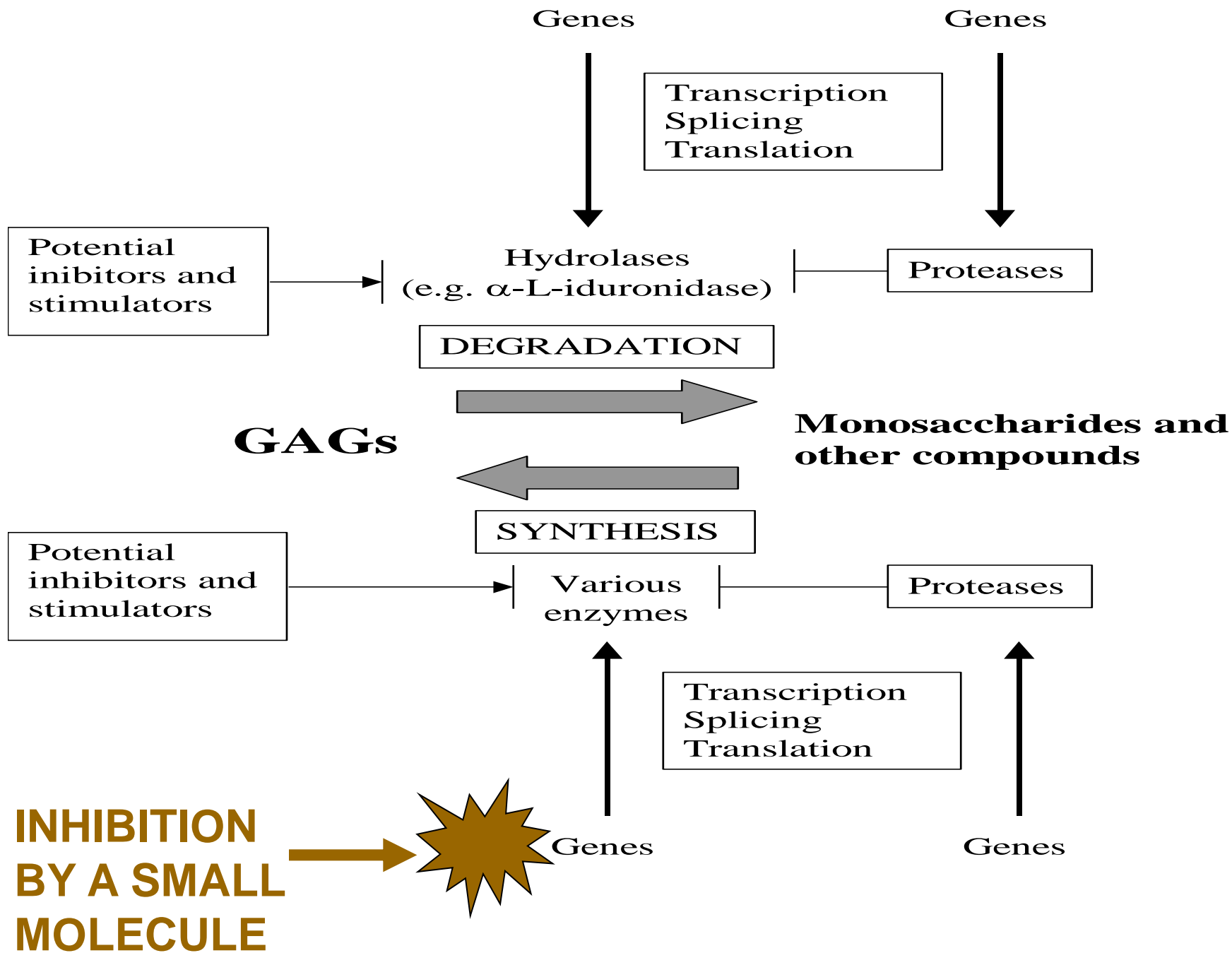
LSD (Synthesis > Degradation)

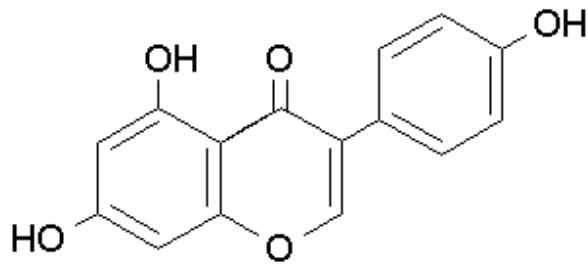
The idea of substrate reduction therapy



SRT for LSD (Synthesis = Degradation)

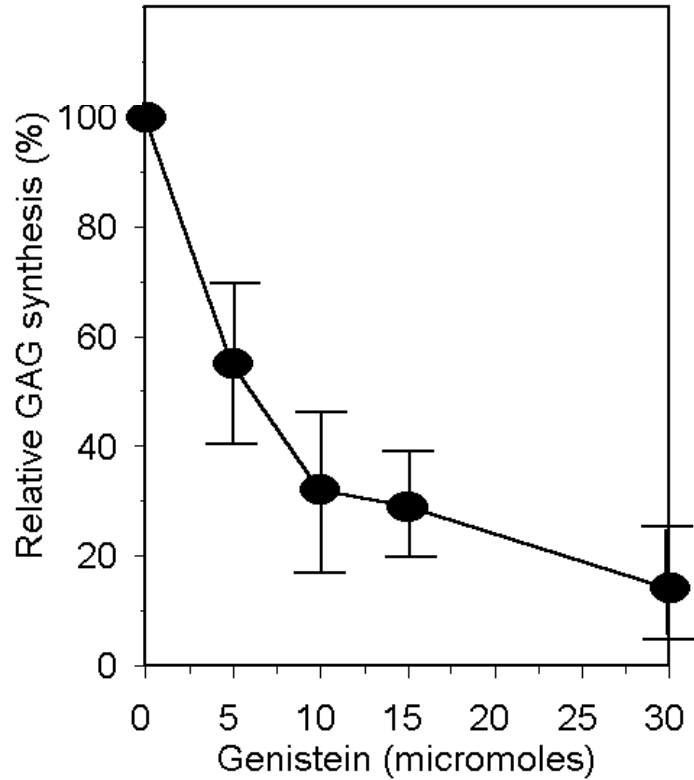




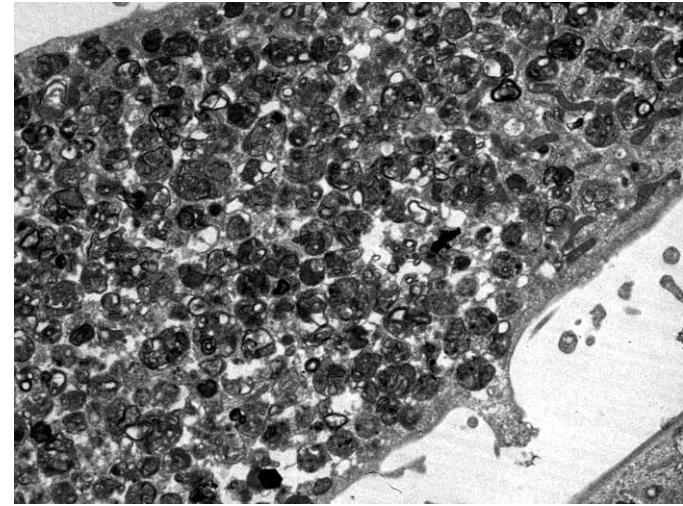


Genistein

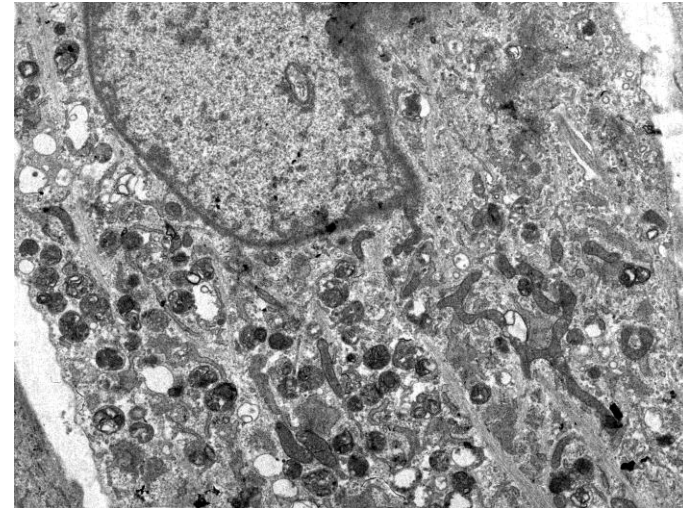
MPS I cells



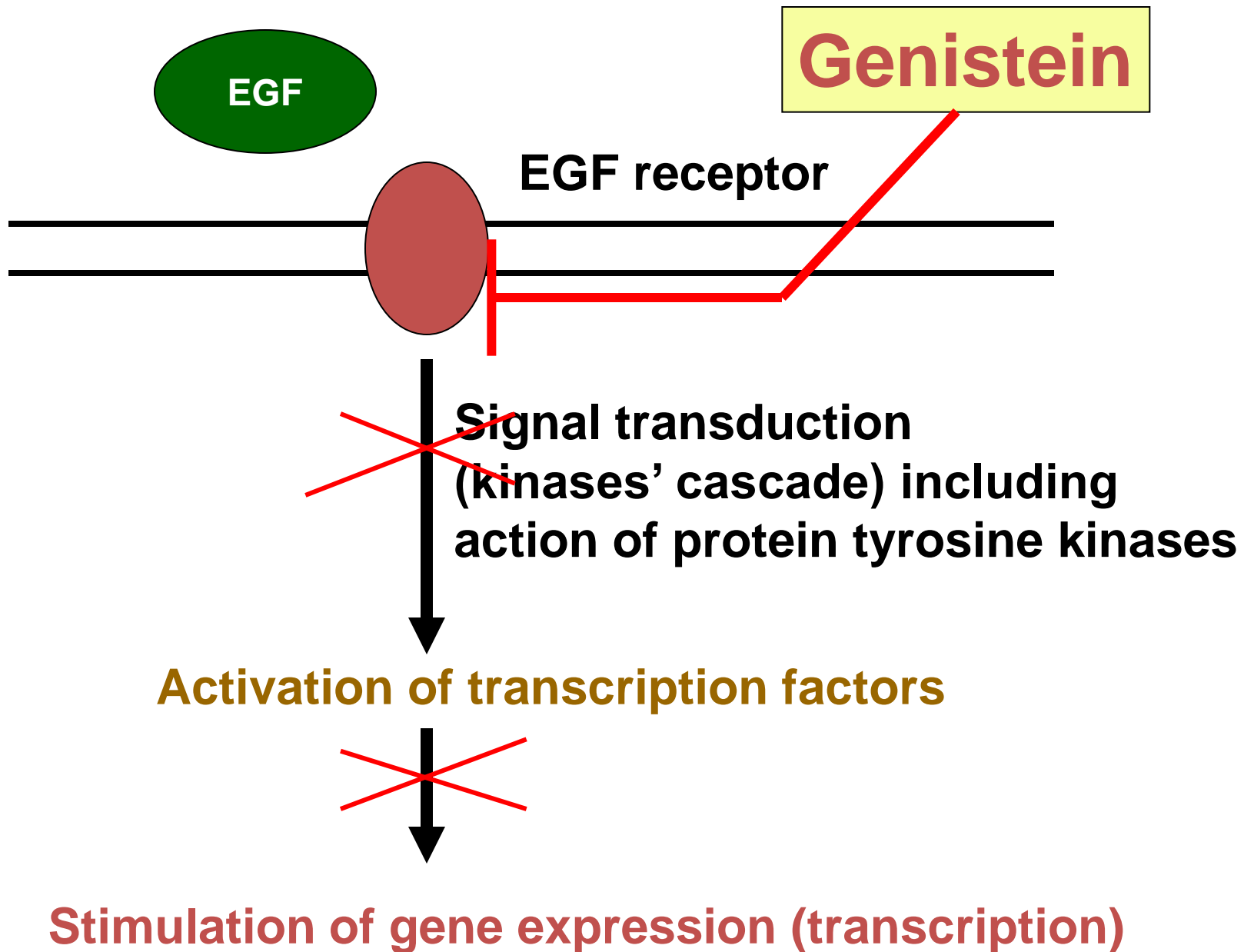
MPS IIIA cell



Genistein

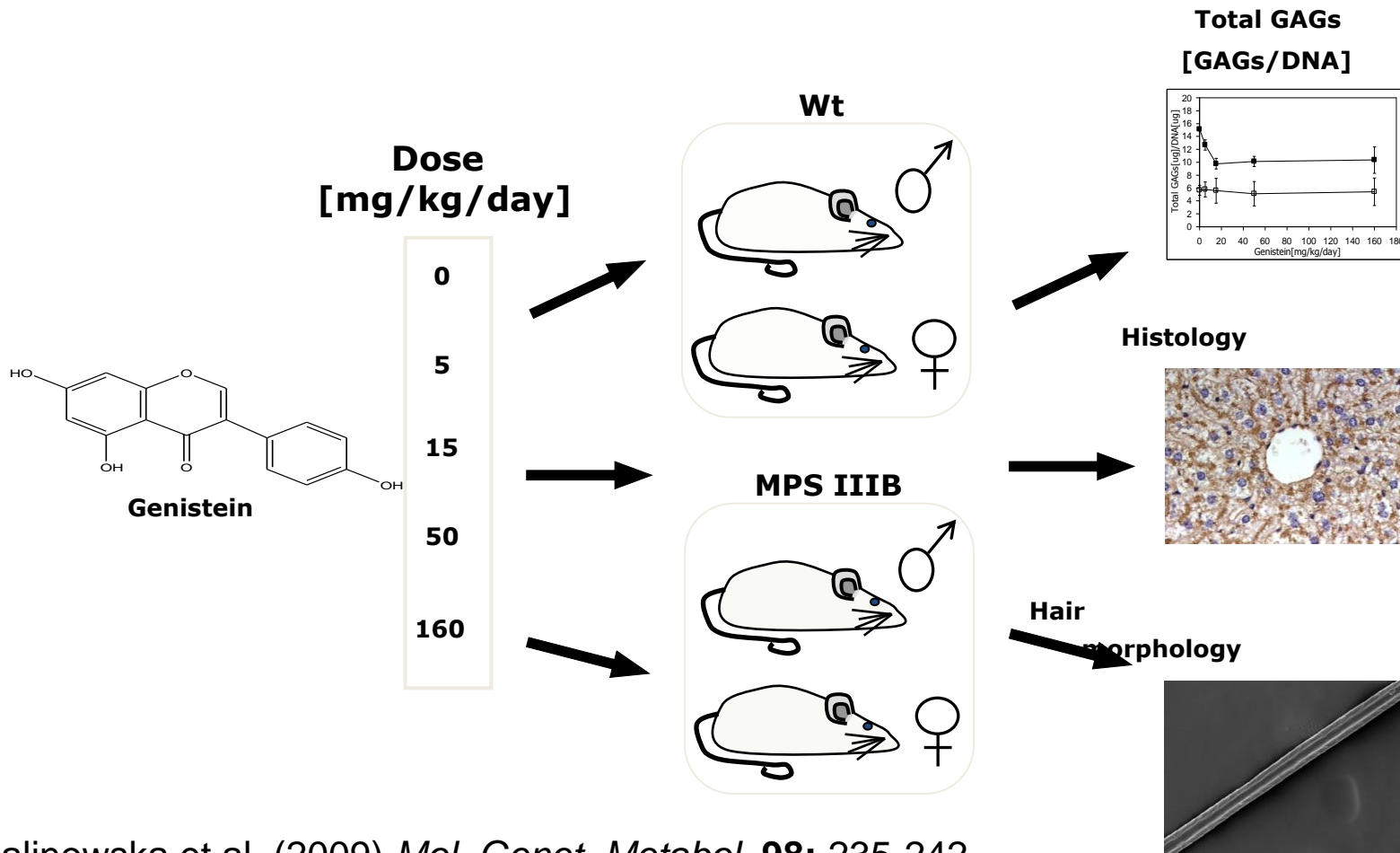


Inhibition of glycosaminoglycan (GAG) synthesis by genistein



Short-term experiment

AIM: To establish the most effective and non-toxic dose of genistein which can significantly reduce GAG storage in MPS IIIB mice and be used as substrate reduction therapy for Sanfilippo syndrome



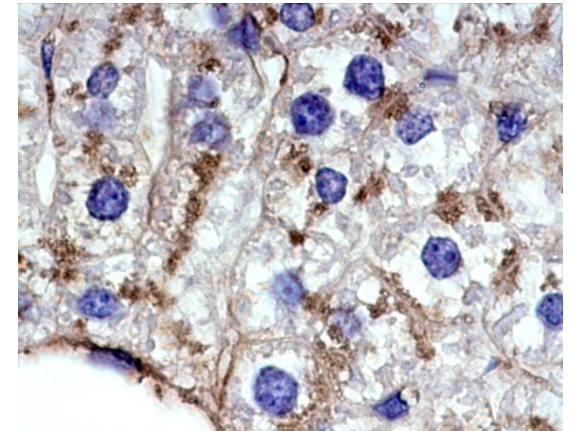
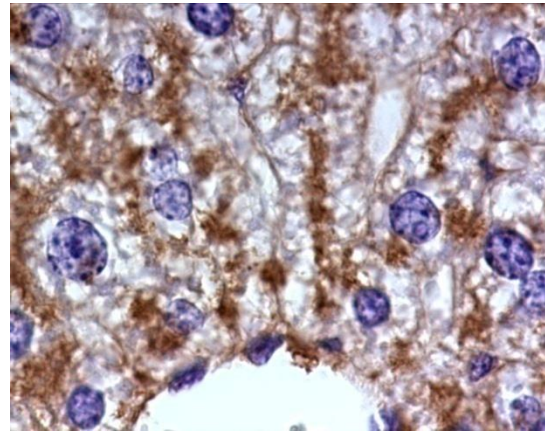
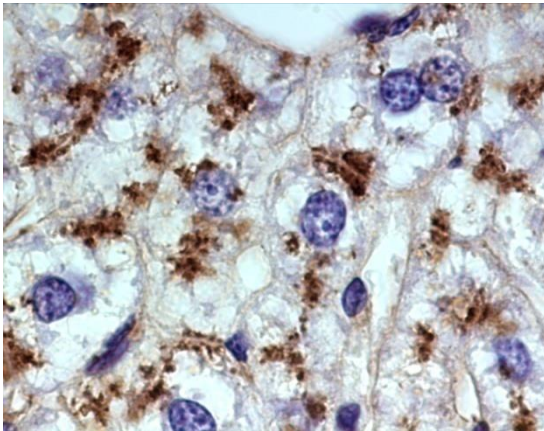
Reduction of GAG storage in liver of MPS IIB mice

Wild-type

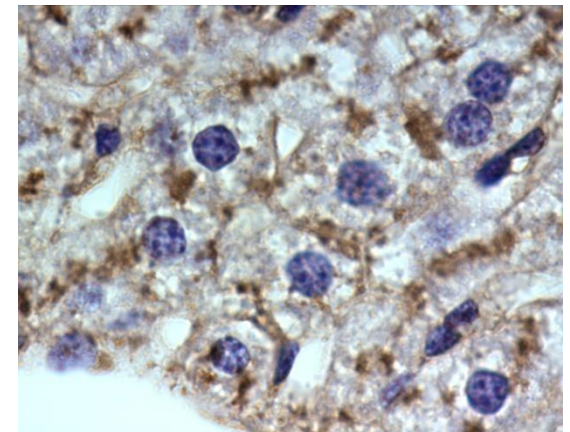
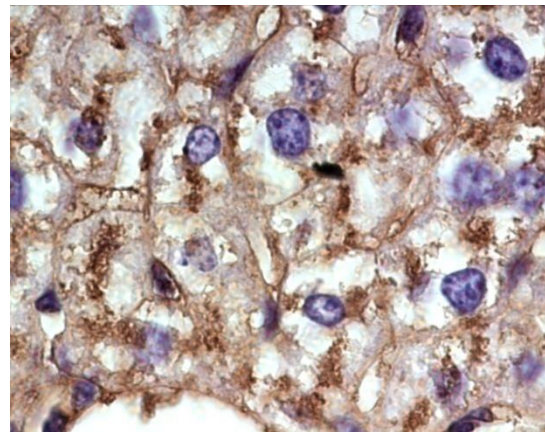
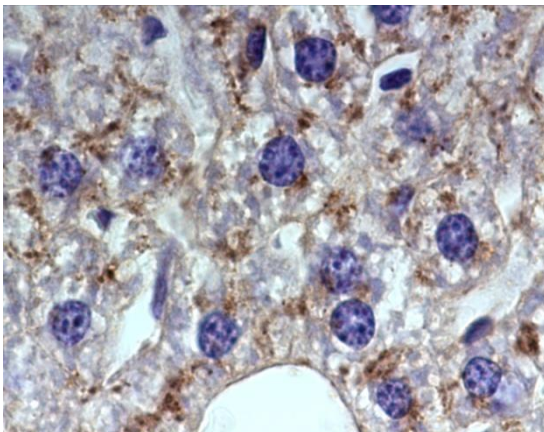
**MPS IIB
untreated**

**MPS IIB
genistein
(160 mg/kg
for 8 weeks)**

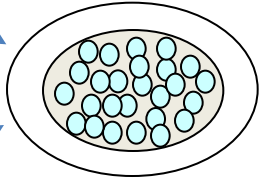
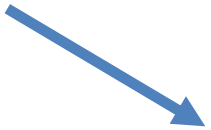
♂



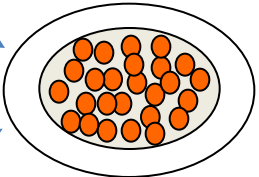
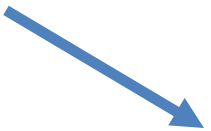
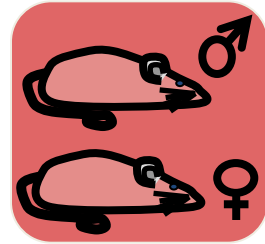
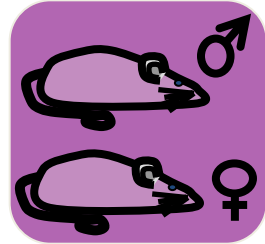
♀



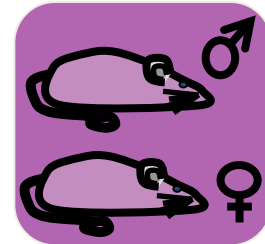
Long-term experiment



Soy free diet



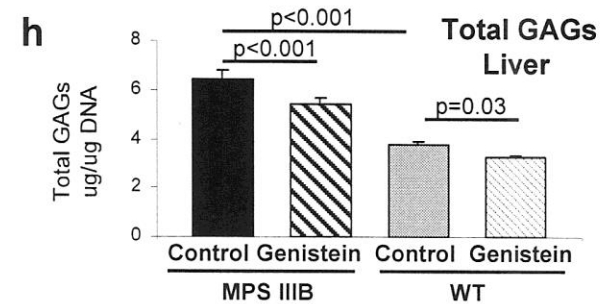
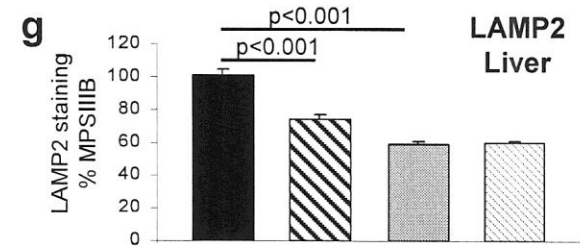
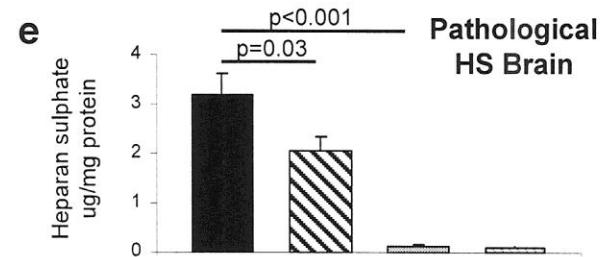
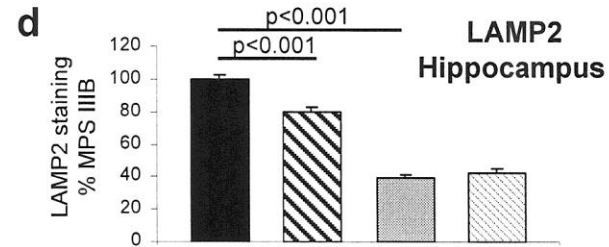
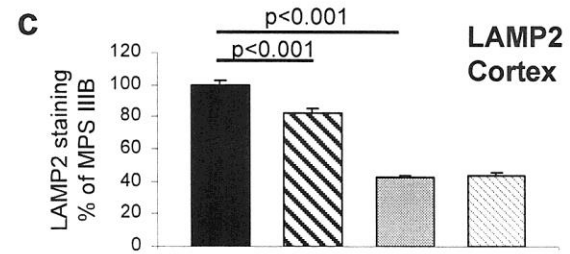
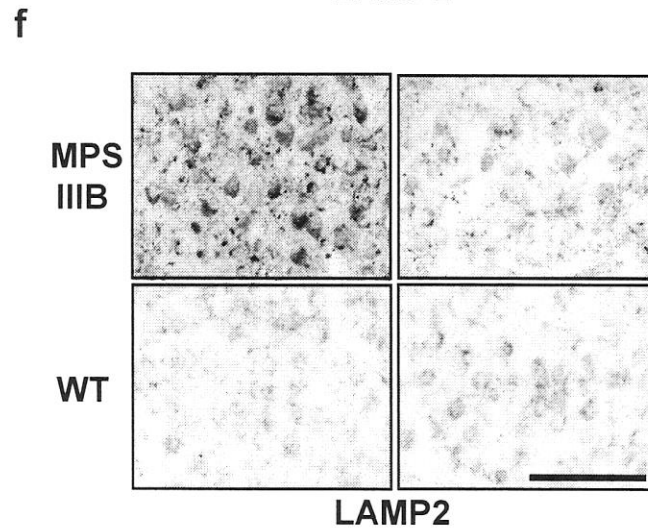
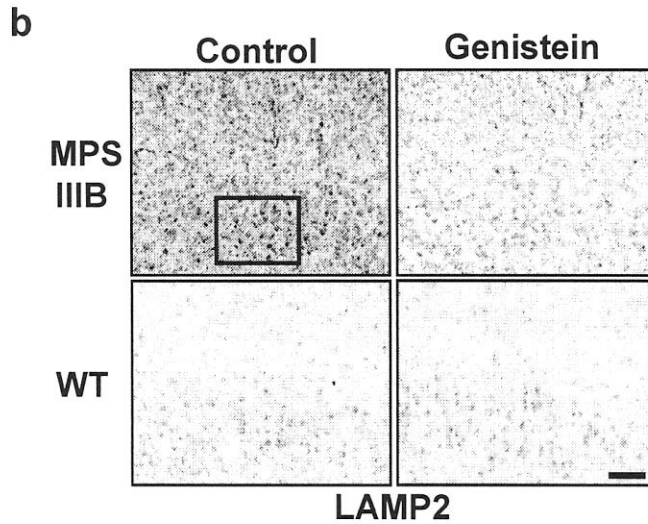
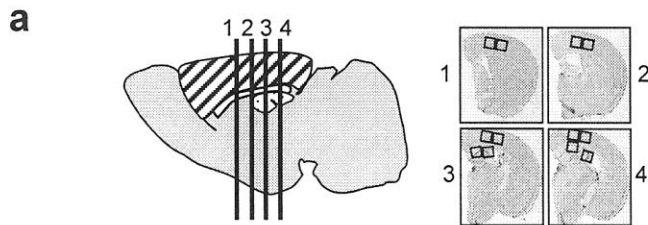
Diet with genistein 160 mg/kg

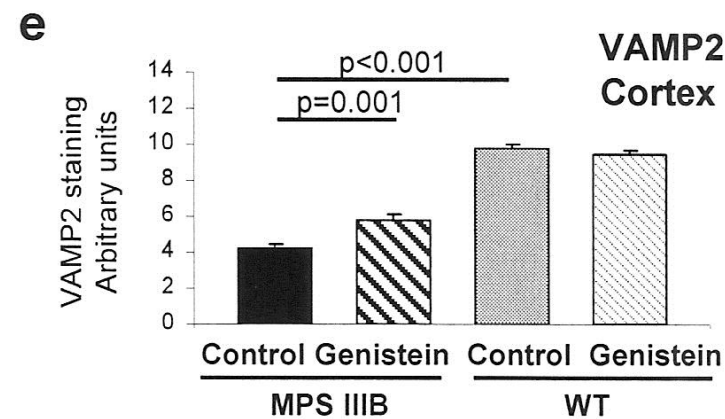
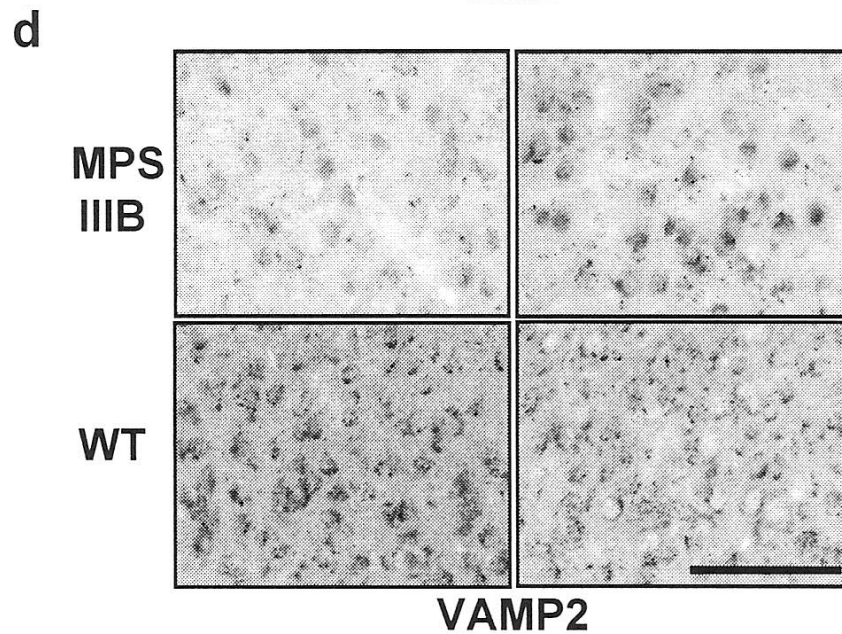
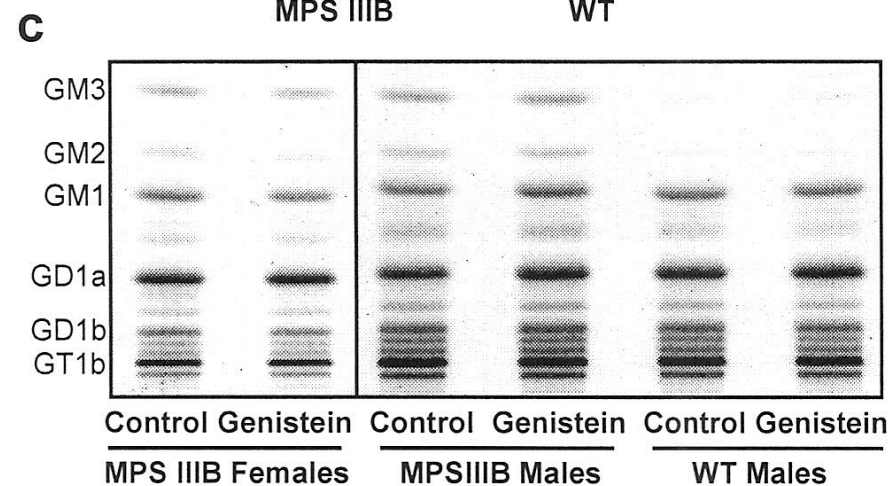
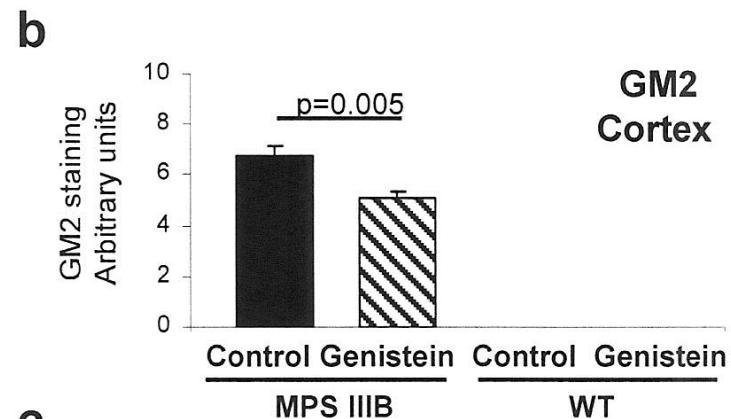
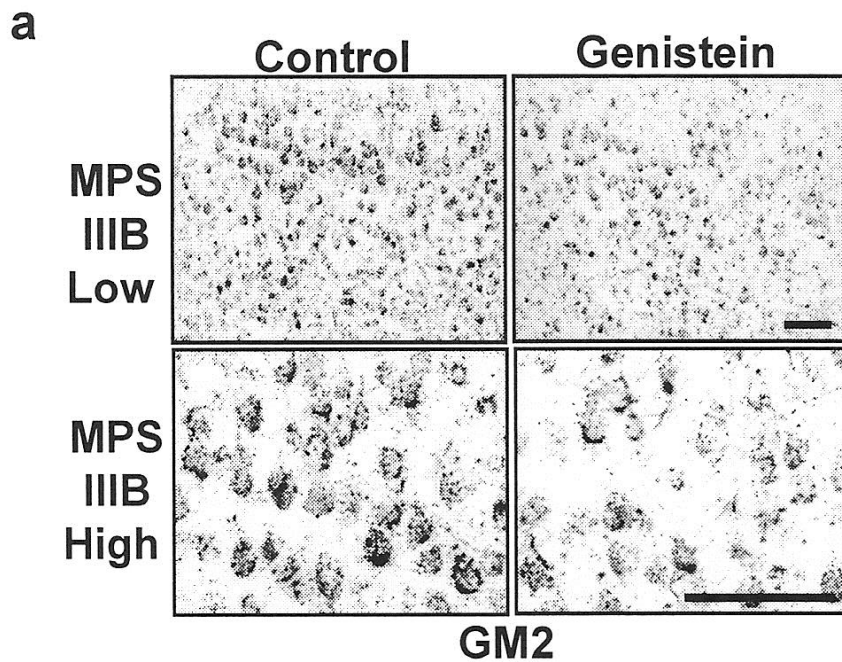


Weight **Blood**
Urine **Hair**

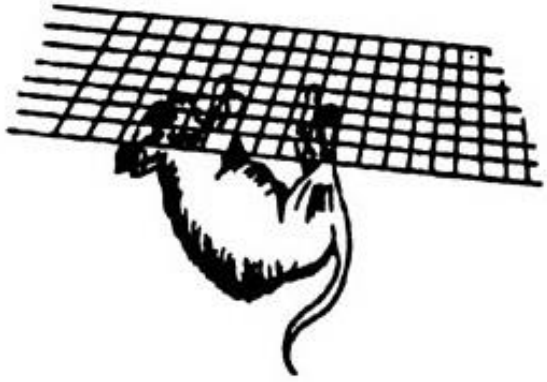
Histology **Biochemistry**
MassSpec

TopScan **Circadian rhythm**
Bar crossing **Inverted Screen**

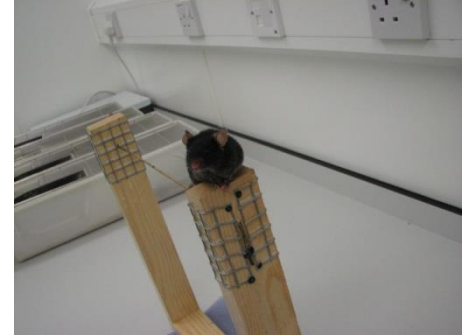




Behavioral tests

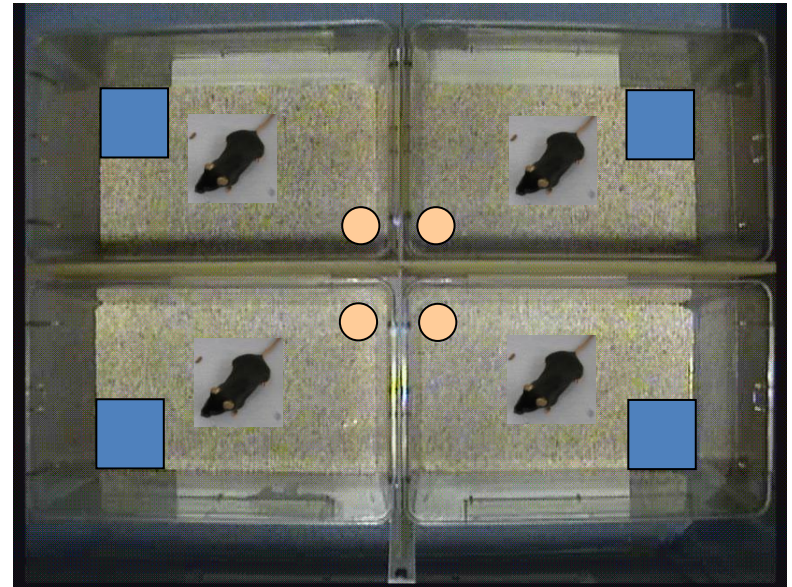


Inverted screen test

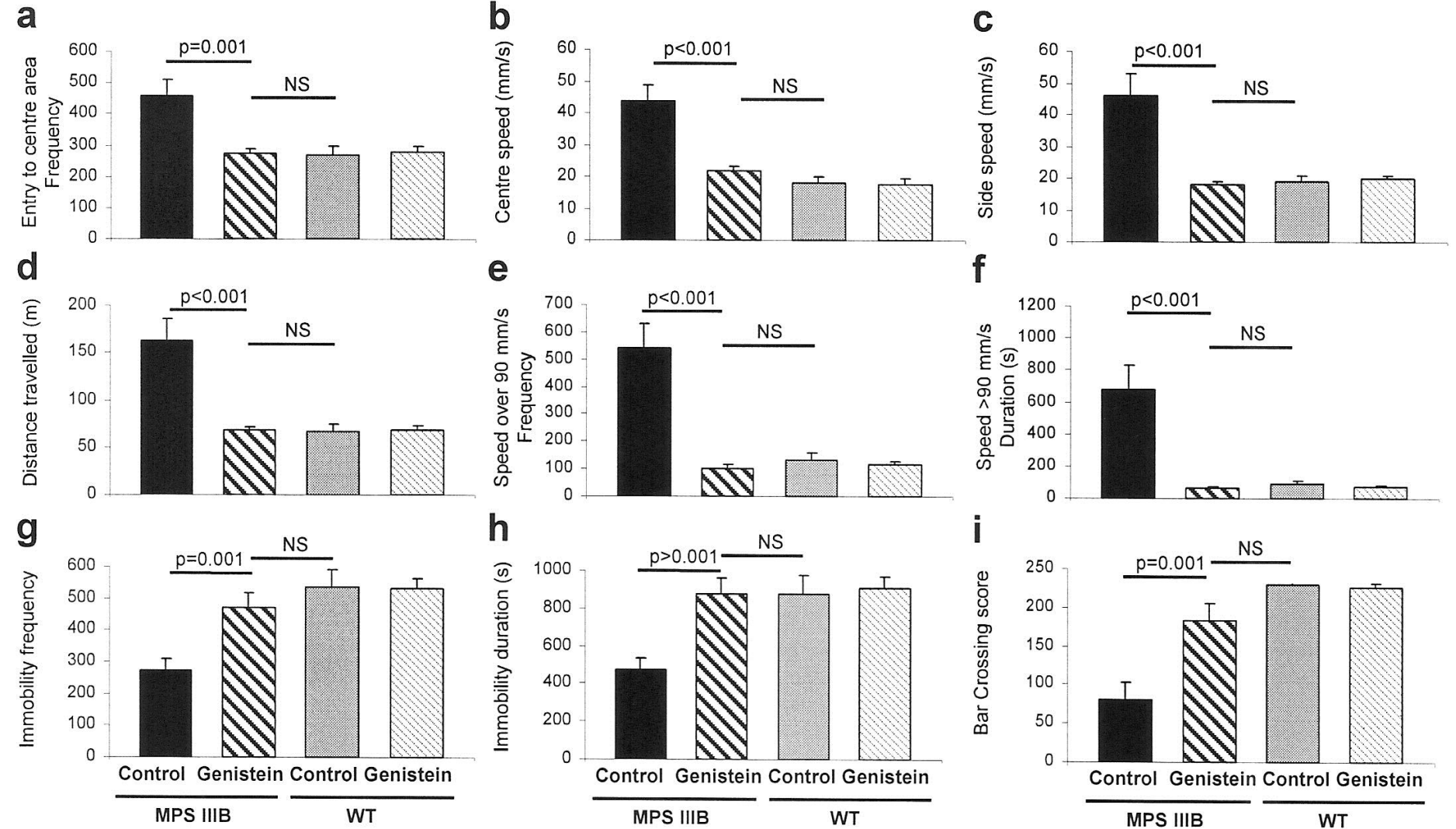


Bar crossing test

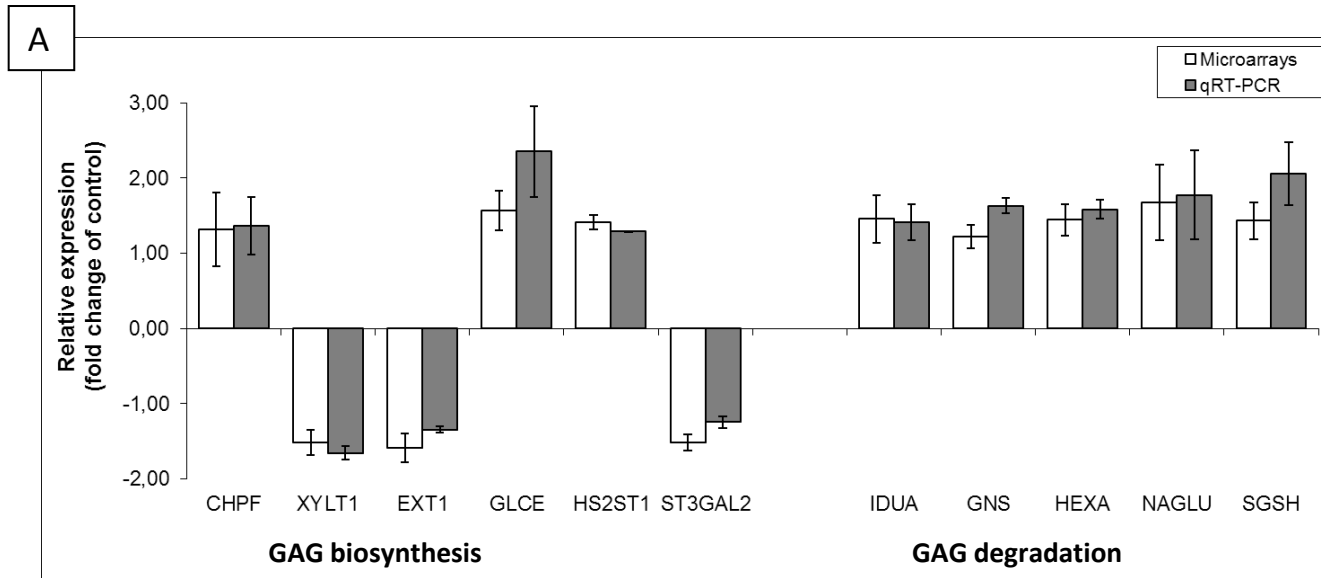
Home cage behavior



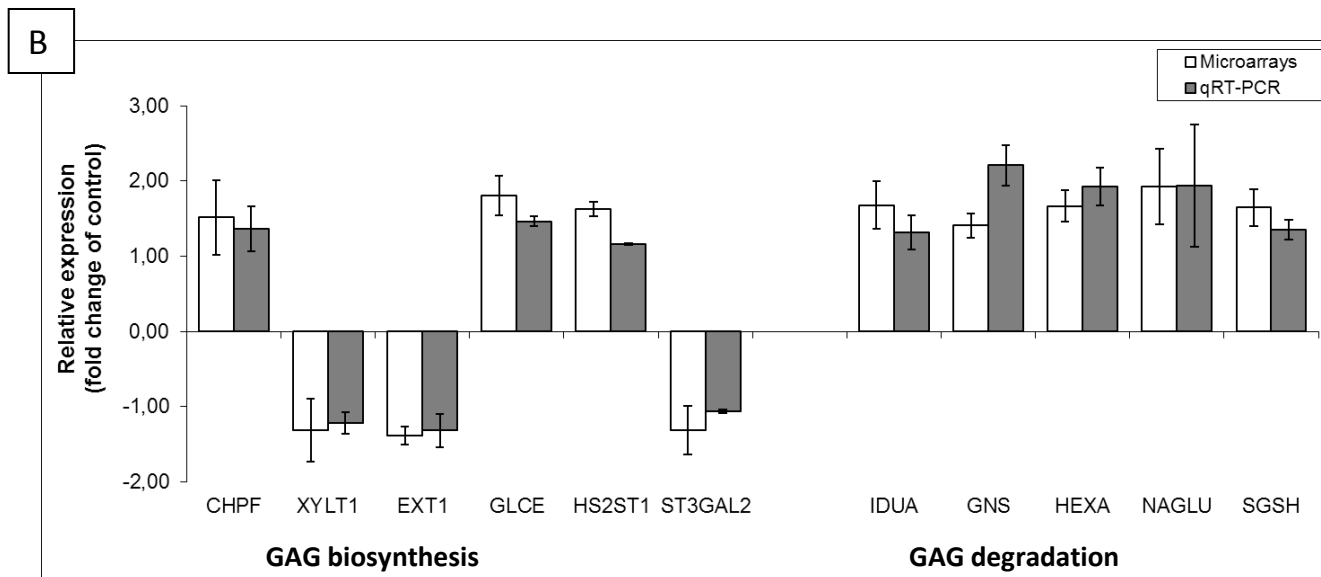
Behavioral tests



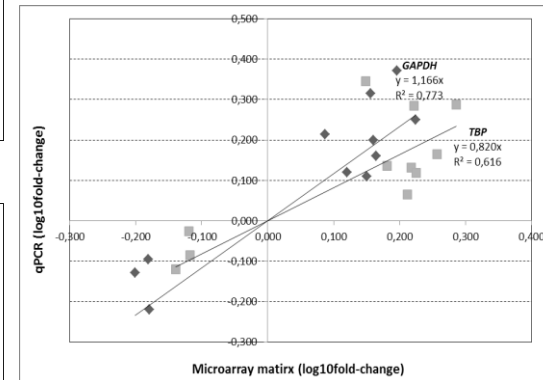
Expression of genes coding for enzymes involved in GAG synthesis and degradation is response to genistein, as assessed by microarray analyses and qRT-PCR

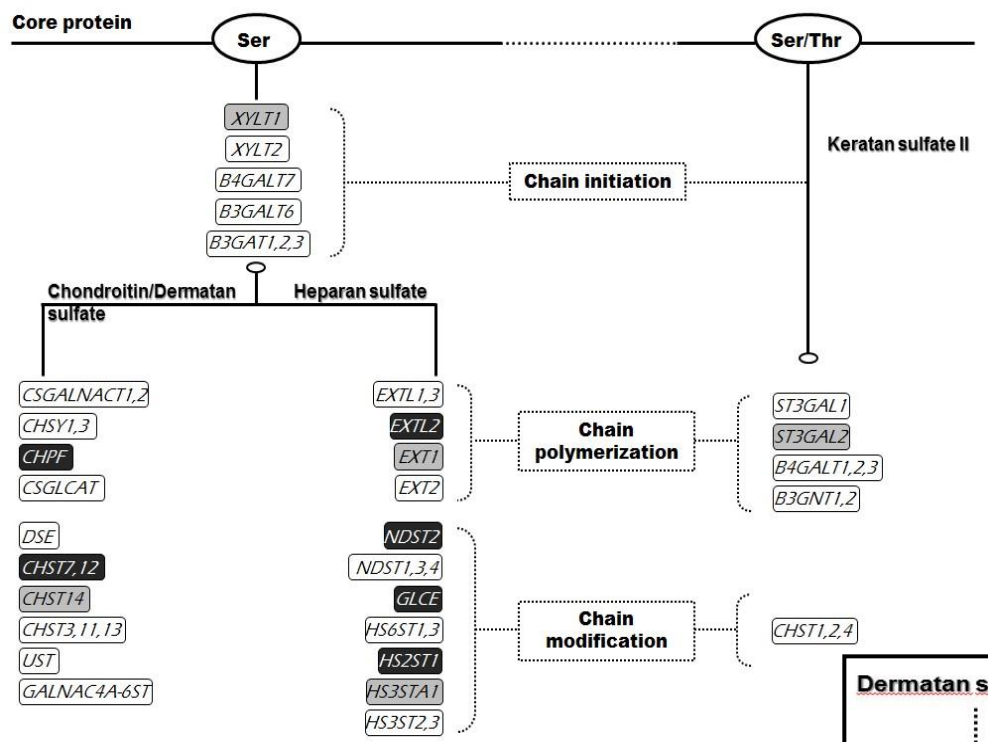


Relative to *GAPDH*
as a control



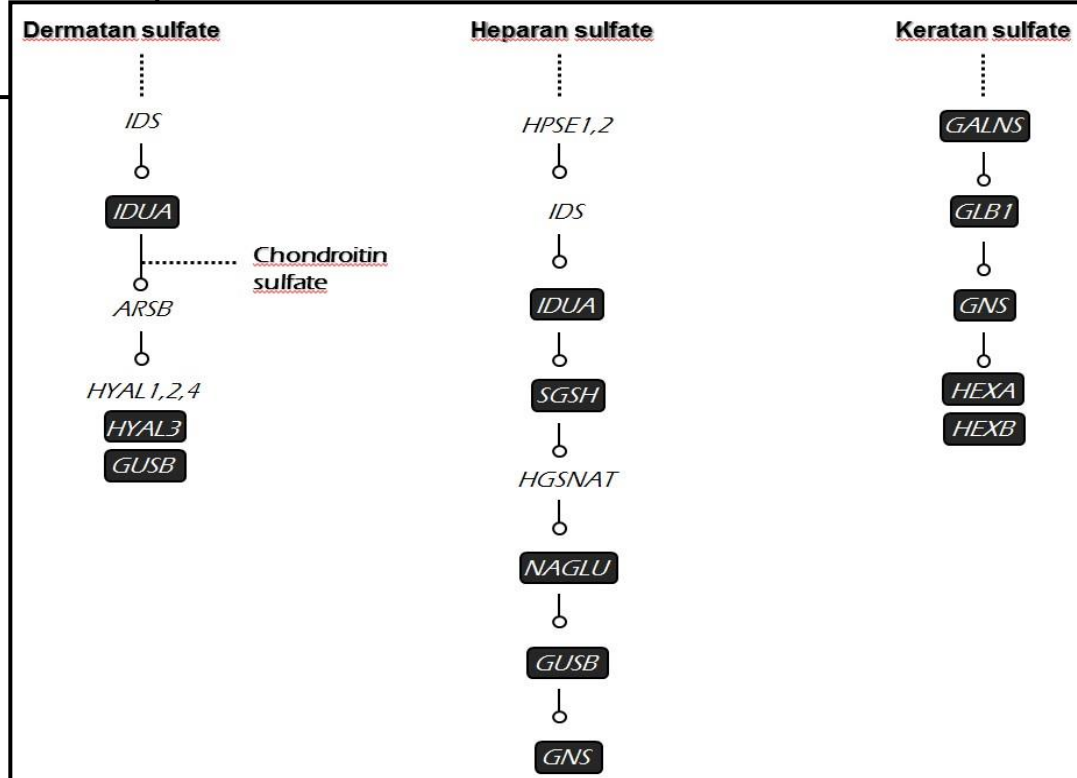
Relative to *TBP*
as a control





GAG synthesis pathways

GAG degradation pathways

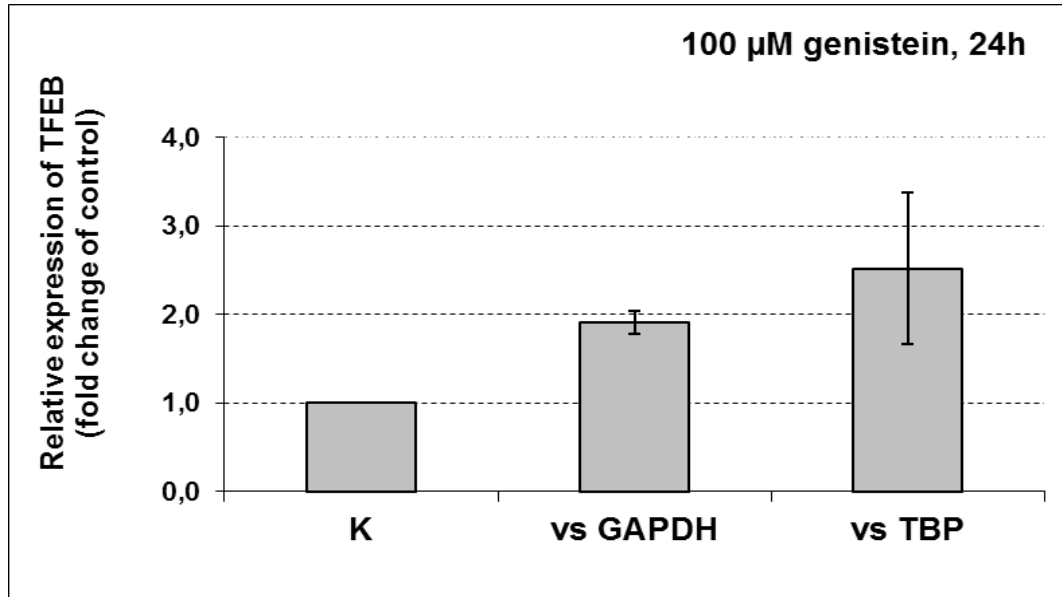


Stimulation by genistein

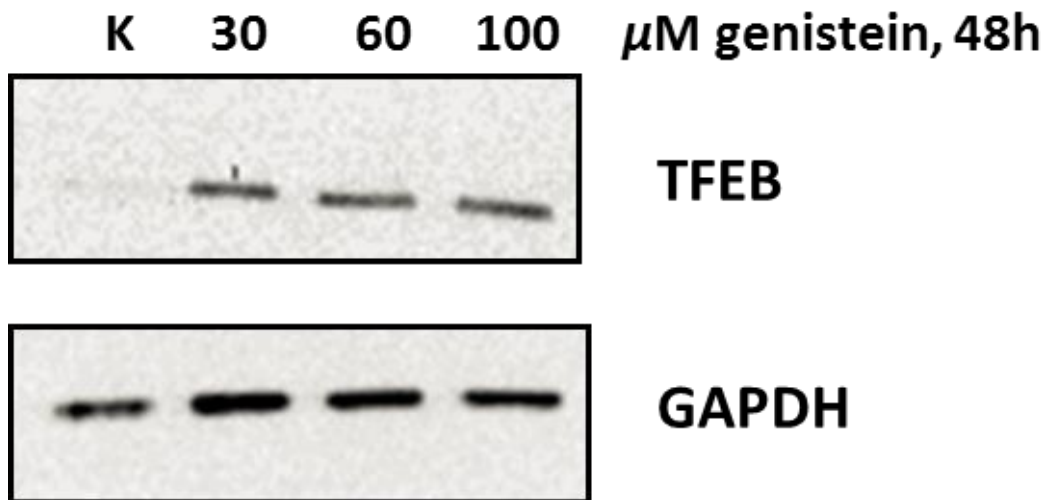
Inhibition by genistein

No strong effect of genistein

Expression *TFEB*, a gene coding for the master positive regulator of lysosomal biogenesis, is stimulated by genistein



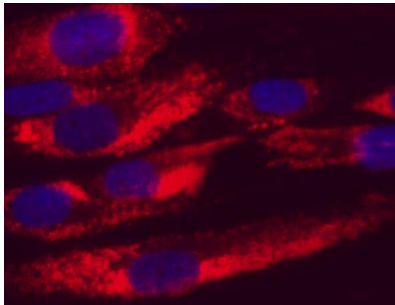
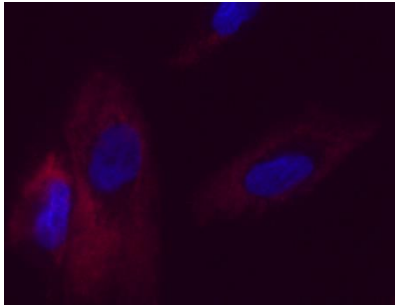
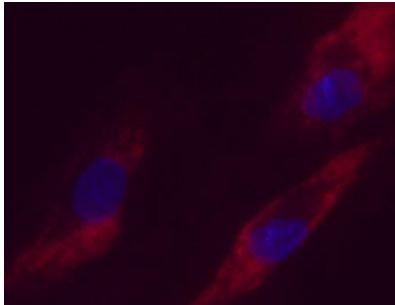
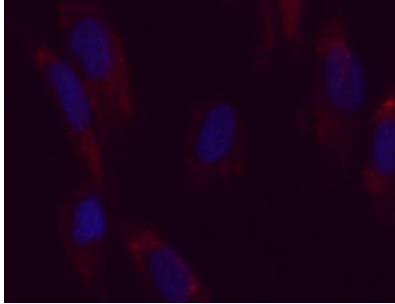
mRNA level



Protein level

Genistein stimulates lysosomal biogenesis in MPS I cells

24 h



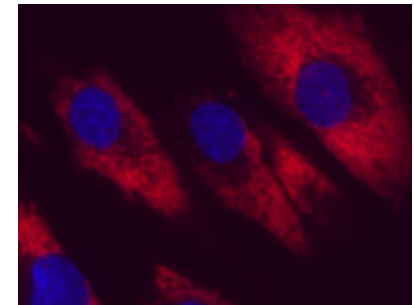
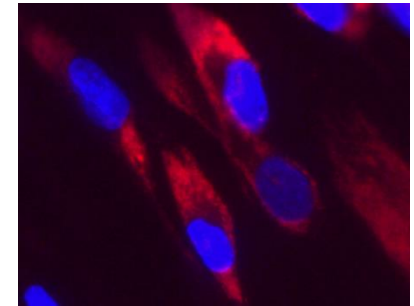
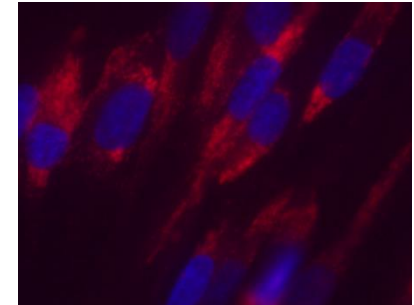
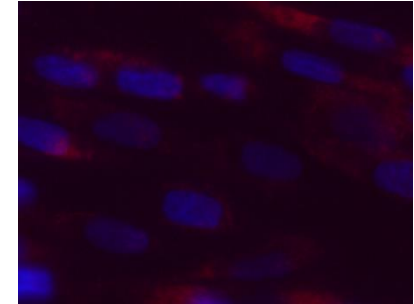
Control – no genistein

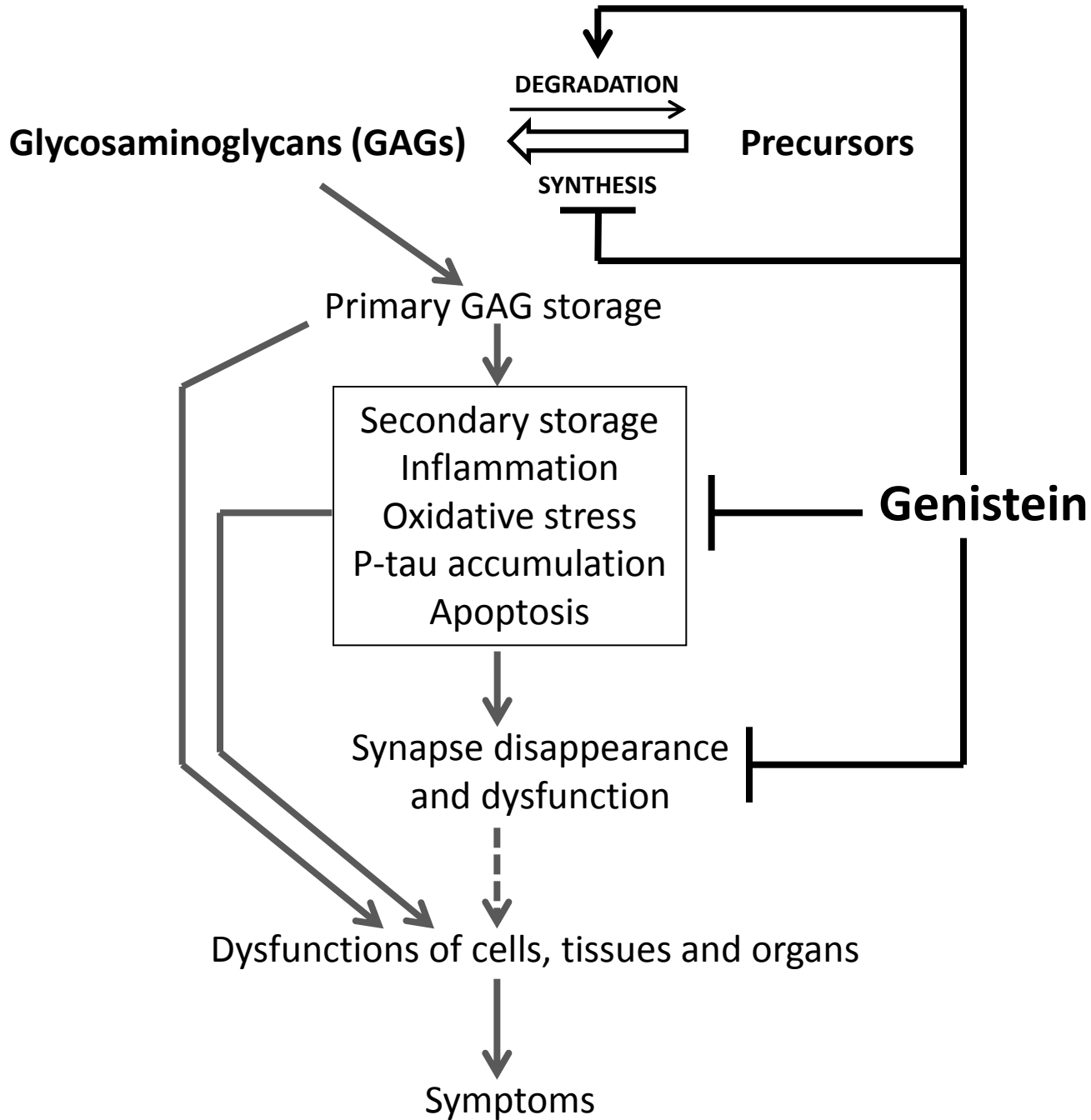
30 μ M genistein

60 μ M genistein

100 μ M genistein

48h





Other applied projects:

- Quick and specific method for identification of Shiga toxin-producing *E. coli* (rapid detection of *stx* genes)
- A novel microbiological test for detection of mutagenic pollution of marine environment
- The use of genistein as a potential compound for treatment of psoriasis
- Chemoprevention against cancer
- The use of phage display system in development of luminescent chemical devices based on nano-particles
- Bacteriophage therapy for treatment of animal infection diseases
- Exploration of marine environment in searching for bioactive compounds