Substrate Reduction, Chaperones and Antisense-Oligonucleotides: Alternatives to Enzyme Replacement Therapy?

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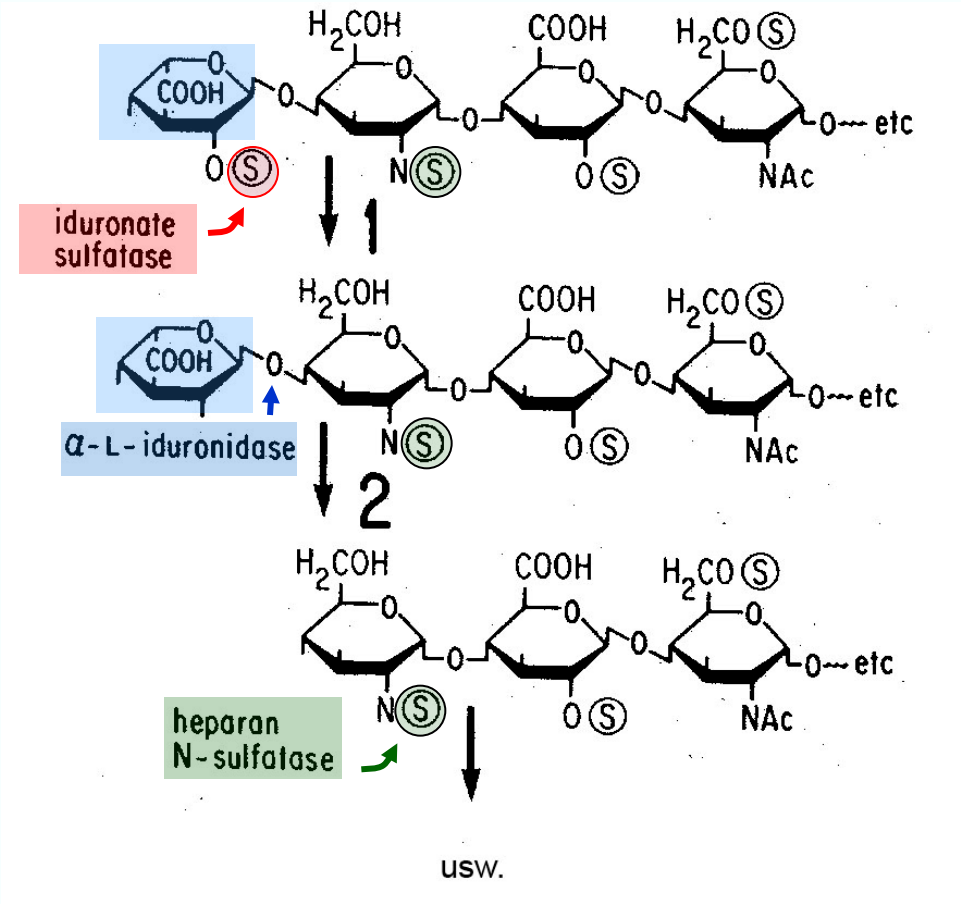
MPS are Lysosomal Storage Dieases

„inherited, deadly constipations of the cell “



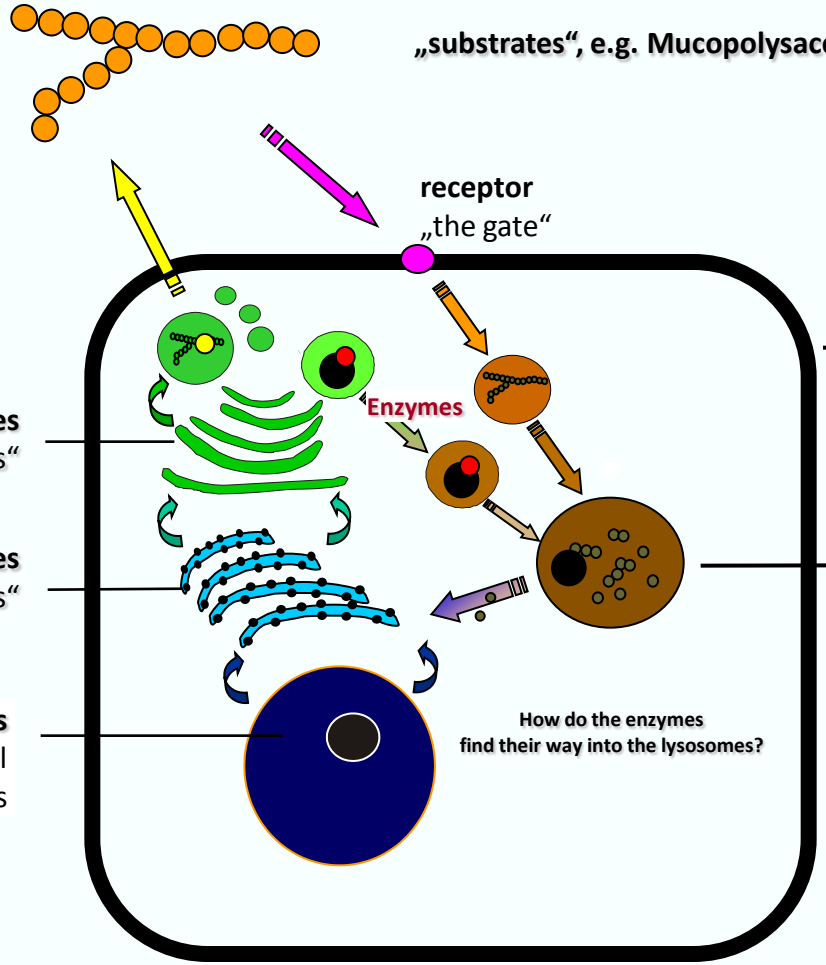
complex sugar chains (Mucopolysaccharides) are stepwisely decomposed by **enzymes („wrenches“)**

If a „wrench“ is defective, the degradation stops, and the partly decomposed **garbage** accumulates in the „gut“ of the cells, in **the lysosomes.**

The cells are slowly killed by this garbage

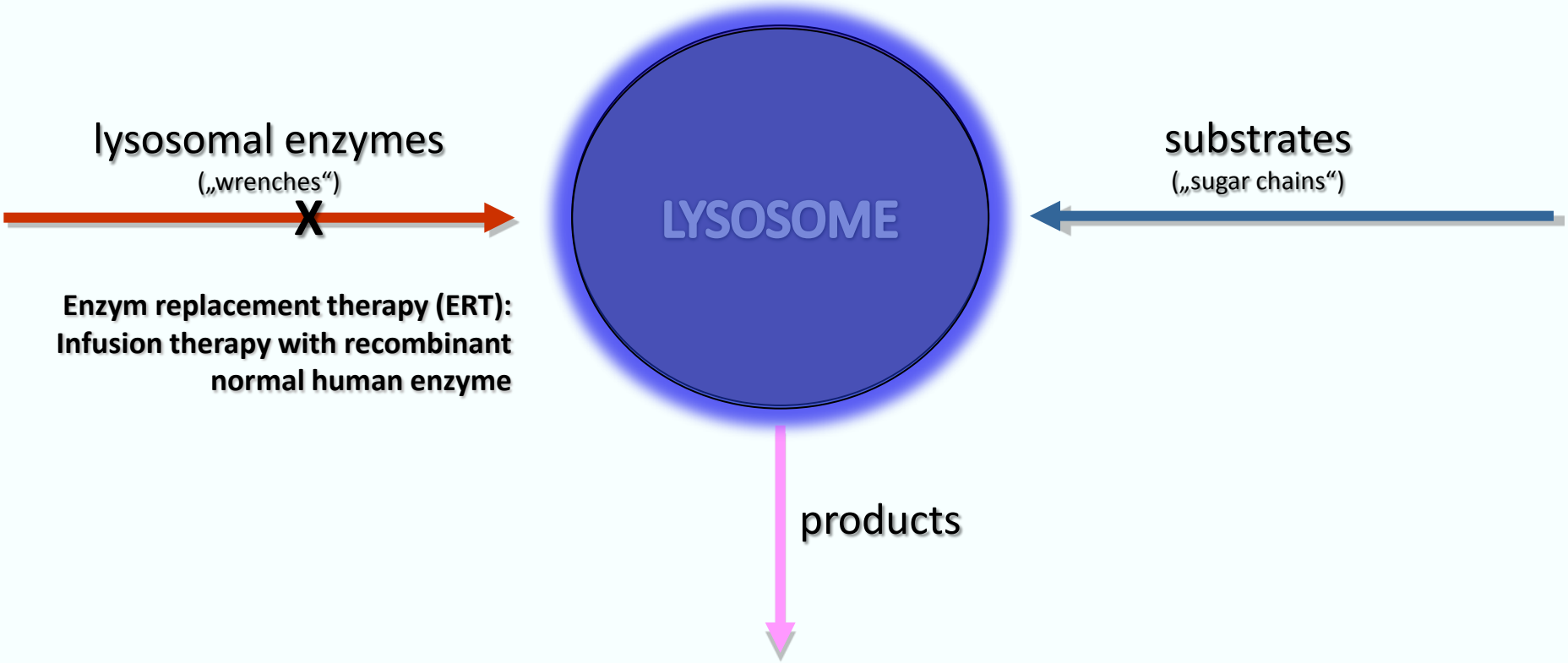
The degradation of mucopolysaccharides

requires many steps



Therapeutic Strategies

for Lysosomal Storage diseases



Enzym replacement therapy (ERT):
Infusion therapy with recombinant normal human enzyme

Results of Enzyme Replacement Therapies

Good results in visceral organs (heart, liver, kidney)
and
connective tissue

Small benefit in bones,

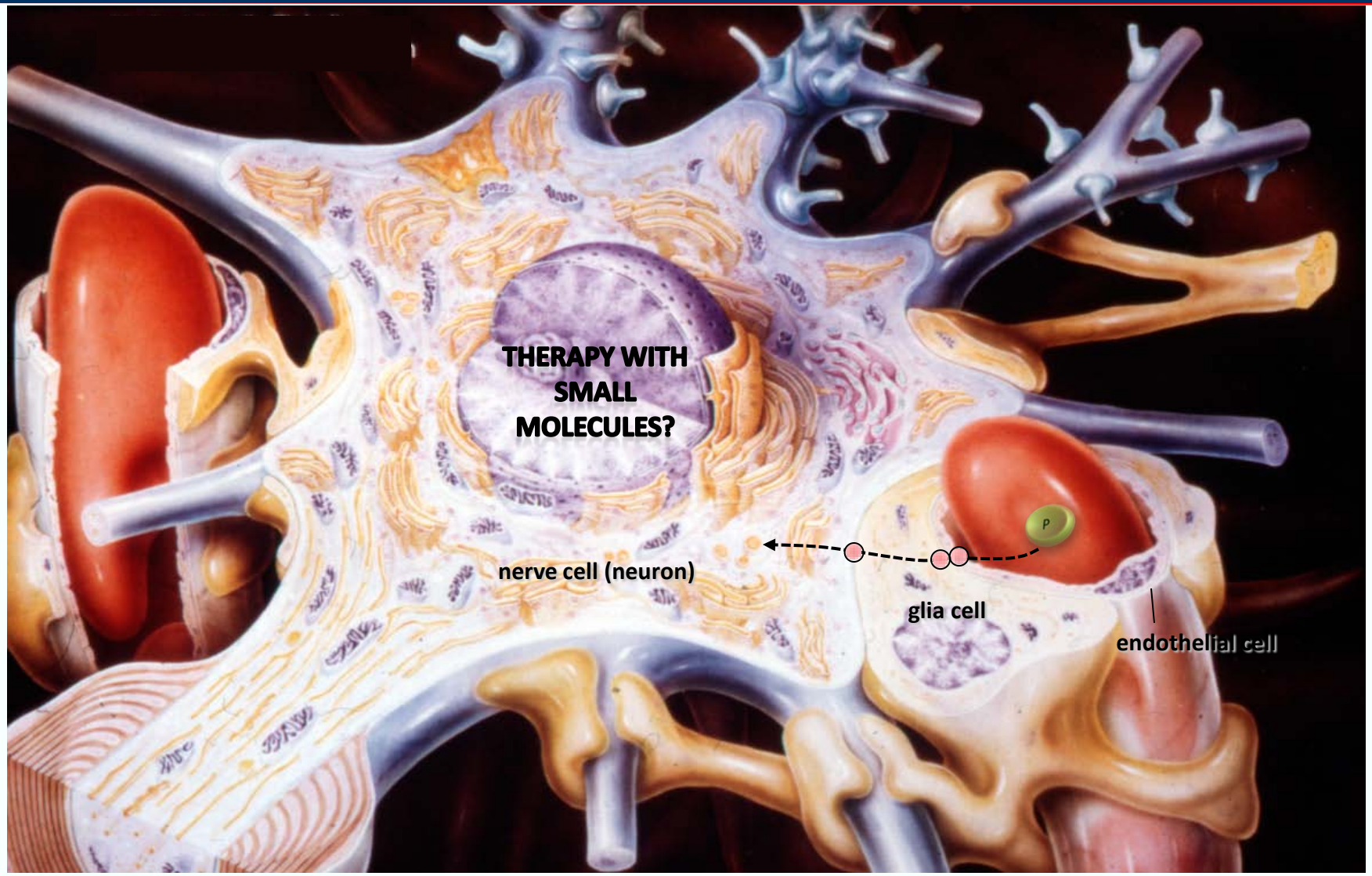
No benefit in brain

Antibodies can prevent any benefit

High costs
€ 300 000 bis 600 000.- per year and patient

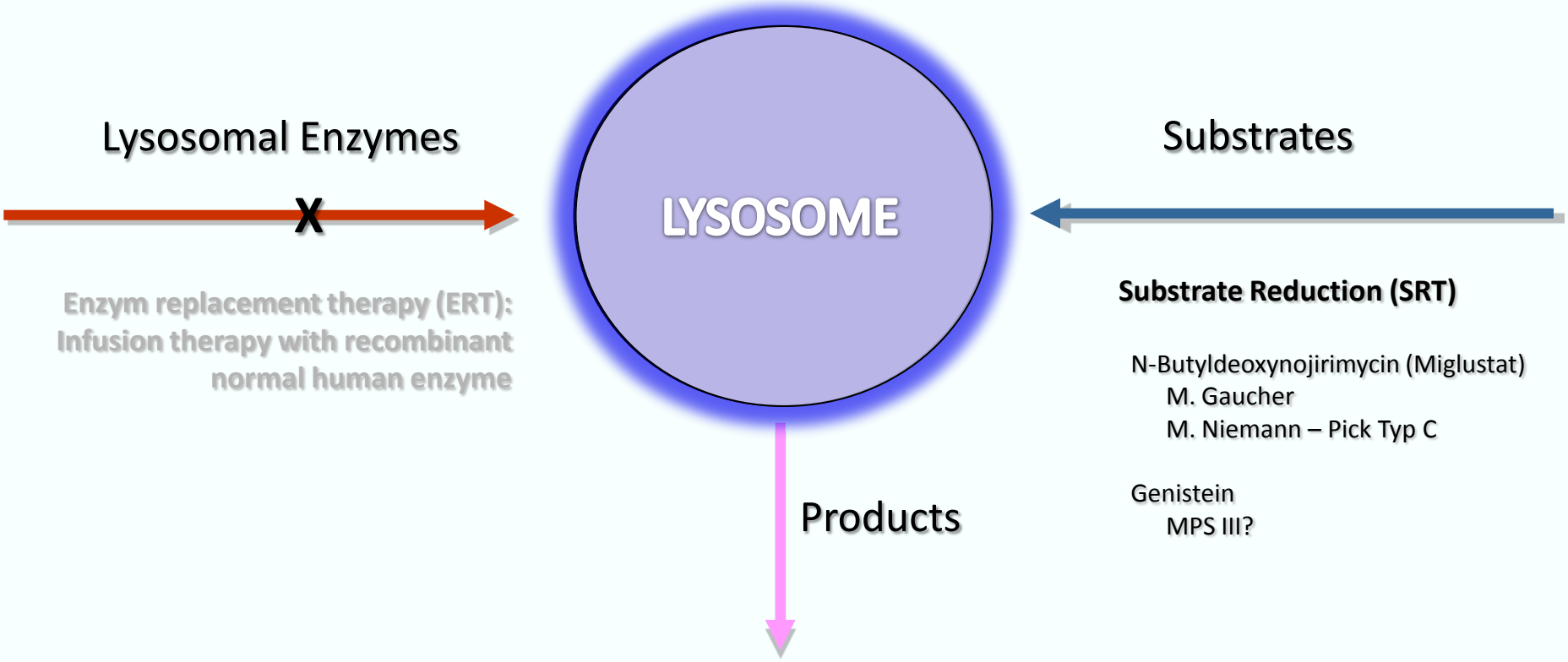
The Blood-Brain Barrier

protects the neurons against viruses and foreign proteins



Substrate Reduction Therapy

for Lysosomal Storage Diseases



Enzym replacement therapy (ERT):
Infusion therapy with recombinant normal human enzyme

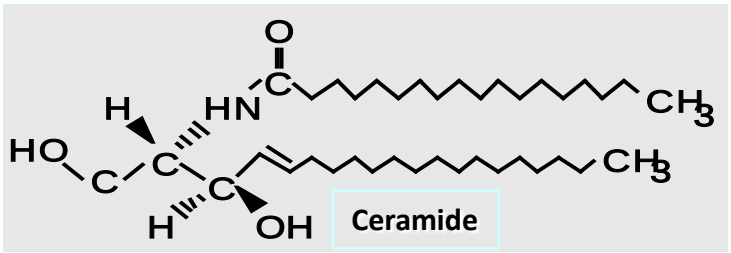
Substrate Reduction (SRT)

N-Butyldeoxynojirimycin (Miglustat)
- M. Gaucher
- M. Niemann – Pick Typ C

Genistein
- MPS III?

Biosynthesis of Glycosphingolipids

Substrate Reduction using Imino Sugars

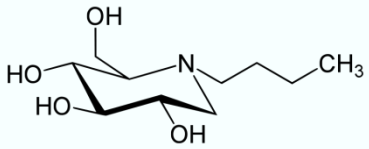


Ceramide

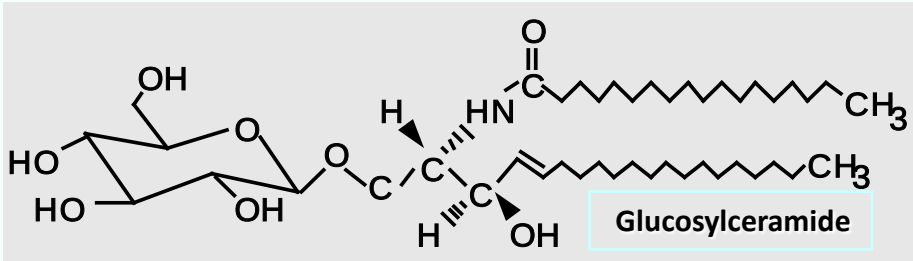
UDP-Glucose

UDP

UDP-Glucose: N-acylsphingosine glucosyltransferase



Miglustat
(N-Butyldeoxynojirimycin)



Glucosylceramide

Storage in Gaucher Disease

Lactosylceramide

Storage, e.g. in Niemann-Pick Disease type C, Gangliosidoses

Ganglio-series

Lacto(neo)-series

Globo- series

MPS III (secondary?)

Substrate Reduction Therapy with Miglustat

approved for Gaucher and M. Niemann-Pick Disease type C

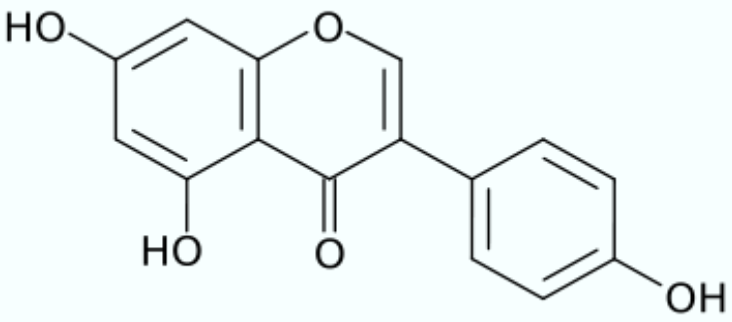
- Helpful for mild or moderately severe **Gaucher disease**
  - Size reduction of liver and spleen
  - Improvement of hematological and biochemical parameters
  - Inreased of lipid fraction in bone marrow
  - prolonged effectiveness
  - Side effects: Diarrheas and initial weight loss

- **M. Niemann-Pick Disease Type C** (approved since January 2009)
  - Improvement of typicla neurological symptoms („sakkadic horizontal eye movements“)
  - Improved swallowing, stabilized hearing, retarded progression of disease

- **MPS III** (Guffon et al J. Pediatr 2011, Jun 8 Epub ahead of print)
  - study on 25 patientsn with MPS III for 12 months
  - Stabilisization of behavior and sozialisation?
  - **No improvement of behavioral problems detectable**

Genistein

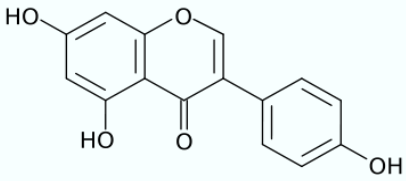
Substrate Reduction for Mucopolysaccharidoses?



- Isoflavonoid from Soja beans
- Previously approved to treat osteoporosis, cardiovascular diseases and others
- **unspecific** enzyme inhibitor
- Decreases the amount of enzymes of the sythetic pathways of mucopolysaccharides **in general** (Piotrowska et al , 2006)
  (decreased gene expression due to an inhibition of tyrosine-specific proteinkinase activity of EGF receptor)
- Side effects, e.g. as a „phytooestrogene“?

Genisteine

preclinical data



- Effective in cultured fibroblasts of patients with MPS I, II, IIIA, IIIB:
  reduced storage of glycosaminoglycans
- Effective in animal models (**MPS II mice**):
  reduced storage in **peripheral organs and evidence for reduced storage in brain**
  (Friso et al , 2010)
- Administration of high doses (30-fold elevated) for 9 months to **MPS IIIB-mice**
  (Malinowska et al. 2010):
  - **Reduced storage of h**eparan sulfate and secondary substances
  - Improved expression of proteins in synaptic vescles
  - **Reduced signs of pathologic imfalmmation processes in brain**,
  - Correction of behavioral disturbances
- **Conclusion:**
  Appropriate for substrate reduction in MPS IIIB or probably other neurodegenerative metabolic diseases?

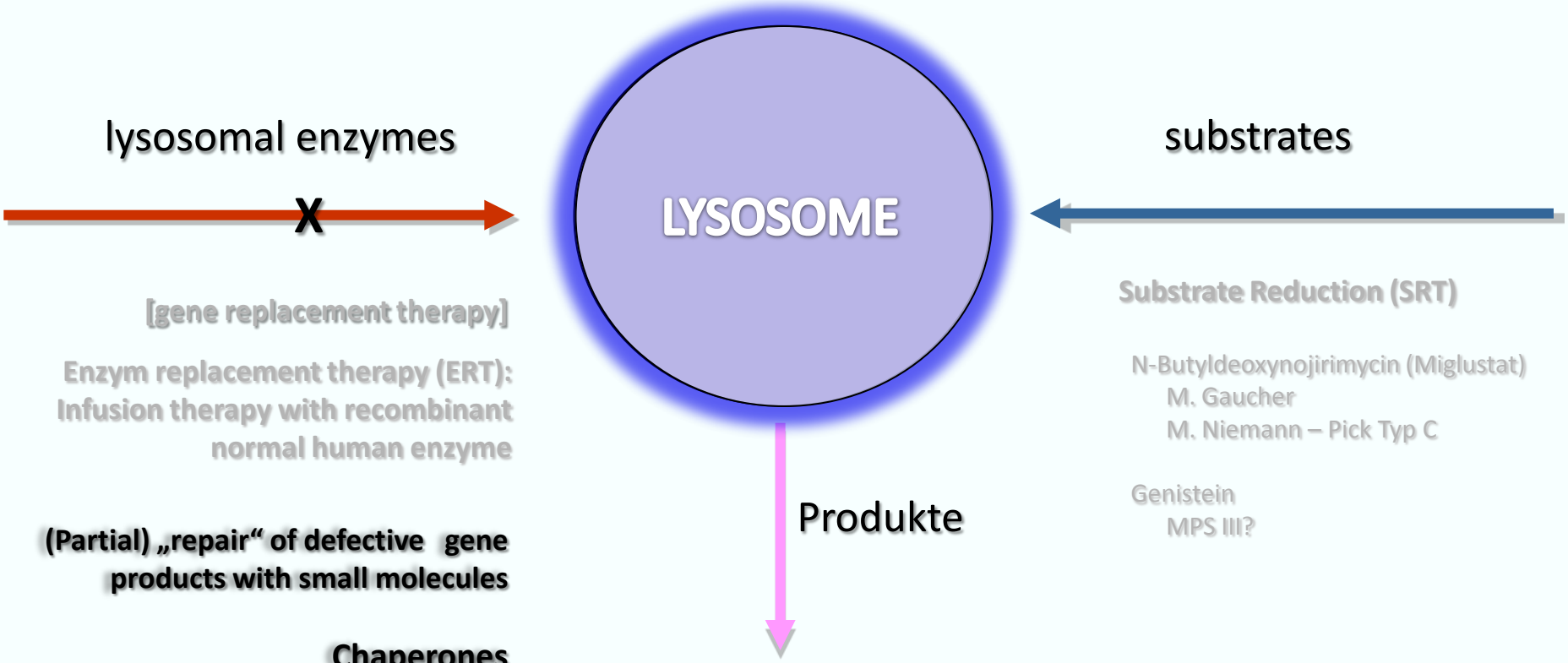
Genisteine

recent clinicl studies

- **Piotrowska et al (2011)**
  - 8 patients with Sanfilippo Syndrome, A or B
  - standardized cognitive tests (Brief Assessment Examination, BAE)
    - After 1 year 7 Pat improved , 1 stabilized;
      after 3 years 2 Pat improved, 3 stabilized , 3 worsened
  - 18 parameters for observations by the parents
    (language, understanding, activity,sleeping problems, etc.)
    - After 1 year all improved;
      after 3 years 5 improved, 3 worsened
  - **Conclusion:** Genisteine-treatment could improve or stabilize the cognitive and behavioral problems of Sanfilippo disease in some patients.
- **Delgadillo et al (2011)**
  - 19 patients (10 males, 9 females) with Sanfilippo Syndrom A, B or C; Genistein for 1 year
  - parameter s:
    „Disability score“ (gait, behavior, Speech, swallowing problems, epilepsy) , morphology of hair, biochemical tests
    - After 1 year: no improvement of Disability Score, reduced frequeny of infections and GI symptoms, galcosaminoglycan levels all increased and highly variable
  - **Conclusion:**
    Genistein does **not improve** the handicaps of patients within the applied score
  - Probably **higher doses** necessary?

Therapeutic Strategies

to repair defective gene products



[gene replacement therapy]

Enzym replacement therapy (ERT):
Infusion therapy with recombinant normal human enzyme

(Partial) „repair“ of defective gene products with small molecules

Chaperones

[Antisense-Nucleotides?]

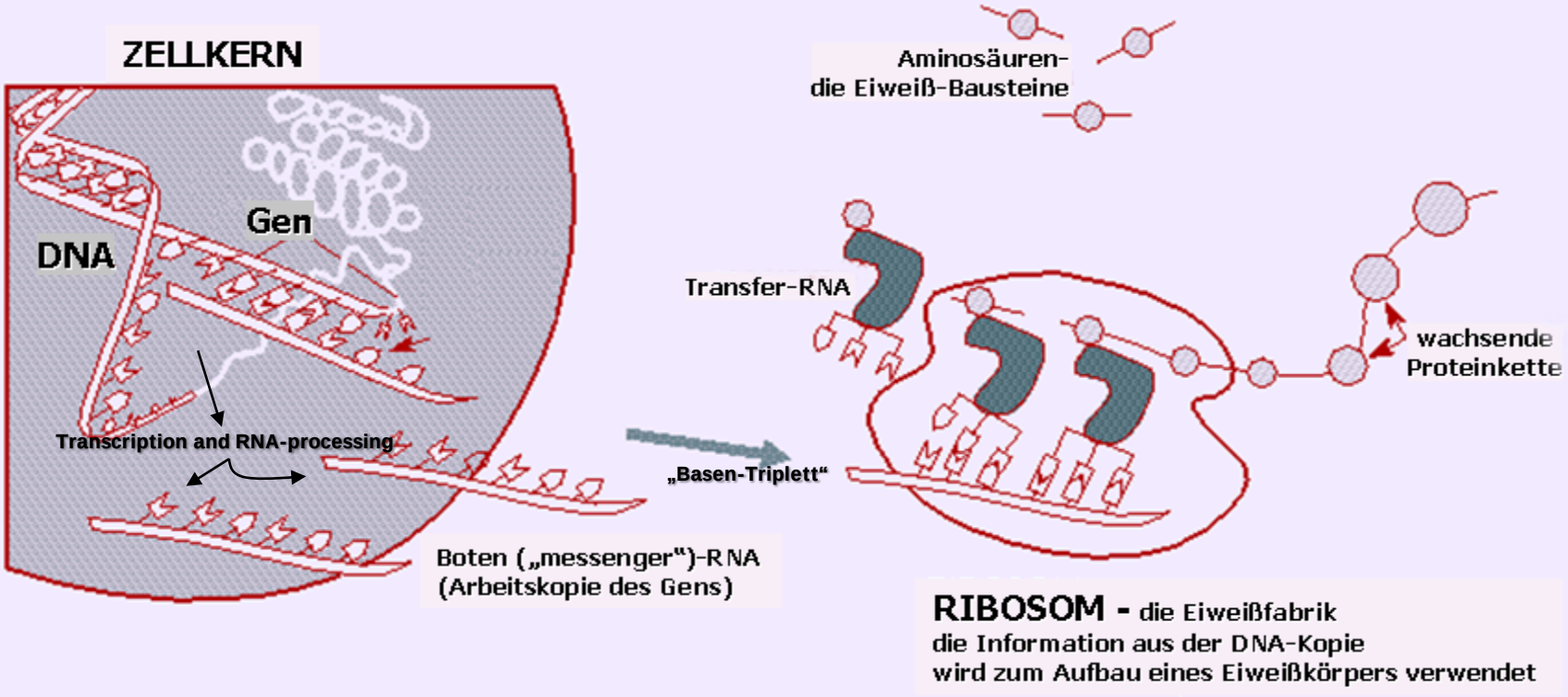
Substrate Reduction (SRT)

N-Butyldeoxynojirimycin (Miglustat)
M. Gaucher
M. Niemann – Pick Typ C

Genistein
MPS III?

How can the genetic infomation be transformed into proteins?

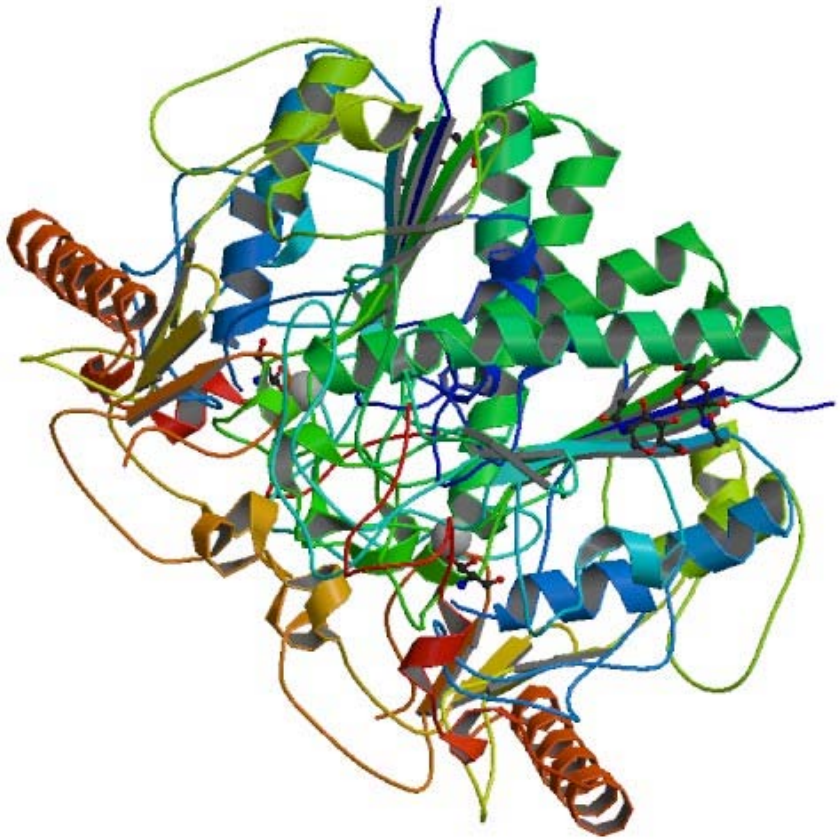
Translation



transcription and RNA-processing
antisense oligonucleotides?

translation, protein folding (and trafficking)
chaperones

Missense-mutations



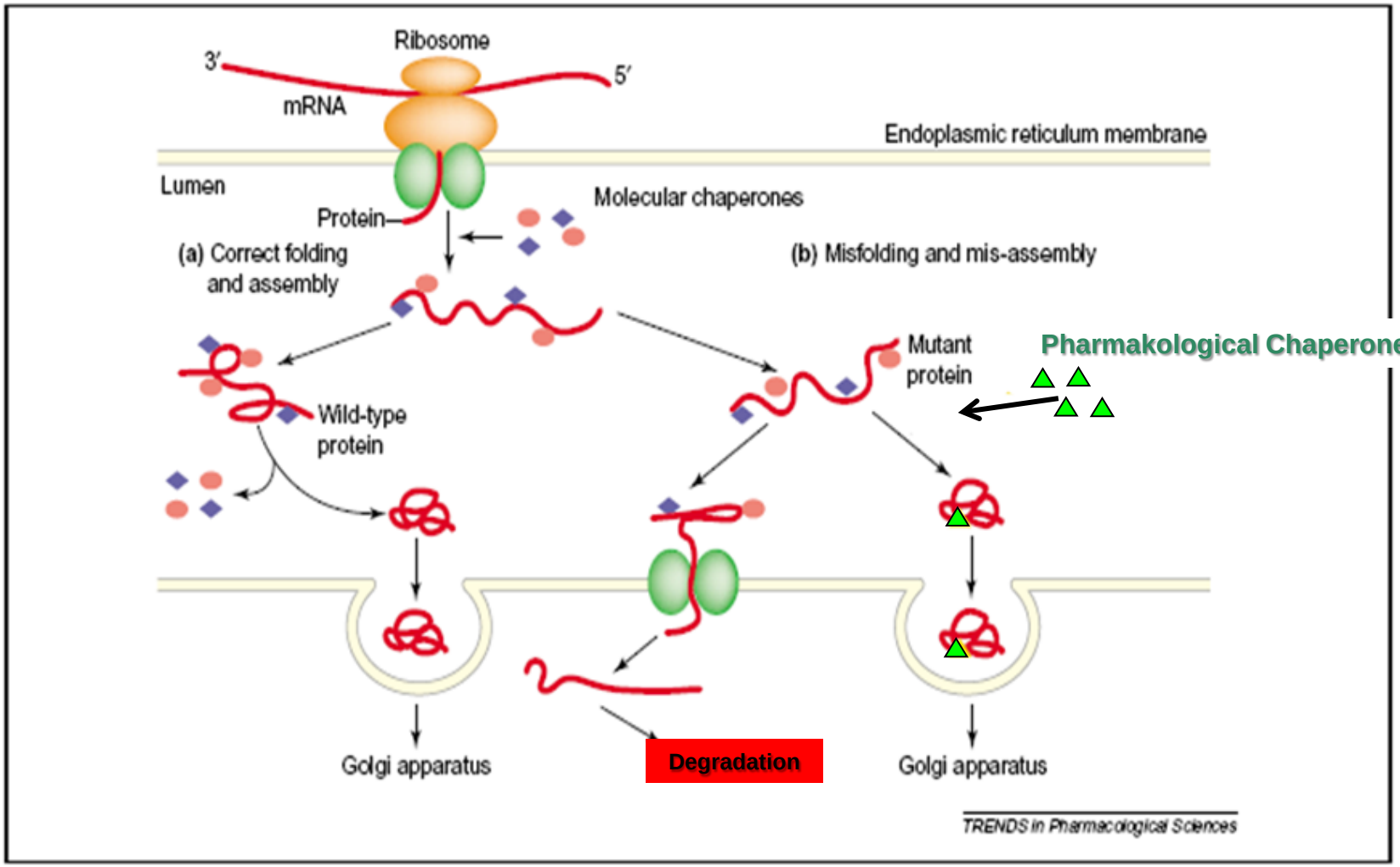
Model of arylsulfatase A

The sequence of bases in the DNA determines the sequence of aminoacids in the proteine chain.

The amino acid sequencs determines the folding of the protein chain.

The principle of chaperone therapy

Proteine synthesis, folding and degradation



Approved therapies and pharmakological Chaperones

for lysosomal storage diseases *)

Disease	Enzyme	Approved Drug	Pharmakological Chaperones	
			Name	Status
Fabry	α-Galactosidase A	Fabrazyme (agalsidase beta) Replagal (agalsidase alfa)	**DGJ (AT1001; Amigal™)** Galactose	**Phase 3** Preclinical
Gaucher	Acid ß-Glucosidase	Cerenzyme (imiglucerase) VPRIV (velaglucerase alfa) Zavesca (Miglustat; NB-DNJ)	**Isofagomine** Ambroxol + 17 substances	**Phase 2** Pilot study Preclinical
GM2 Gangliosidosis	Acid ß-Hexosaminidase	None	**Pyrimethamine** + 6 substances	**Phase 2** Preclinical
Pompe	Acid α-Glucosidase	Myozyme (alglucosidase alfa) Lumizyme	**Desoxynojirimycin (DNJ; AT2220)** NB-DNJ (Miglustat) NO-DNJ	**Phase 2** Preclinical
MPS IIIC	Heparansulfat-AcetylCoA: GlNAc-Transferase	None	Glucosamine	Preclinical
GM1 Gangliosidosis Morquio B	**Acid ß-Galactosidase**	**None**	**6 Substances, 3 from our group**	Preclinical

*) simplified, according to: *Valenzano et al (2011) Assay and Drug Development Technologies, 9/3, 213-235*

Steps in preclinical investigations

Mol Ther. 2009 June; 17(6): 964–971.
Published online 2009 March 17. doi: 10.1038/mt.2009.53

PMCID: PMC2835191

The Pharmacological Chaperone N-butyldeoxynojirimycin Enhances Enzyme Replacement Therapy in Pompe Disease Fibroblasts

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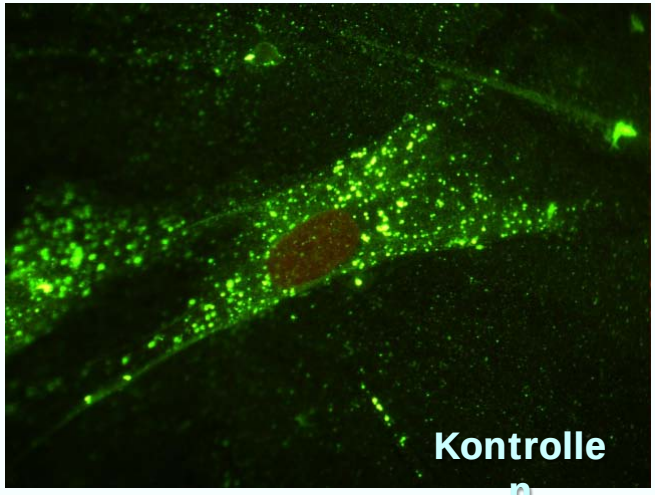
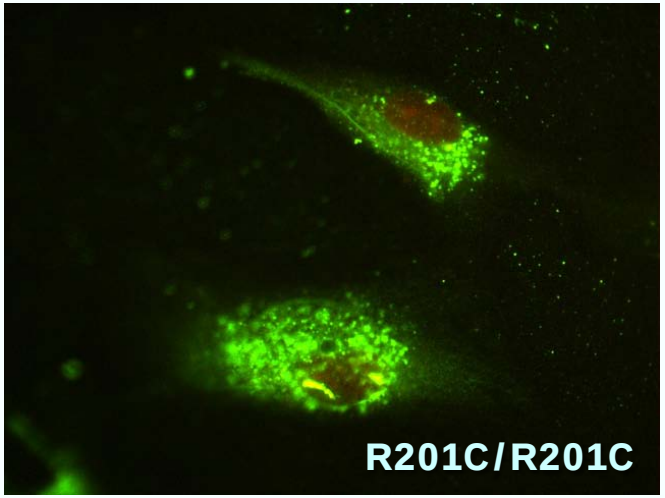
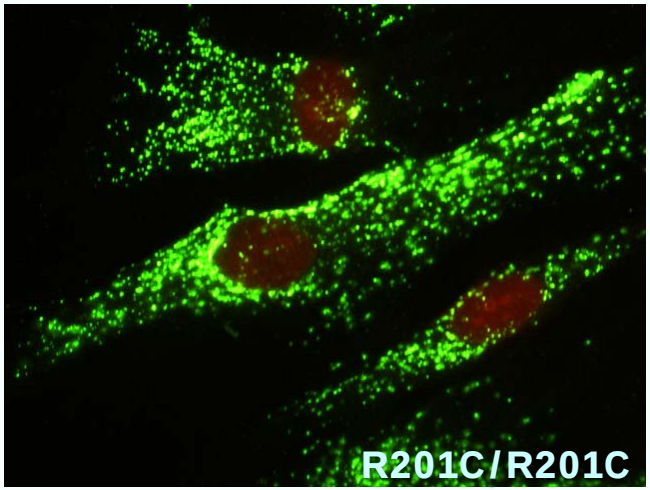
Immunofluorescence with specific antibodies against ß-galactosidase

(Fantur et al (2010) Genet Metab 100, 262-268)



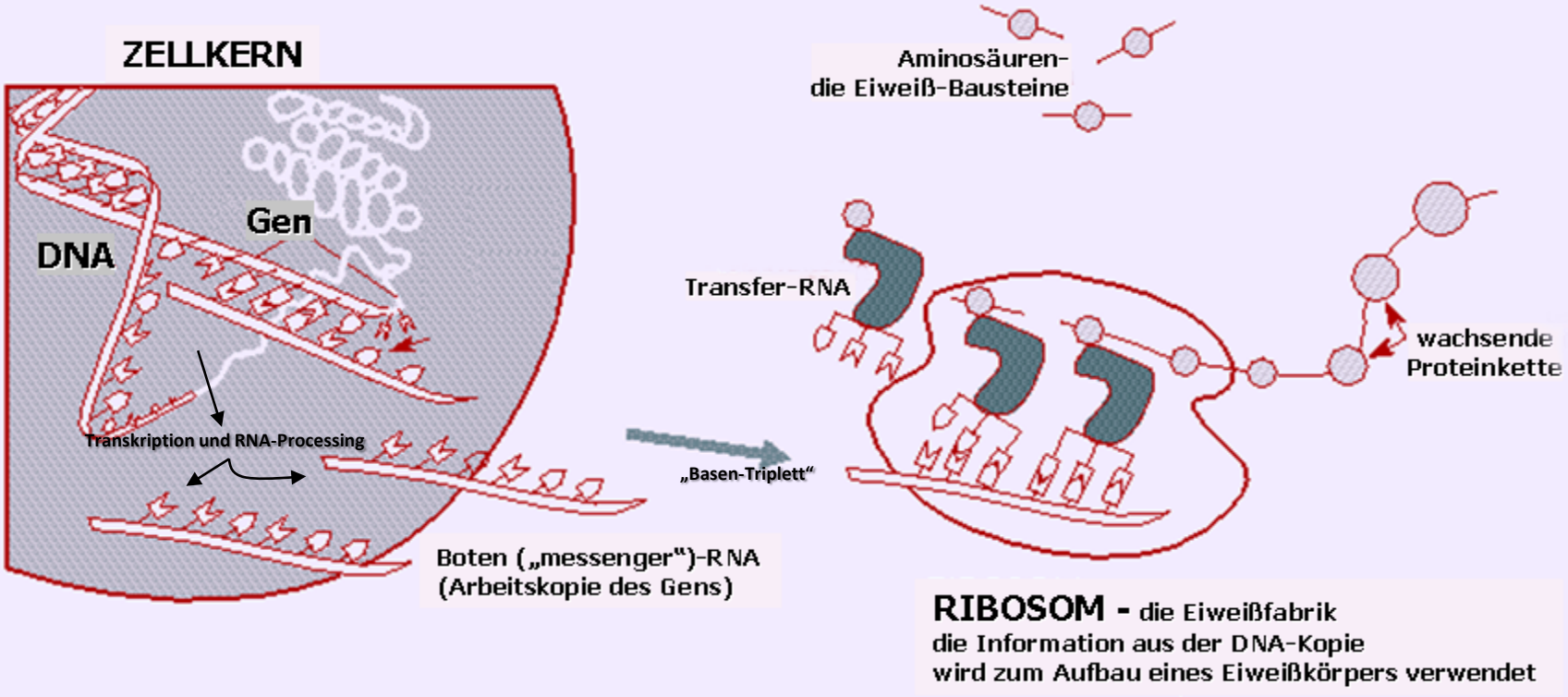


4 Tage Kultur in Gegenwart von 500µM DLHex-DGJ



How can genetic infomation be transformed into proteins?

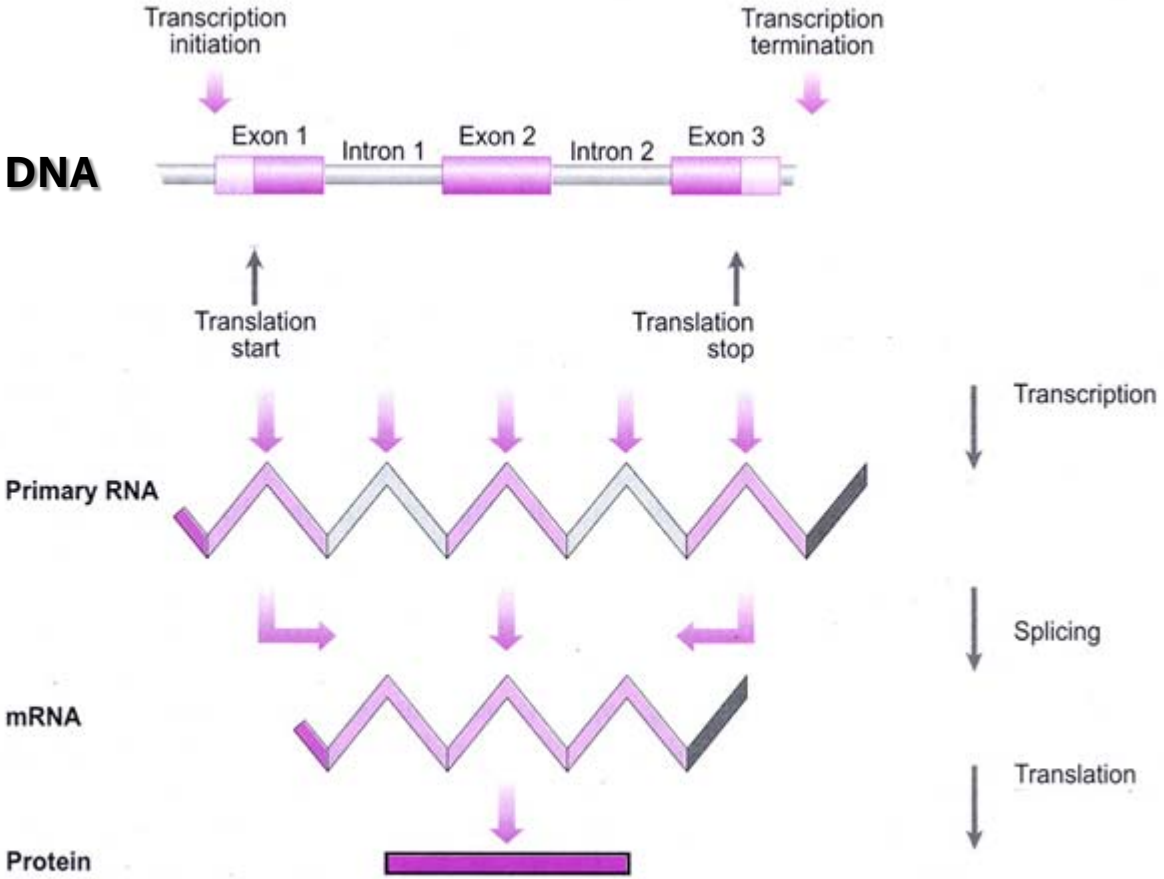
Transcription and RNA Processing



Transcription und RNA-Processing

Translation

Transcription and RNA - Processing

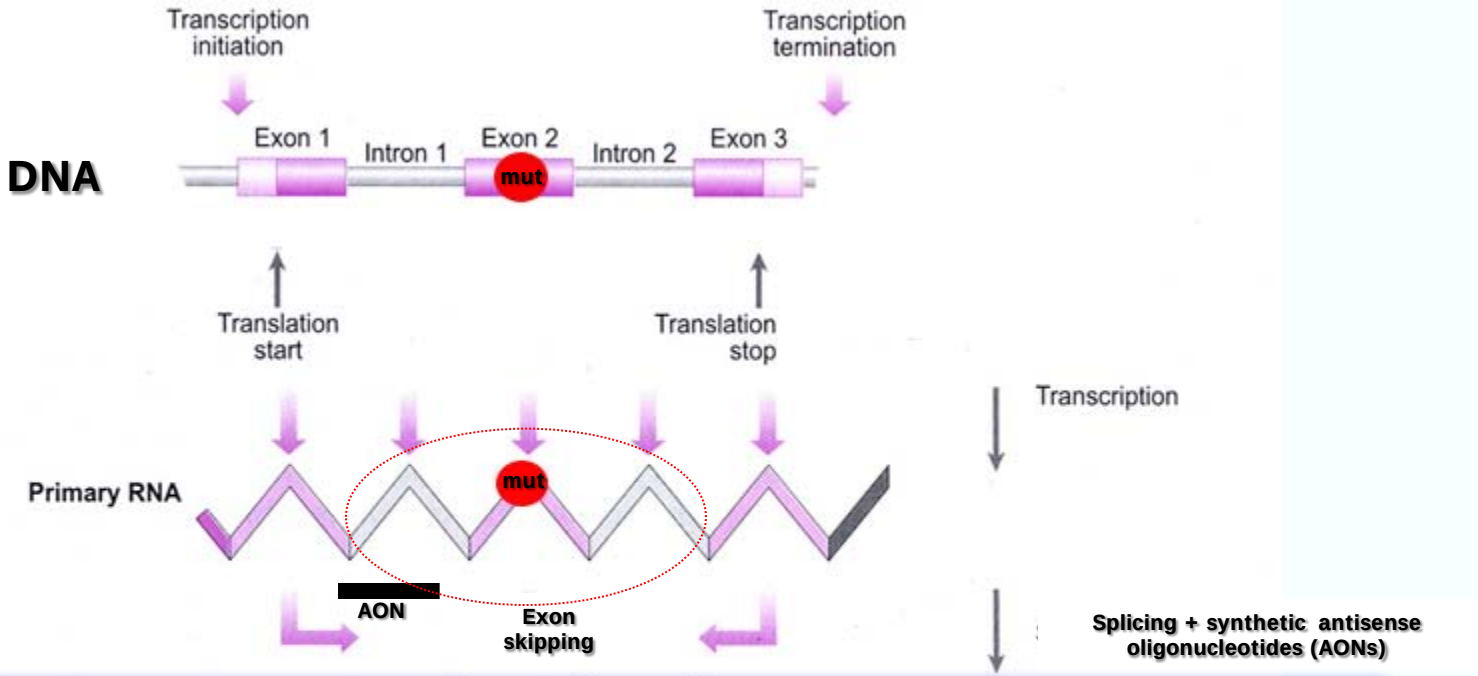


Mutations in introns results in erronous assembly of mRNA („Splice-site mutations“)

„Antisense-Nucleotides“ can repair this assembly

Duchenne-muscular-dystrophy

„Antisense – mediated Exon skipping“: the defective exon is removed



Applicable for Lysosomal Storage diseases?

Summary

- The **blood-brain barrier** prevents the uptake of recombinant enzymes and thus a successful use of enzyme replacement therapy in the central nervous system.

- Current efforts try to find **small molecules** which can enter the brain and may be of benefit for the treatment of lysosomal storage diseases

- **Substrate reduction therapy** is based on
  - A **specific inhibitory effects** of imino sugars on a single defined enzyme in the synthesis of glycolipids, currently approved for **Gaucher** and **M. Niemann-Pick disease, type C**
  - Isoflavonoids (Genisteine and others) inhibit the de novo synthesis of mucopolysaccharides **indirectly.**
    An approval is already available for non-lysosomal diseases,
    the clinical benefit is currently still unclear.

- A **repair of altered gene products** requires mutation-specific approaches
  - **chaperones** repair the negative effects of missense mutations by stabilizing the conformation and thus restoring the function of mutant enzymes.
  - Currently, numerous novel chaperones are tested in preclinical and clinical trials. However **not all mutations** can be treated with chaperons.
  - **Antisense-nucleotides** may be helpful for future methods correcting defects of **RNA-Processing**.