Biotechnological approaches to diagnosis and treatment of rare genetic disorders

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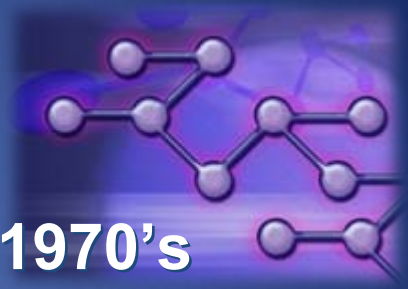


- Biotechnology Industry originated in the 1970's
- More than 200 new therapies and vaccines
- Over 400 potential drugs currently in Clinical Trials
- Hundreds of medical diagnostic tests

From concept to realization

- Around 30.000 known diseases worldwide
- Worldwide Investment in R & D steady rise over past 10 years
- Number of innovative medicinal products on the global market was only 40 per annum.
- On average development of new drug takes 8 -12years and 895 mio Euros
- From the decision to develop a drug to finished product requires around 800 steps
- Only ONE out of the 5.000 to 10.000 substances tested gets as far as Market Authorization
- Todays challenge : development of Orphan Drugs

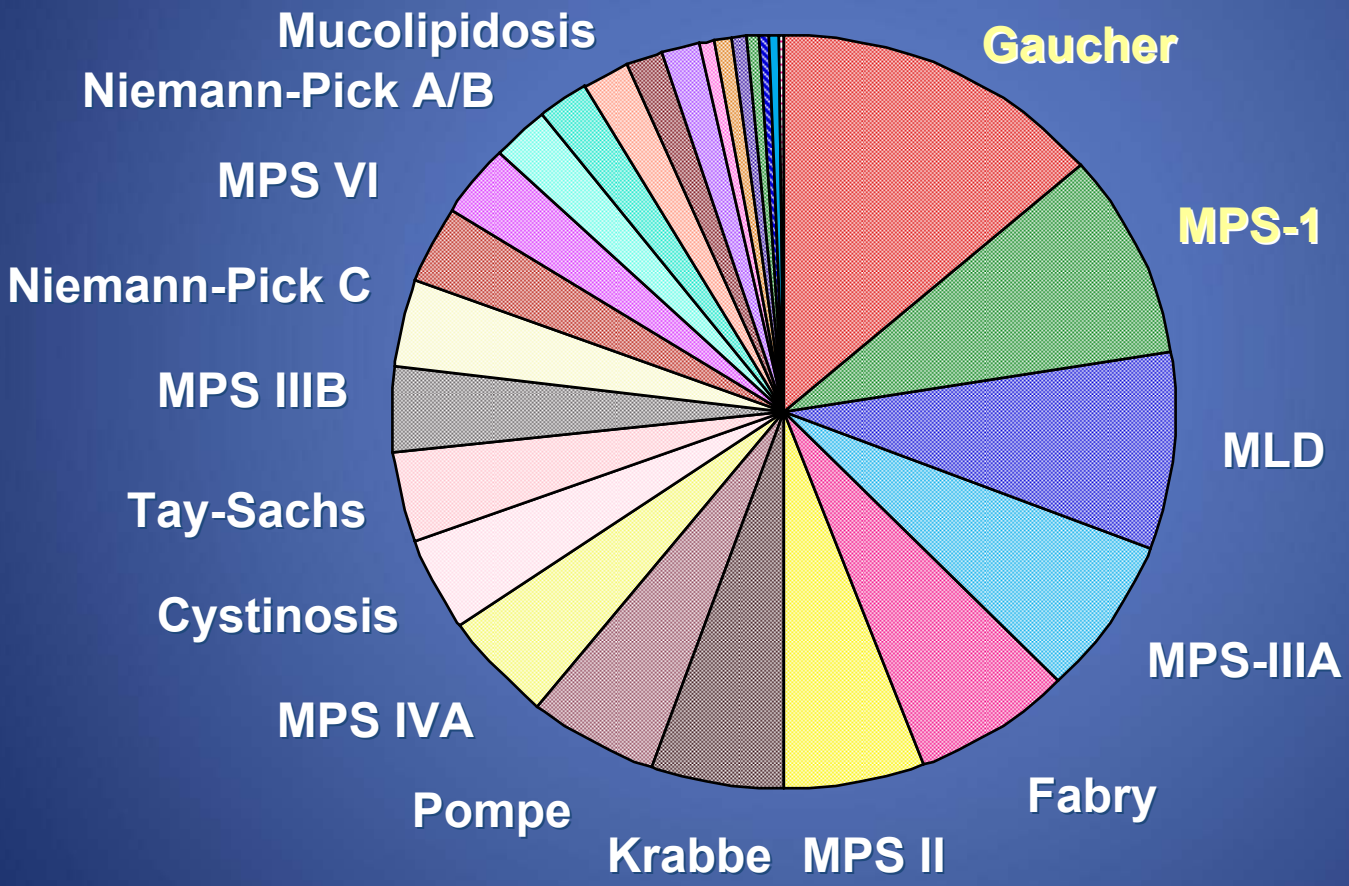
❖ **Biotechnology – today:**

- Isolation
- Insertion into a vector
- Transformation
- Test to isolate genetically modified organism

❖ **Further sophistication:**

- Recombinant DNA technology in Human cells
- Gene activation techniques

Propensity of Lysosomal Storage Disorders

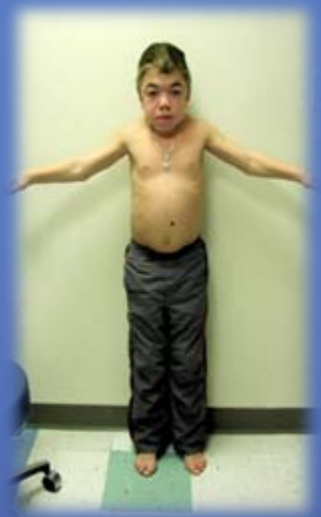


Biotechnological approches to diagnosis and treatment of rare genetic disorders

Disease	Product	Source	Company	Missing Enzyme
Gaucher Disease	Cerezyme®	CHO cells/carbohydrate remodelling	Genzyme	glucocerebrosidase
Gaucher Disease	GA-GCB	Human cell line: gene activation / mannosidase inhibition	Shire	glucocerebrosidase
Fabry Disease	Fabrazyme™	CHO cells	Genzyme	α galactosidase A
Fabry Disease	Replagal™	Human cell line: gene activation	Shire	α galactosidase A
Hurler Syndrome	Aldurazyme™	CHO cells	Biomarin	α–L-iduronidase
Hunter Syndrome	Elaprase	Human cell line	Shire	iduronate sulfatase
MPS VI	Naglazyme	CHO cells	Biomarin	arylsulfatase B
Pompe	Myozyme	CHO cells	Genzyme	acid α–glucosidase

What is Hunter Syndrome (Mucopolysaccharidosis type II /MPS II)?

A rare X-linked genetic disorder which predominantly affects males deficiency of lysosomal enzyme iduronate-2-sulfatase (I2S) results in tissue accumulation of glycosaminoglycans (GAG), Multi-systemic, heterogeneous disease, results in severe morbidity and early mortality Symptoms include heart, airway, bone and liver abnormalities and CNS involvement in severe cases

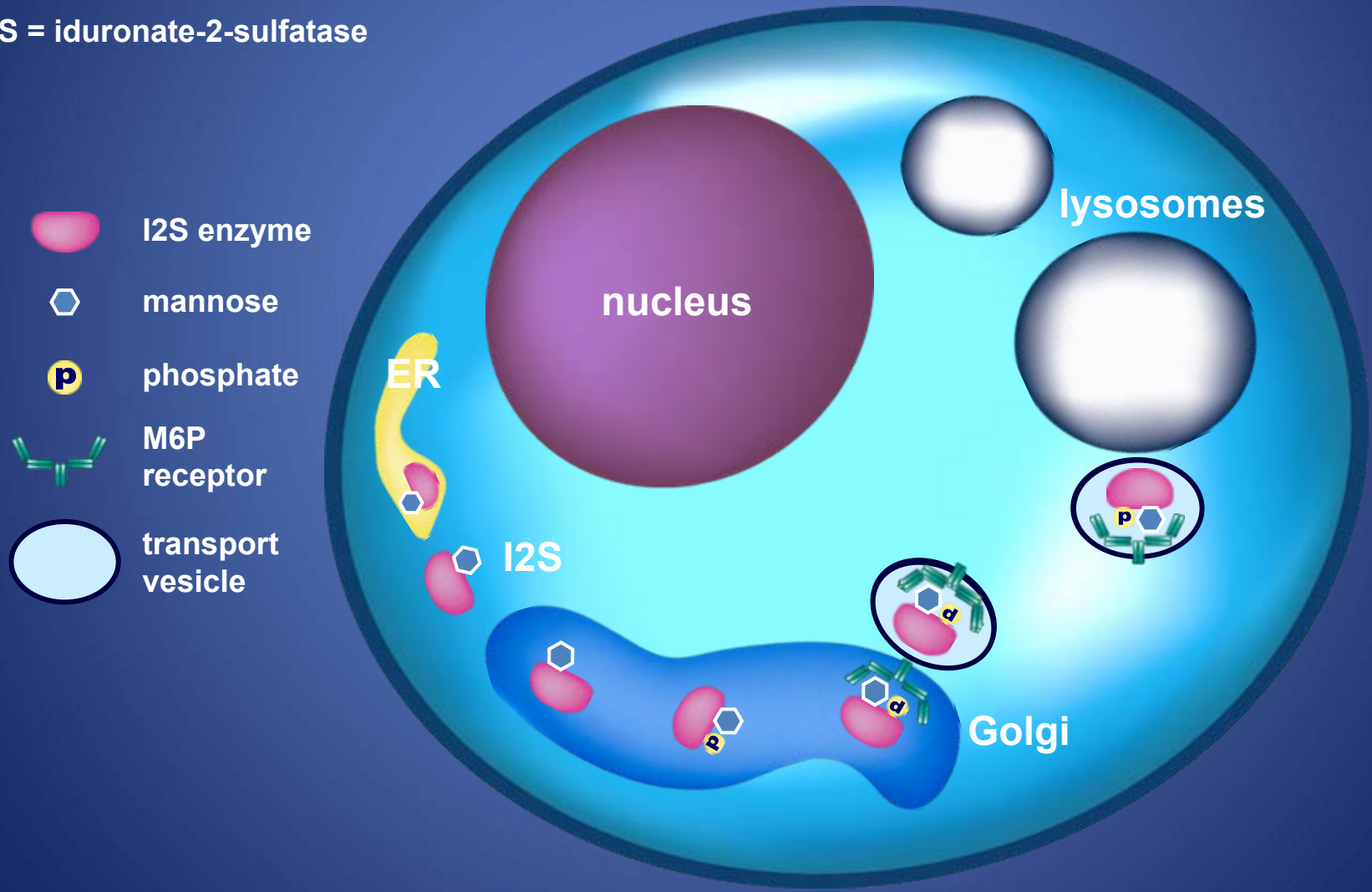


What is Elaprase?

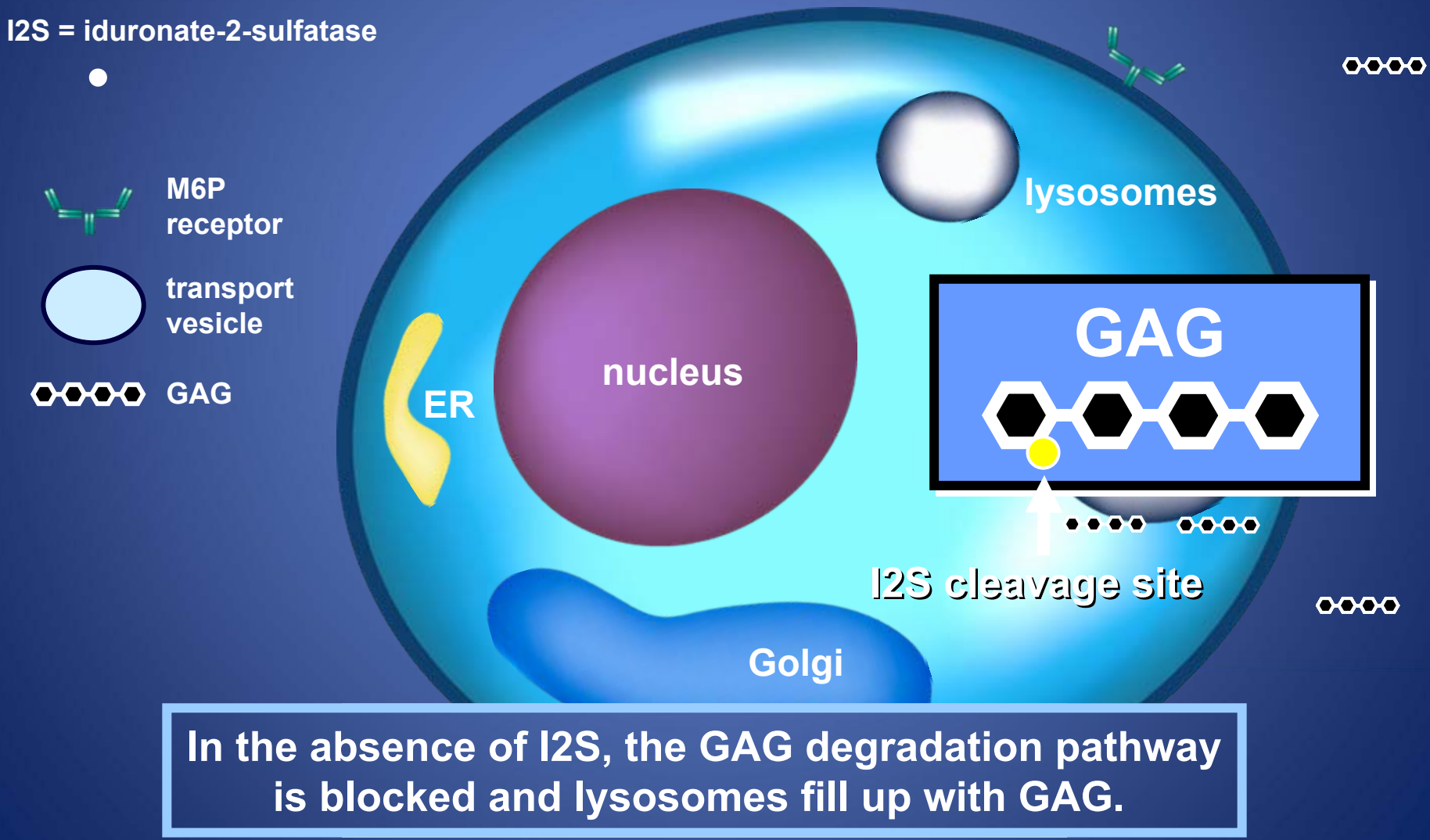
Human iduronate-2-sulfatase produced by genetic engineering technology an ERT which in clinical studies has been shown to either stop or ameliorate the clinical manifestations of the disease

Normal I2S Cellular Processing

I2S = iduronate-2-sulfatase

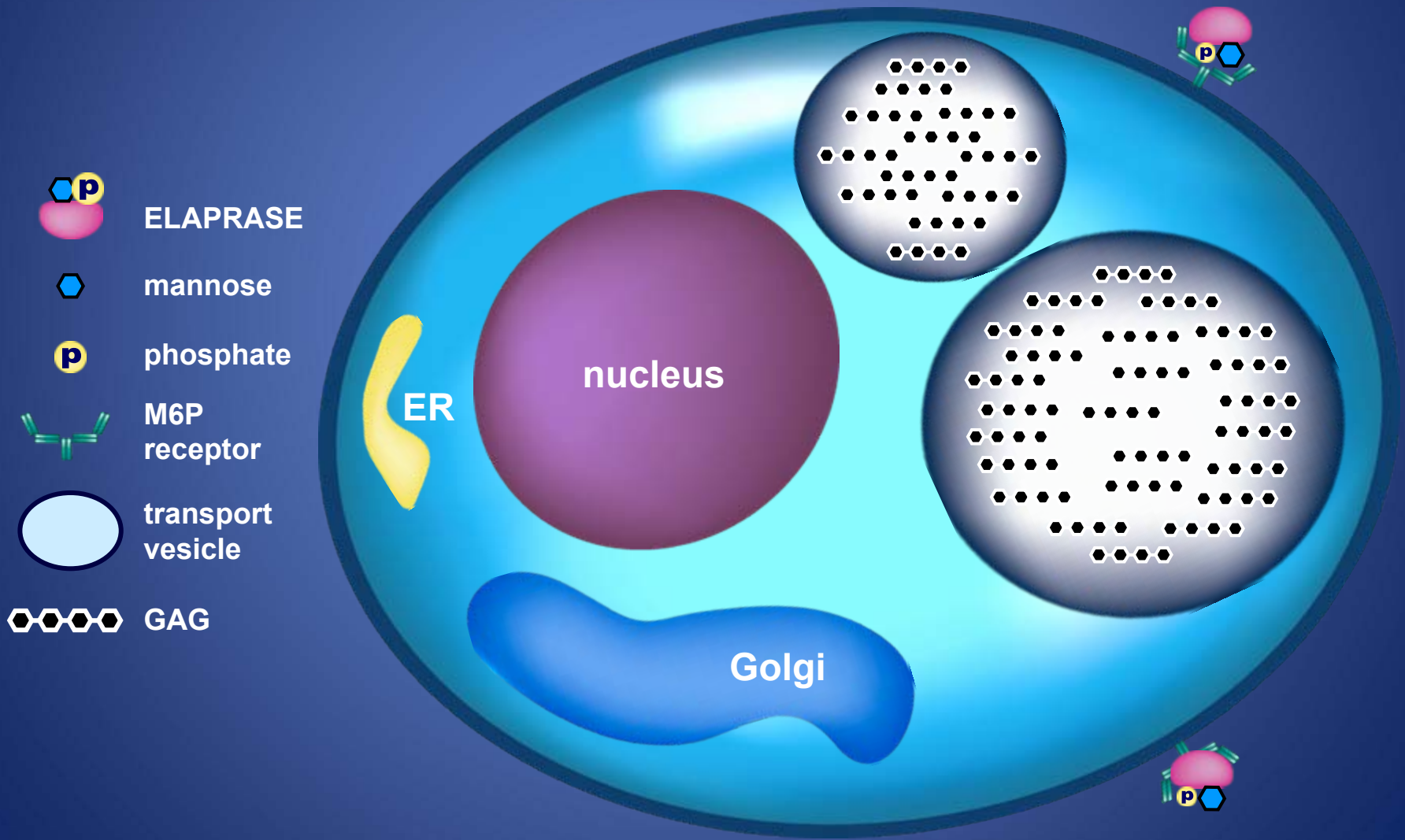


GAG Accumulation



In the absence of I2S, the GAG degradation pathway is blocked and lysosomes fill up with GAG.

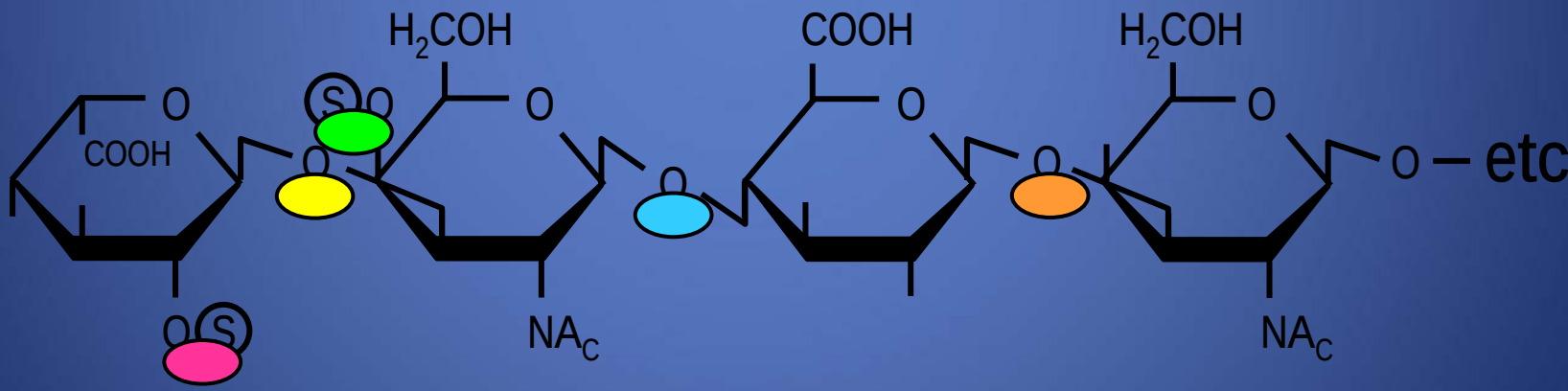
Mechanism of Action of ELAPRASE™ (idursulfase)



Degradation of dermatan sulfate[1]

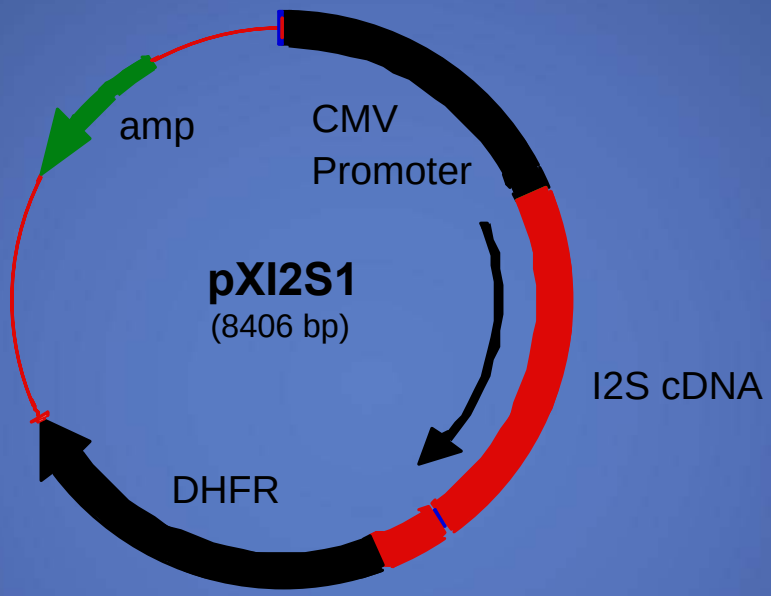
- **I2S enzyme**
- **α-L-iduronidase**
- **N-acetylgalactosamine 4-sulfatase**
- **β-hexosaminidase A, B, S**
- **β-glucuronidase**

I2S = iduronate-2-sulfatase
NAc = N-acetylgalactosamine



[1]Neufeld & Muenzer. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3421.

Engineering a Human Cell Line for Manufacturing Recombinant Human Iduronate-2-Sulfatase (I2S)



- An I2S production cell line was created by transfection of human HT-1080 cells with the pXI2S1 plasmid using an electroporation technique.
- Cell lines expressing I2S were isolated by stepwise methotrexate (MTX) selection (DHFR gene) and cloning.
- From this process a clonal cell line was isolated, expanded in culture and further passaged to create cell banks suitable for manufacturing I2S

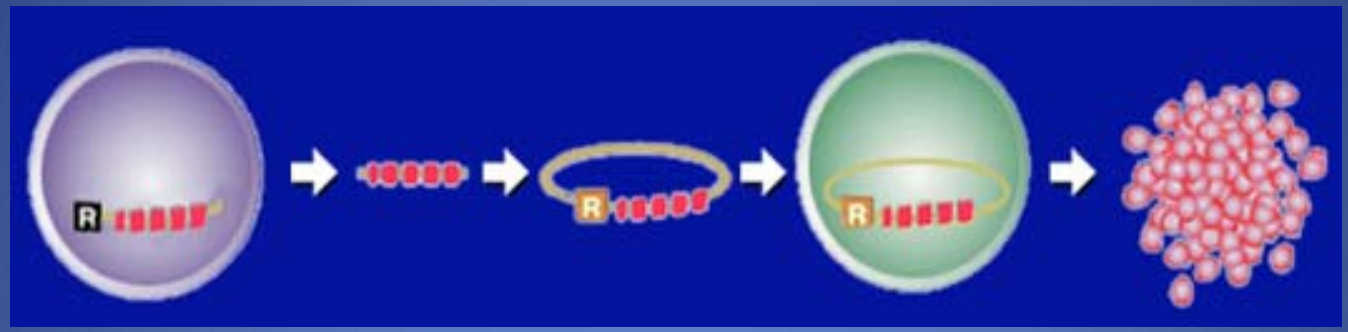
Manufacturing: From concept to realization

- Cell line development
- Cell fermentation
- Down stream processing
- Product release and documentation

A bioprocess in a cell development room

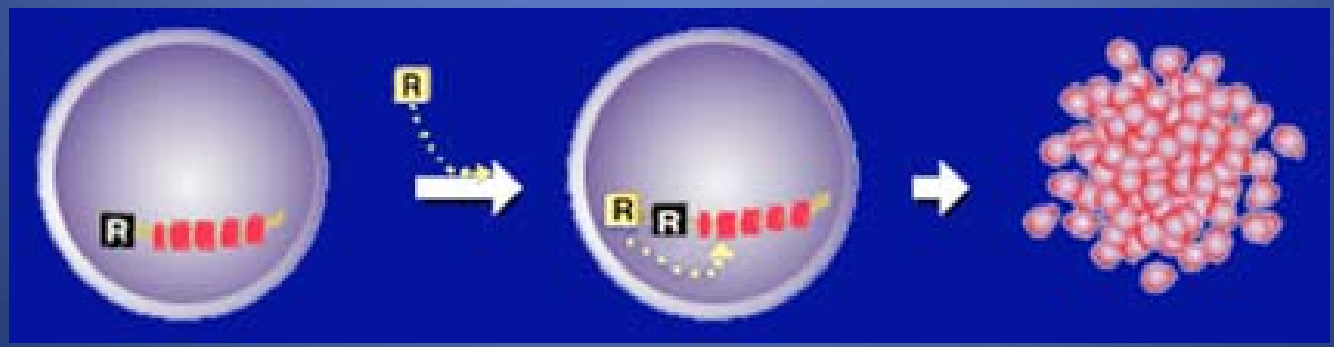
Protein Production Technology

Traditional Approach



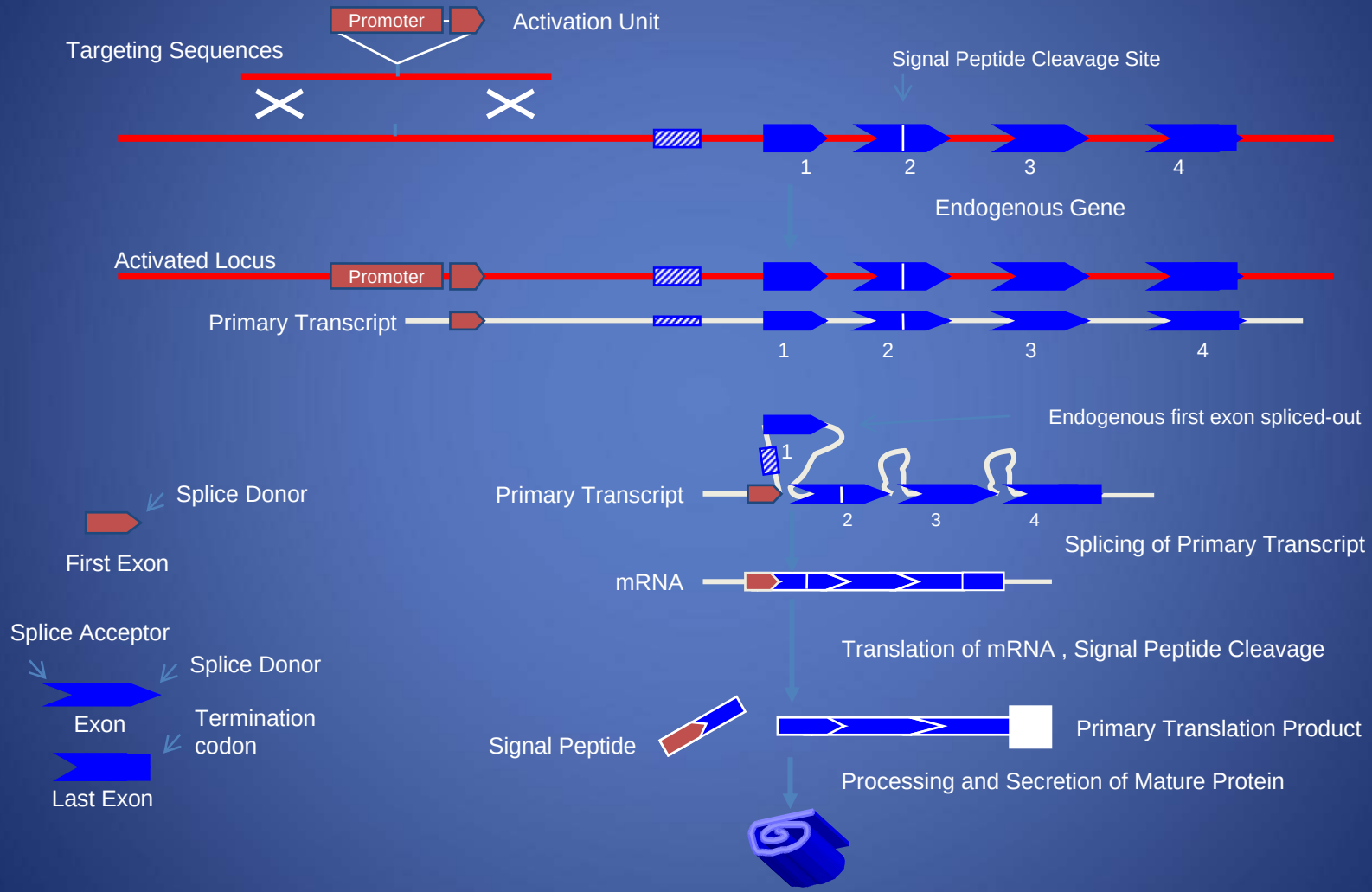
Human Cell Hamster cell

Shire novel method



Human Cell Human Cell

Gene Activation : Detailed View

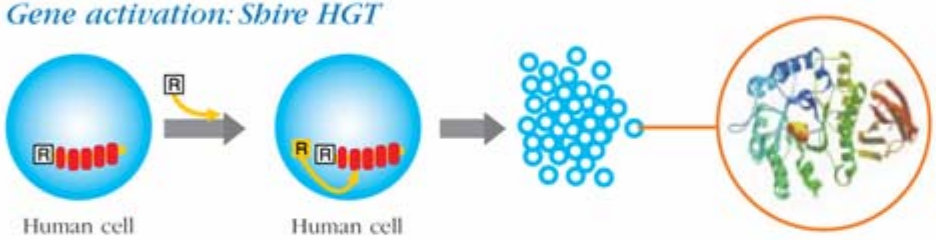


Agalsidase alfa:

Human cell-derived Enzyme Replacement Therapy

- Produced by unique gene activation technology
- The only human cell-derived agalsidase

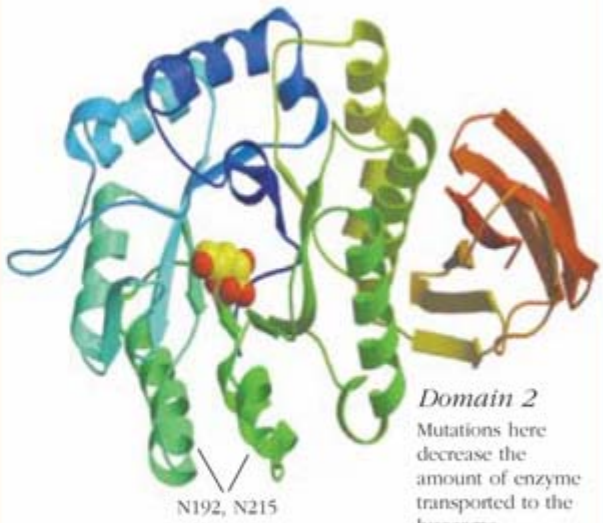
Gene activation: Shire HGT



Classical approach

R regulatory DNA sequences function as an on switch

Structure of human alpha-galactosidase[1]



Domain 2

Mutations here decrease the amount of enzyme transported to the lysosome

Domain 1

Most frequent mutations here are at N192 and N215; such mutations disrupt the targeting of the glycoprotein to the lysosome

1. Garman SC, Garboczi DN. *J Mol Biol* 2004; **337**: 319-35.

Human cell line *vs.* CHO production

Only human cells can provide truly human glycosylation - which is important for

- **Proper biodistribution and cellular targeting**
- **High specific activity of the protein**
- **Low immunogenicity. Non-human cells add on sugar moieties which act as antigenic epitopes.**

Importance of Glycosylation in ERT

- **Sialic acid residues**
  are essential for minimising hepatic uptake (via asiaglycoprotein receptors), resulting in enhanced distribution to target organs (kidney, heart)

- **Mannose-6-phosphate (M6P) residues**
  are essential for M6P-receptor mediated internalisation and lysosomal targeting

- **Human cells**
  produce an appropriate ratio of sialic acid to M6P residues

Importance of Fully Human Glycosylation for Safety

- Unlike non-human cell lines, human cells do not form antigenic sugar epitopes, thus minimising risk of immune mediated reactions

- Clinical safety profile is consistent with human glycosylation
  - low level IgG Ab titres which decreases with time
  - mild infusion reactions which decrease with time
  - no evidence of immune complex deposition
  - allows rapid infusion time
  - safe for home infusion therapy

Diagnosis

Enzyme determinations/molecular genetic diagnosis

- **Hunter (MPS II)**

  I2S activity in white blood cells or skin fibroblasts.

- **Fabry**

  Males: Alfa-galactosidase A in plasma, leukocytes or fibroblasts

  Females: Very low levels confirm diagnosis. Often molecular genetic diagnosis is required

Diagnosis

Enzyme determinations/molecular genetic diagnosis

- **Mb. Gaucher**

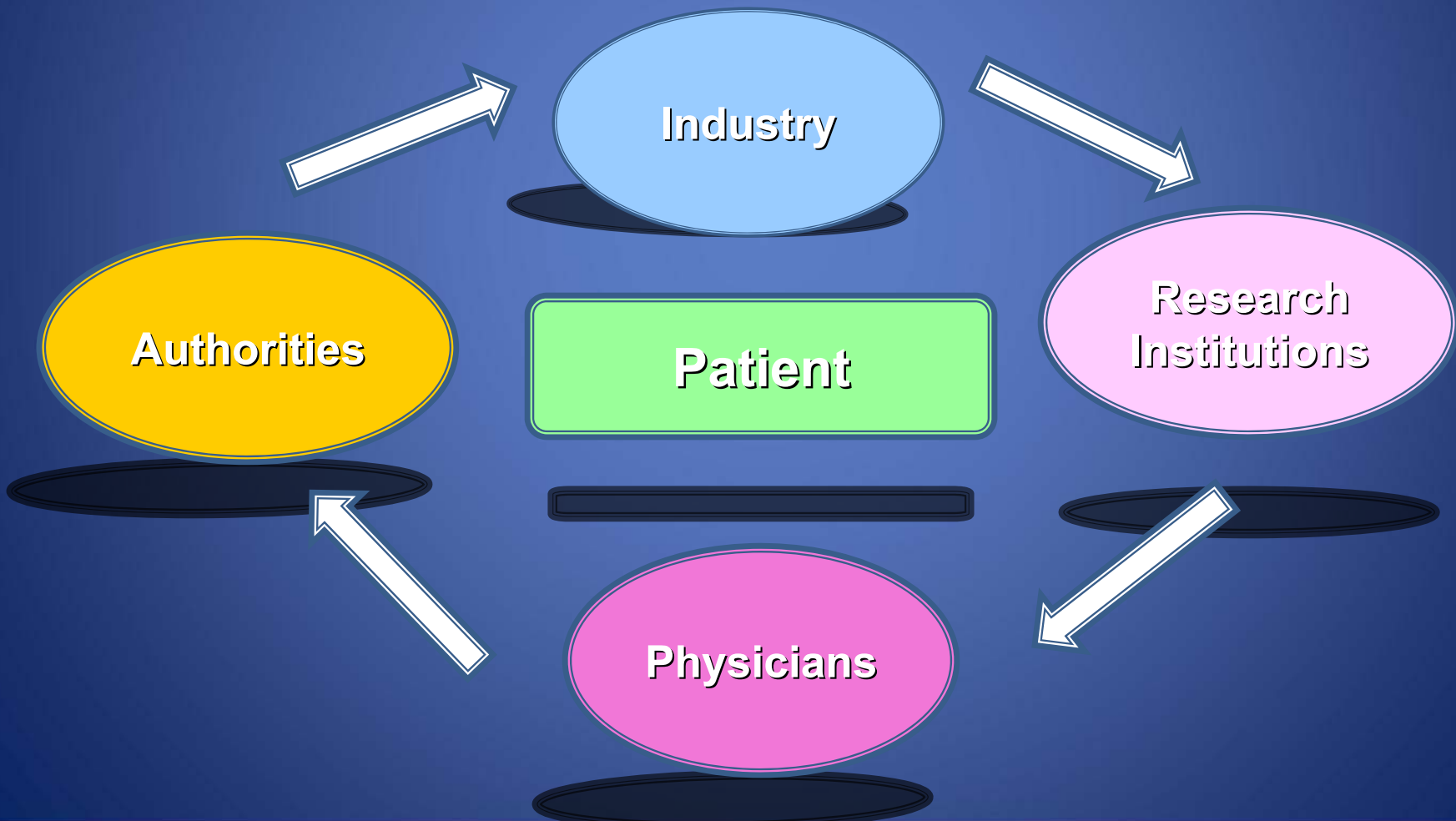
  Glucocerebrosidase in white blood cells or skin fibroblasts

  Molecular genetic diagnosis provides diagnostic verification

- **MPS I**

  Alfa-L-iduronidase in cell extracts of peripheral blood leukocytes or cultured skin fibroblasts

- Diagnosis for rare, debilitating, life-threatening diseases require the cooperation of all involved parties



In summary:

- Development of new methods for 2nd and 3rd generation of Biotechnology products
- Society drives and regulates technologies
- Invest in technologies tailored to help countries building capacity in communities, through education,training etc.
- Envision a future in which Biotechnology harnessed responsibility to help all nations and all people