UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Principles of Molecular Disease:

Lessons from the Hemoglobinopathies

Postgraduate course Human Genetics
2/12/2021

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The effect of mutations on protein function

- mutations resulting in a **LOSS** OF FUNCTION of the protein
- mutations resulting in a **GAIN** OF FUNCTION of the protein
- mutations resulting in a **NOVEL PROPERTY** by the protein
- mutations resulting in gene EXPRESSION at the **wrong time or place**

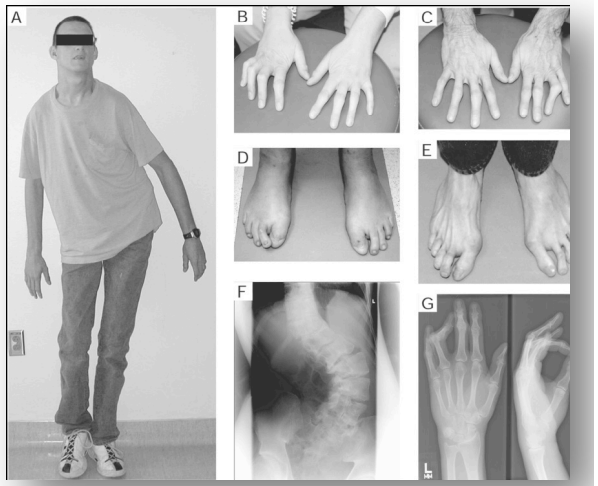
LOSS-OF-FUNCTION MUTATIONS

- *deletion* of the entire gene (and eventually also contiguous genes)
  examples: microdeletion syndromes, monosomies (Turner), α-thalassemias

- *chromosomal rearrangements*

- *premature stop* codon (nonsense or frameshift mutations)

- missense mutations may *abolish protein function*
  e.g. Catshl syndrome: loss- of – function FGFR3



FGFR3
p.R621H

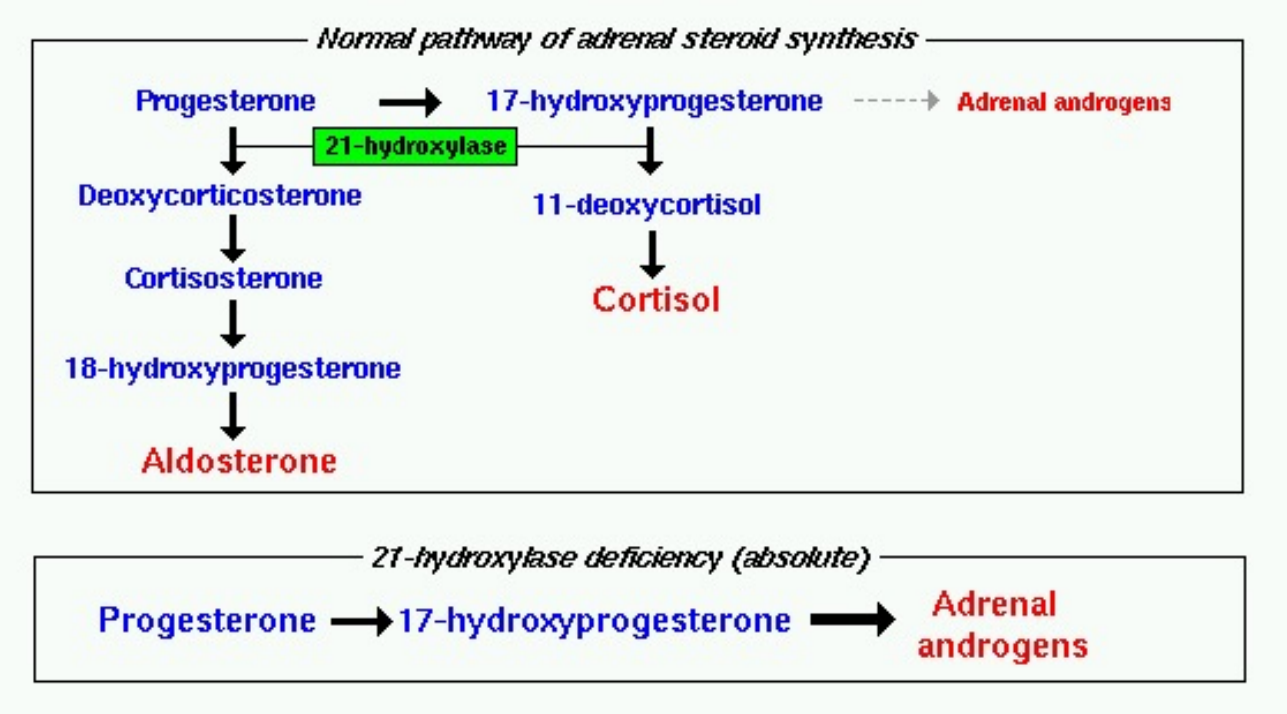
Severity of disease ~ **amount** of function lost

LOSS-OF-FUNCTION MUTATIONS

- missense mutations

Severity of disease ~ **amount** of function lost

E.g. Congenital adrenal hyperplasia



LOSS-OF-FUNCTION MUTATIONS
- missense mutations

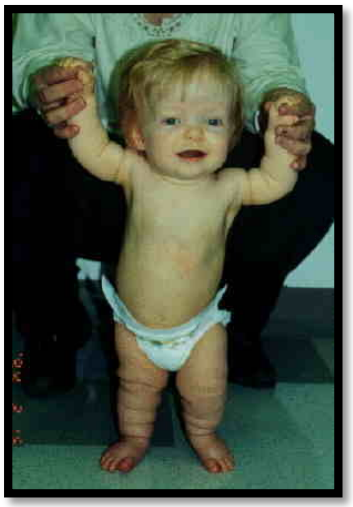
Severity of disease ~ **amount** of function lost

E.g. Congenital adrenal hyperplasia

Enzyme Activity	Phenotype	*CYP21A2* Mutation
0%	Severe (classic)	Whole-gene deletion (null mutation) Large gene conversion p.Gly111ValfsTer21 p.[Ile237Asn;Val238Glu;Met240Lys] p.Leu308PhefsTer6 p.Gln319Ter p.Arg357Trp
Minimal residual activity (<1%)		c.293-13A>G or c.293C>G
2%-11%		p.Ile173Asn
~20%-50%	Mild (non-classic)	p.Pro31Leu p.Val282Leu p.Pro454Ser

GAIN-OF-FUNCTION MUTATIONS

= mutations that enhance one or more of the normal functions of the protein



- mutations that enhance one normal function of the protein
  f.e.: the G380R mutation in
  FGFR3 causing achondroplasia

- mutations that increase the production of a normal protein
  in its normal environment
  f.e.: trisomy 21 (Down syndrome) *note: Alzheimer*
  duplication of *PMP22* in Charcot-Marie-Tooth disease type 1A
  chromosomal duplications in cancer

NOVEL PROPERTY MUTATIONS

= (missense) mutations ➔ novel property of the protein +/- normal function

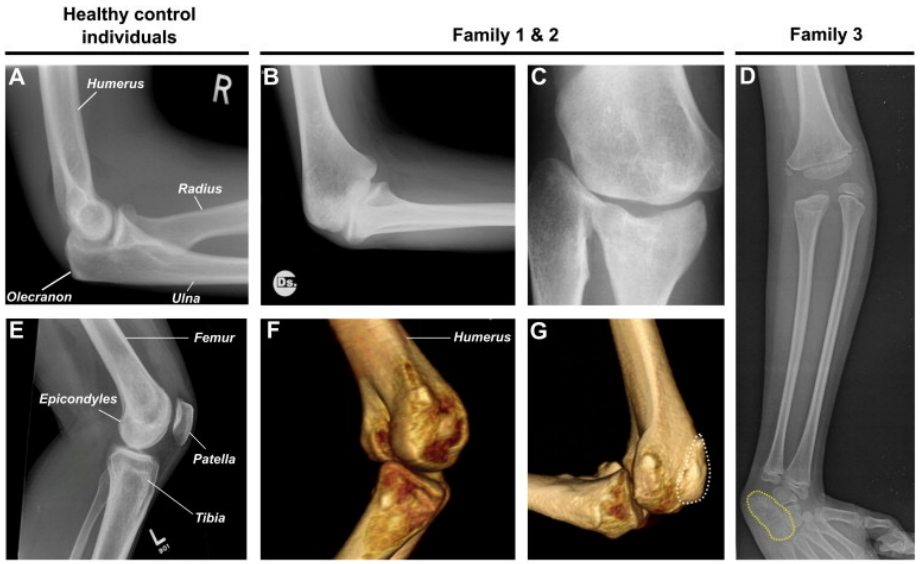
infrequent (most AA substitutions either neutral or detrimental)

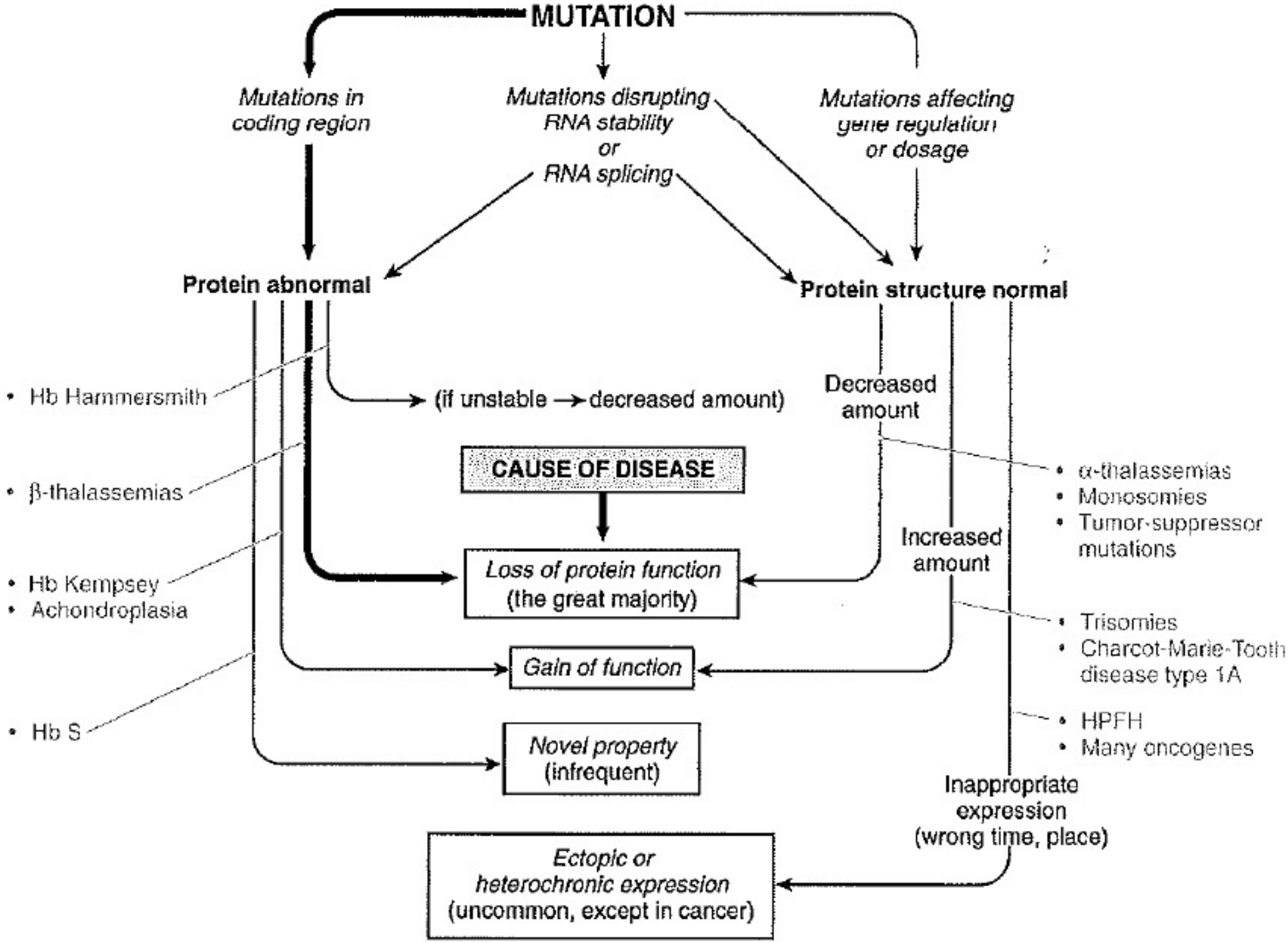
e.g. sickle cell disease

MUTATIONS ASSOCIATED WITH HETEROCHRONIC OR ECTOPIC GENE EXPRESSION

= mutations that alter the regulatory regions of a gene

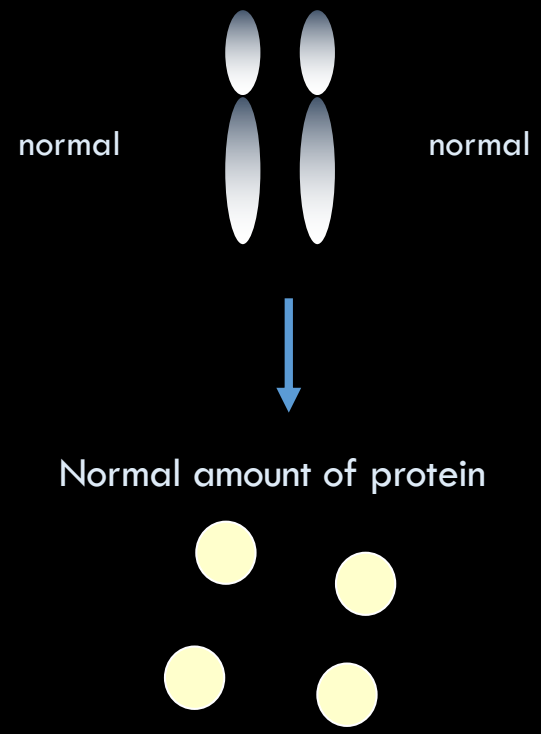
Examples:
- oncogene mutations in cancer
- hereditary persistence of HbF (continued expression of γ-globin)
- PITX1
  Liebenberg syndrome



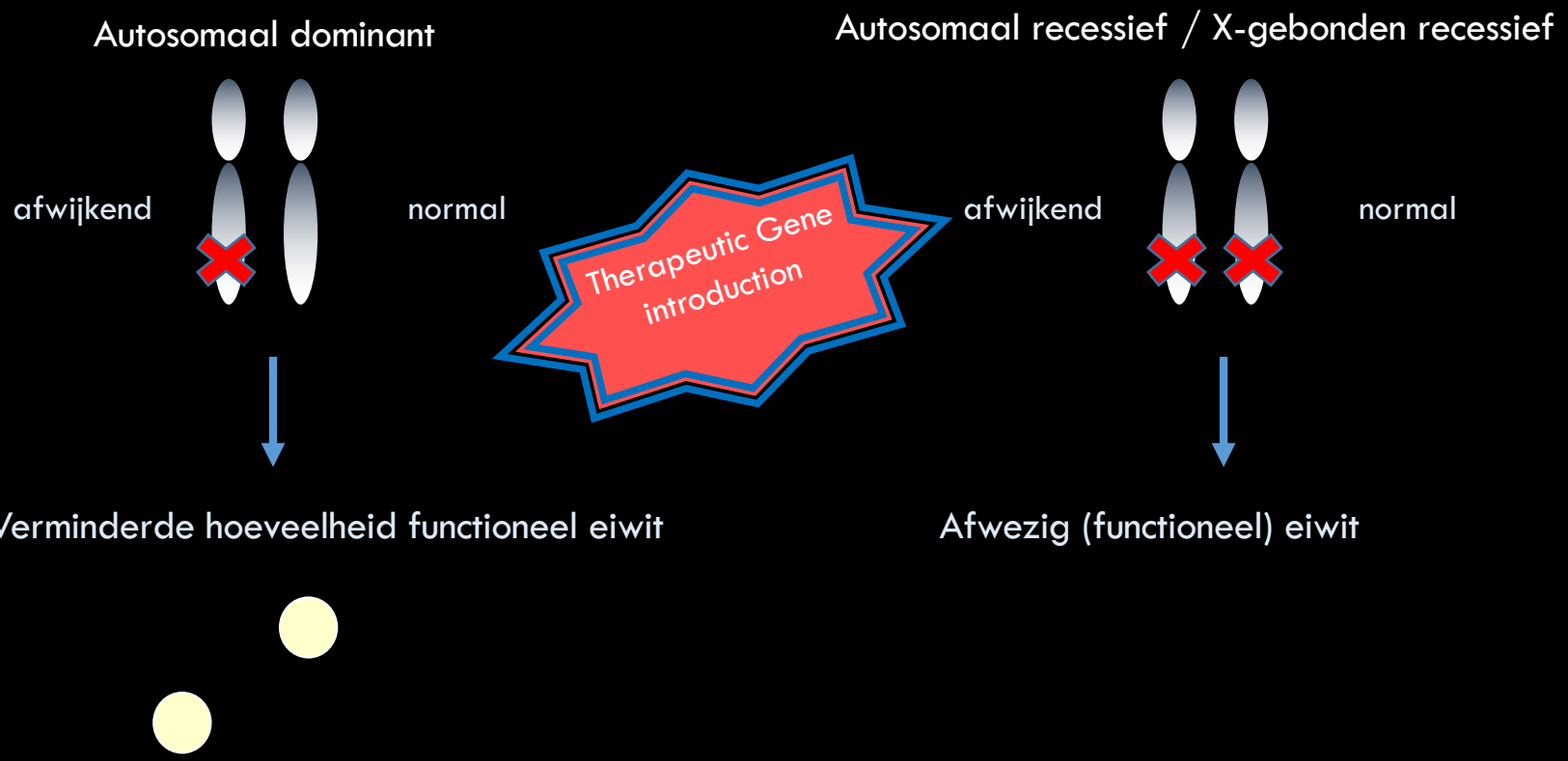

MUTATION
Mutations in coding region
Mutations disrupting RNA stability or RNA splicing
Mutations affecting gene regulation or dosage
Protein abnormal
Protein structure normal
• Hb Hammersmith
(if unstable → decreased amount)
Decreased amount
CAUSE OF DISEASE
• β-thalassemias
• α-thalassemias
• Monosomies
• Tumor-suppressor mutations
Increased amount
• Hb Kempsey
• Achondroplasia
Loss of protein function (the great majority)
• Trisomies
• Charcot-Marie-Tooth disease type 1A
Gain of function
• Hb S
• HPFH
• Many oncogenes
Novel property (infrequent)
Inappropriate expression (wrong time, place)
Ectopic or heterochronic expression (uncommon, except in cancer)

Mutations causing a shortage of gene product

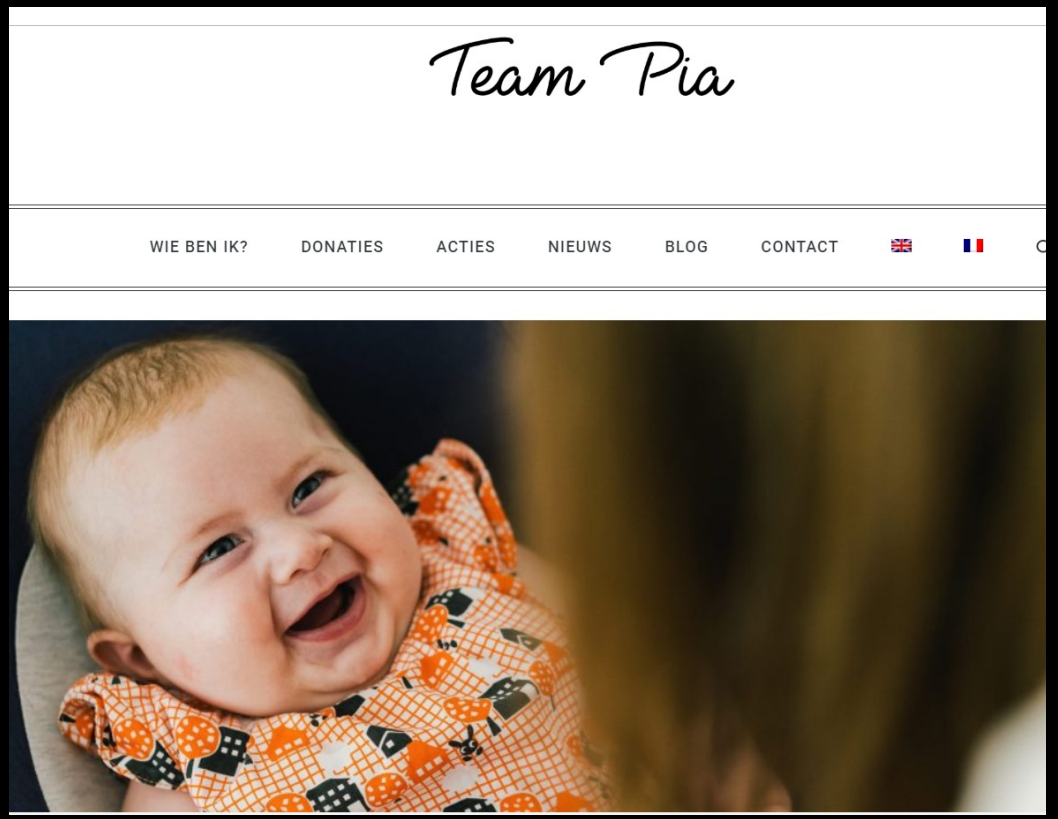
Normal



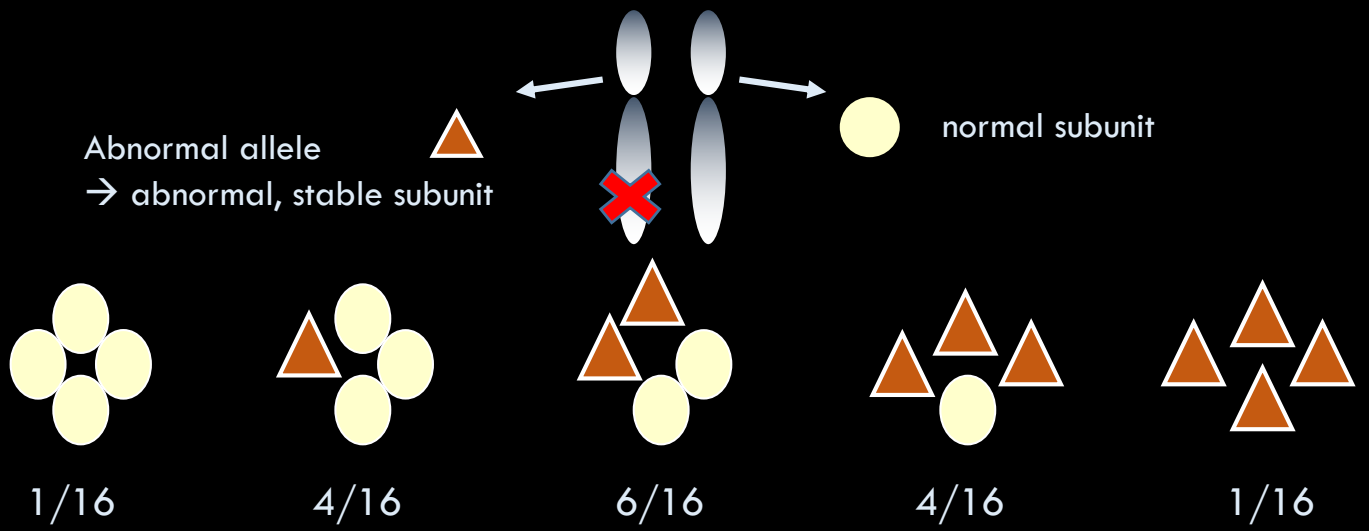
Gendefecten die leiden tot een eiwittekort



Gene introduction

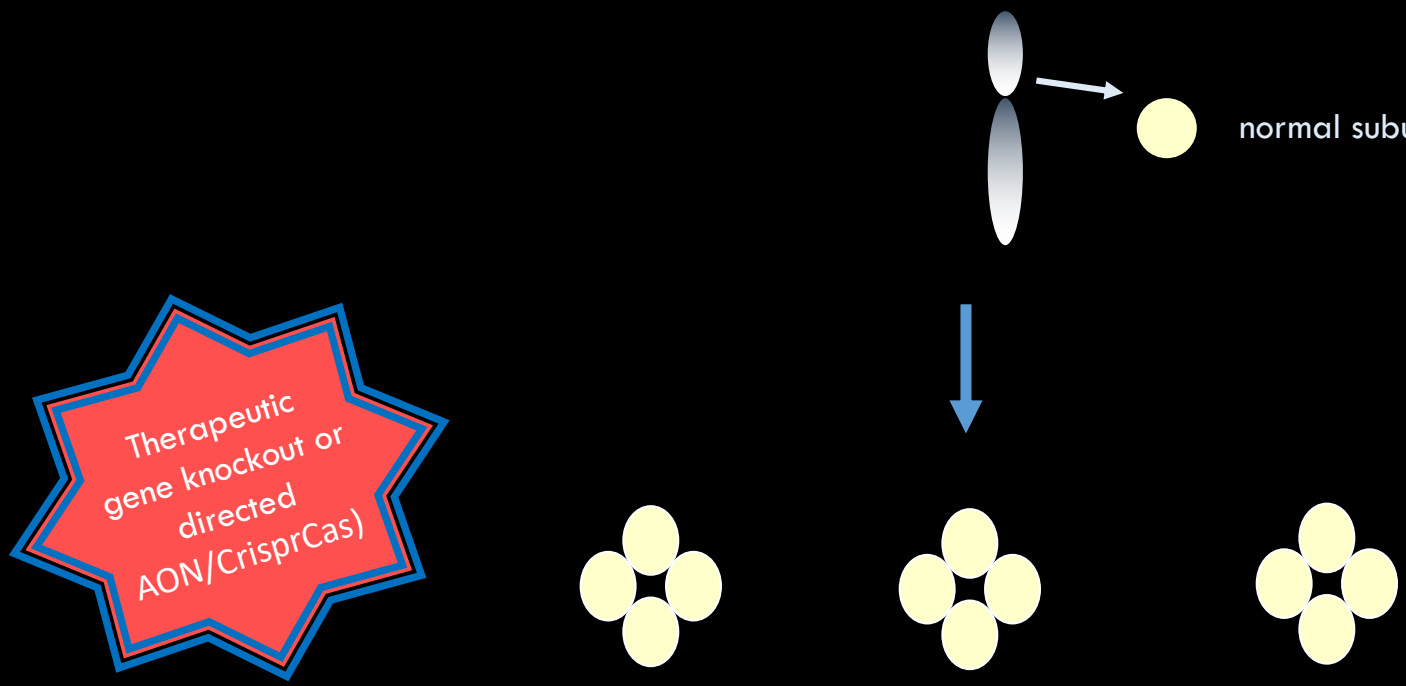


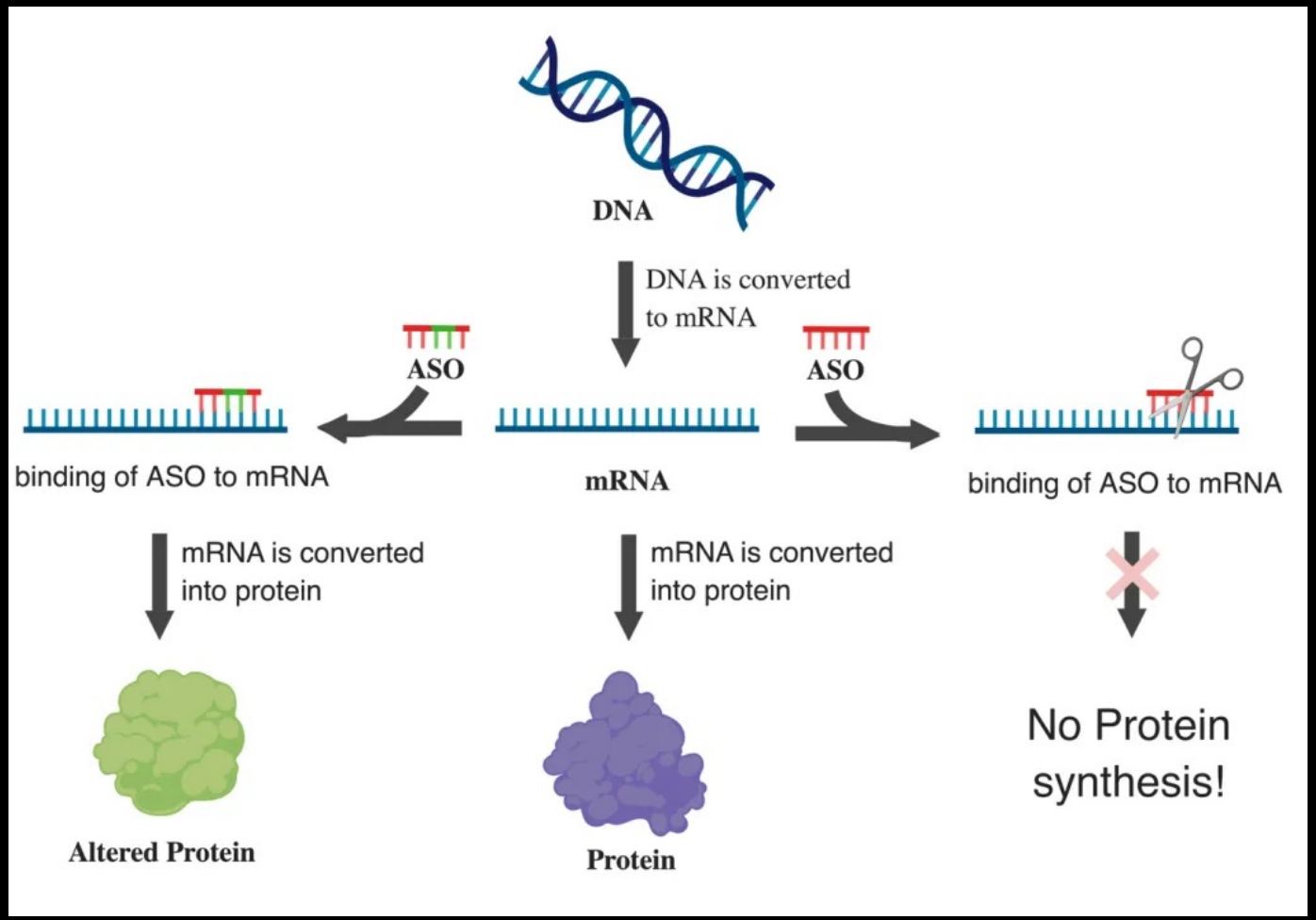
Mutant proteins that is stable and forms multimers: dominant negative effect



Only 1/16 of complexes are functional

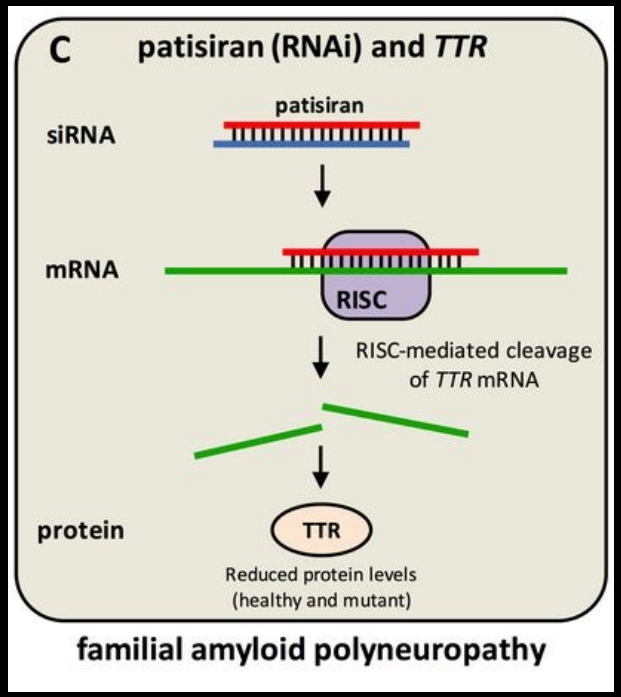
Mutant proteins that is stable and forms multimers: dominant negative effect



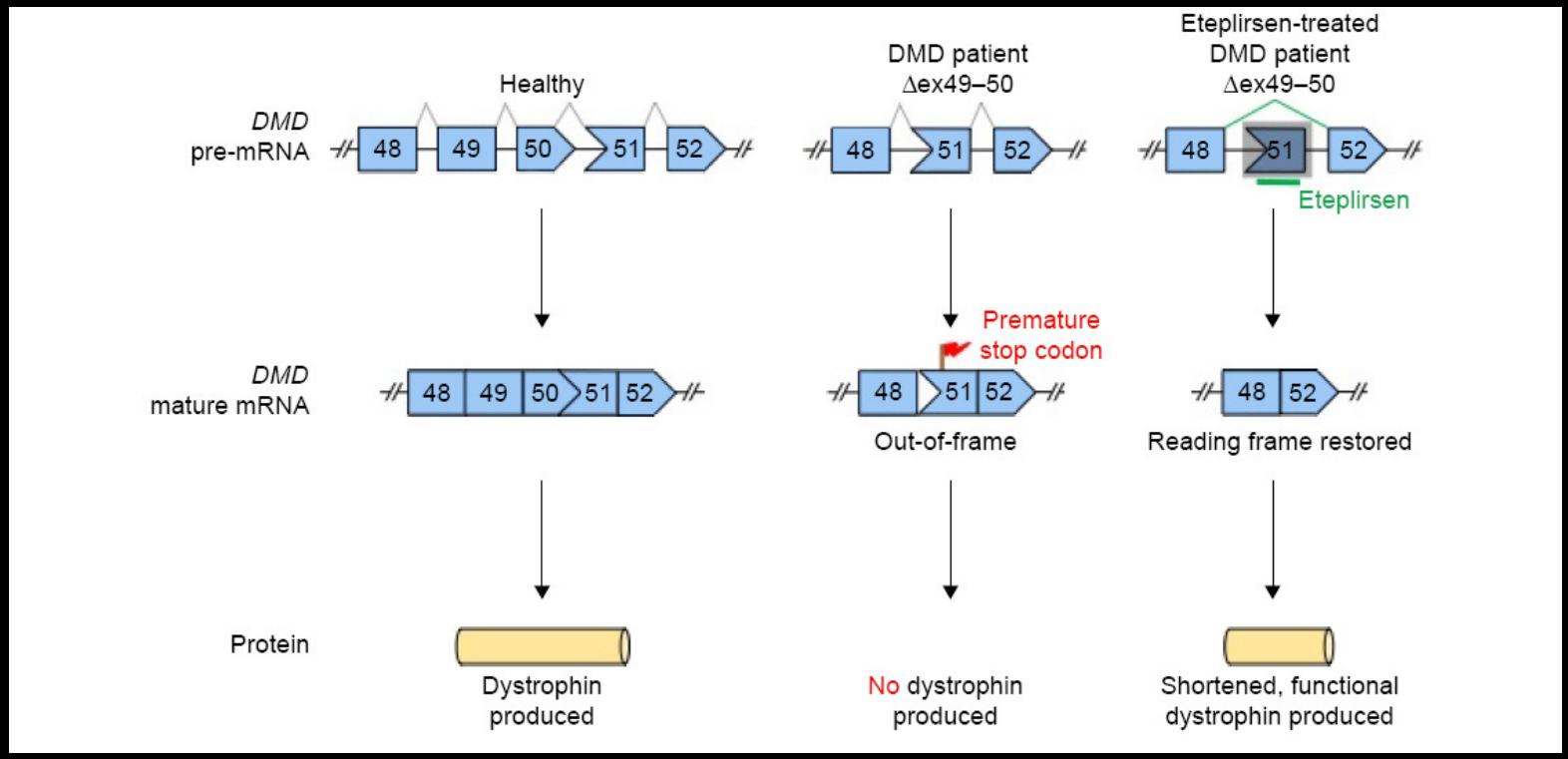

DNA
DNA is converted to mRNA
ASO
ASO
binding of ASO to mRNA
mRNA
binding of ASO to mRNA
mRNA is converted into protein
mRNA is converted into protein
No Protein synthesis!
Altered Protein
Protein

L. Nitschke

Antisense oligonucleotides – Familial Amyoidosis



Nieuwe doorbraken: Antisense oligonucleotiden - Duchenne



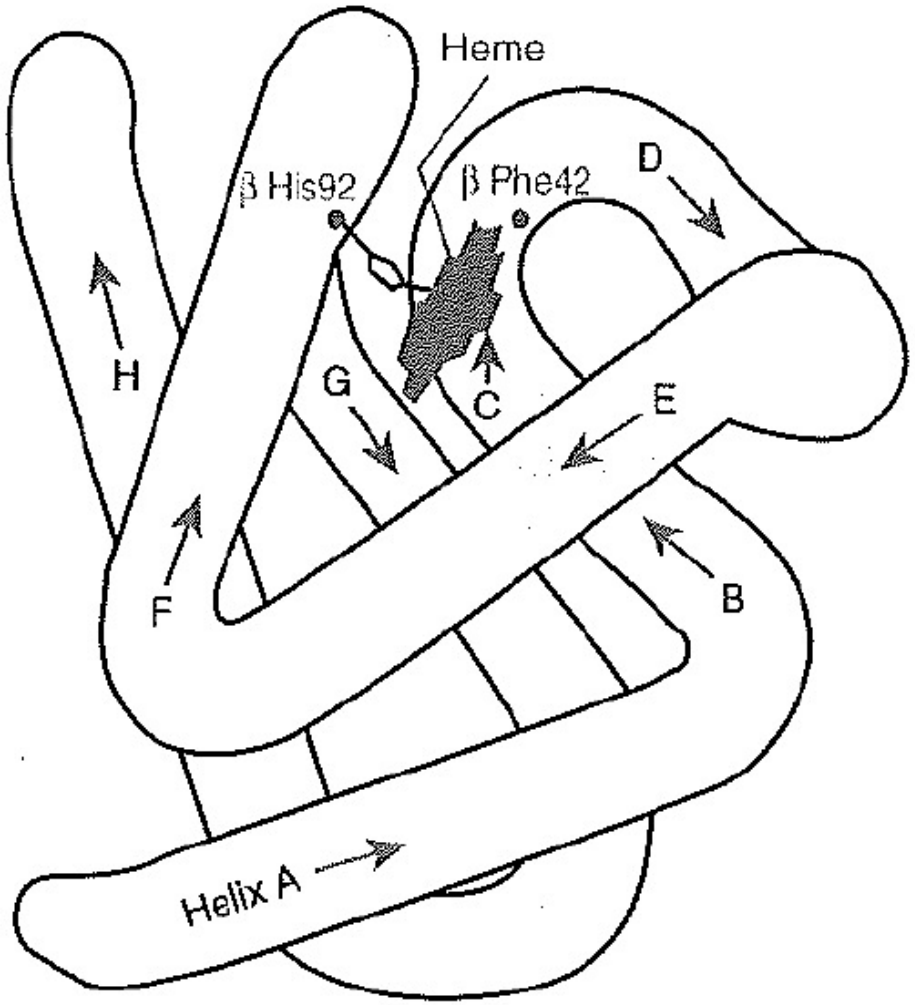
K. Lim et al, 2017

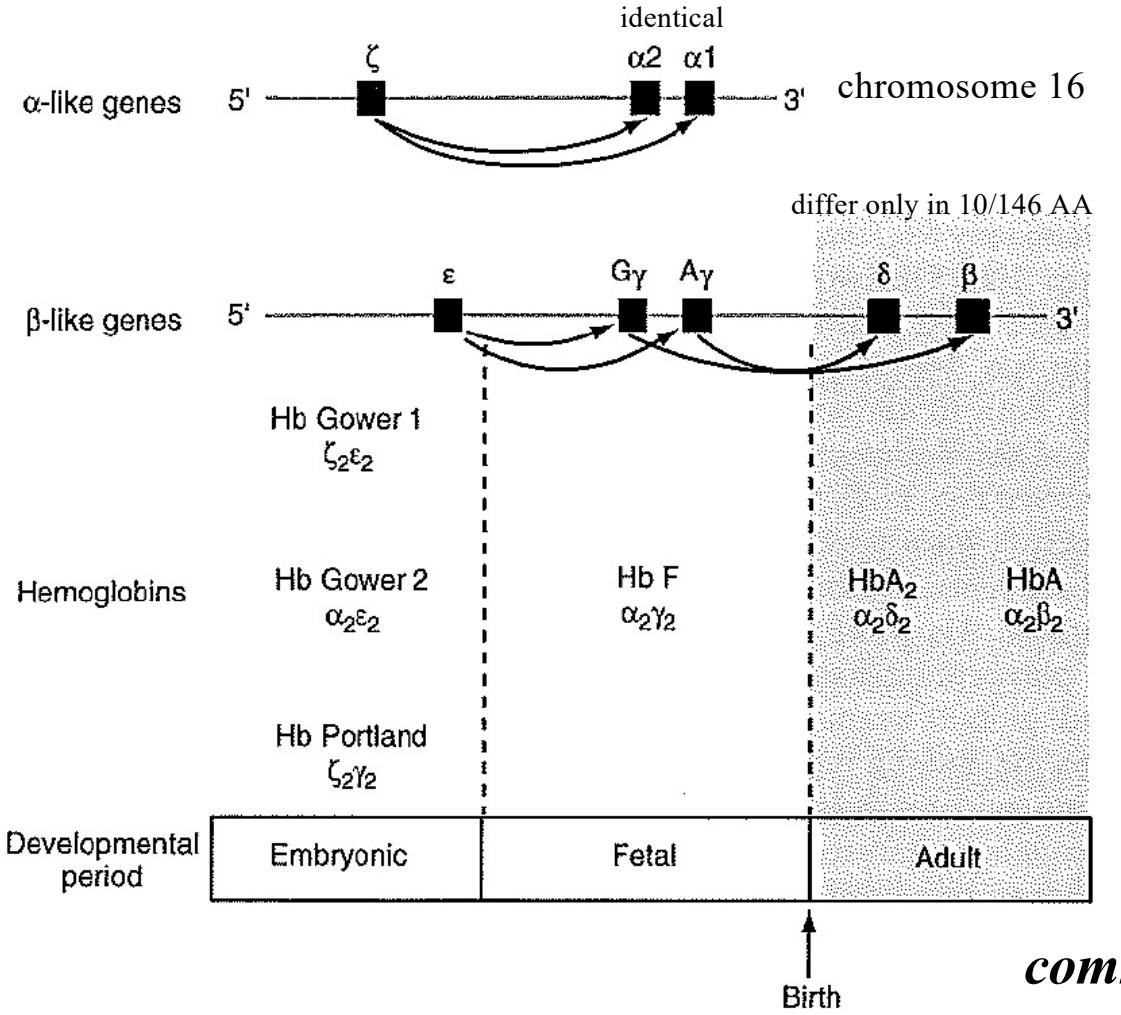
Hemoglobinopathies

- most **common** single-gene disorders in humans
- more than 5% of the world's population is carrier of an abnormal globin gene

Hemoglobin

- 4 subunits: 2 α (like) and 2 β (like) - chains
- each subunit is composed of :
  - a polypeptide chain (*globin*)
  - a prosthetic group (*heme*): iron-containing pigment that combines with O_2
- highly conserved structure
- Hb A (adult hemoglobin): α_2 β_2


Heme
β His92
β Phe42
D
H
G
C
E
F
B
Helix A

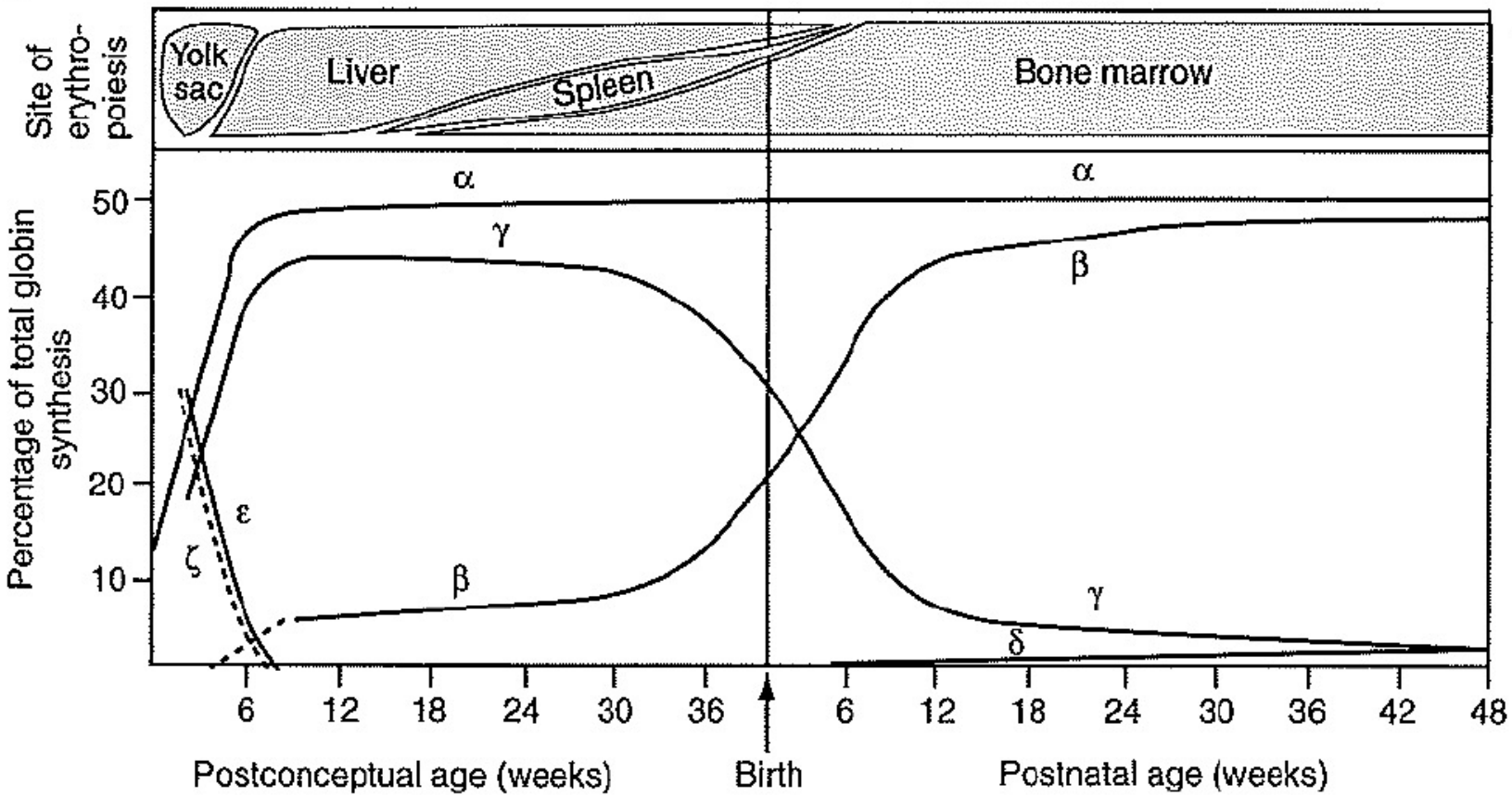


In adult life:

>97% HbA
2% HbA_2
<1% HbF

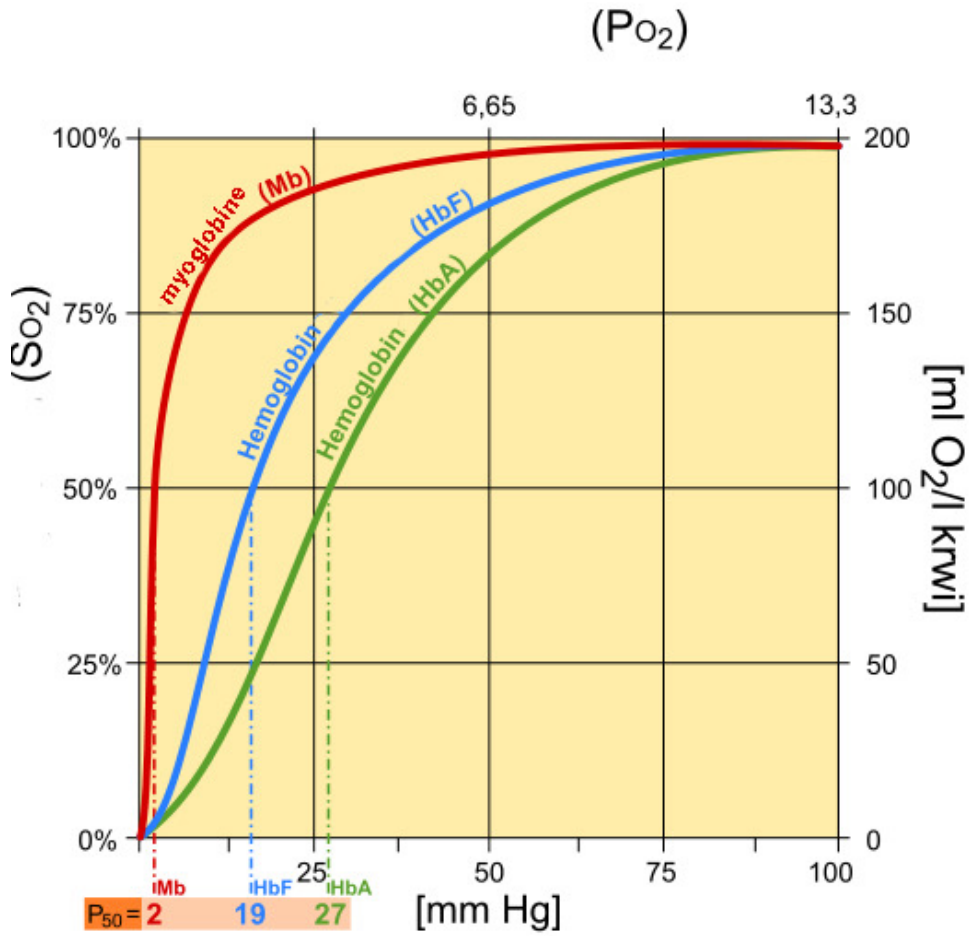
common ancestral gene

Globin switching

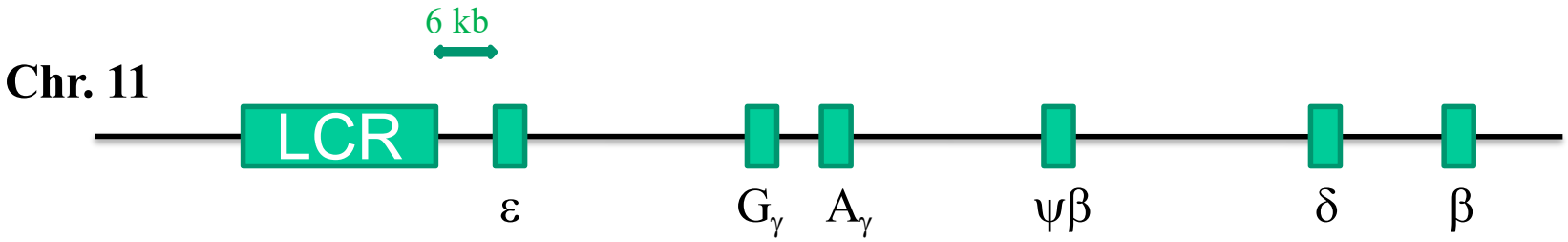


Globin switching

WHY?



B Globin cluster



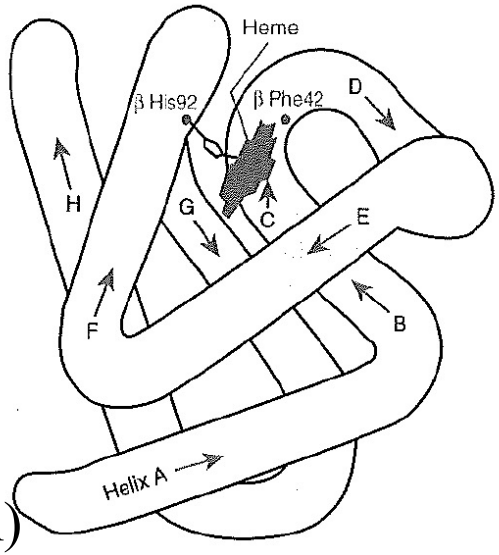
- expression of β-globin gene controlled by **nearby promoter** and **LCR**
- locus control region (LCR): required for the expression of any gene in the β-globin cluster
- deletions of LCR results in εγδβ- thalassemia

MUTATIONS AFFECTING THE GLOBIN CHAINS

1. Mutations that alter the **structure** of the globin protein
2. Reduced **availability** of one or more globin chains (Thalassemias)
3. mutations that impair the globin developmental **switching**

1. STRUCTURAL VARIANTS

- usually due to **point mutations** in one of the globin genes
- more than 400 abnormal hemoglobin variants have been described
- only about 50% are clinically significant
- three classes:
  - mutants that cause *hemolytic* anemia
  - mutants that alter *oxygen transport*
  - mutants that reduce the *abundance* of the globin chain (thalassemias)



Structural Variants that cause Hemolytic Anemia

- the mutant makes the Hb tetramer **unstable**

  → *loss-of-function*

  e.g.: Hb Hammersmith (β-chain Phe42Ser mutation)

- the mutant gives the globin chain an unusual **rigid** structure

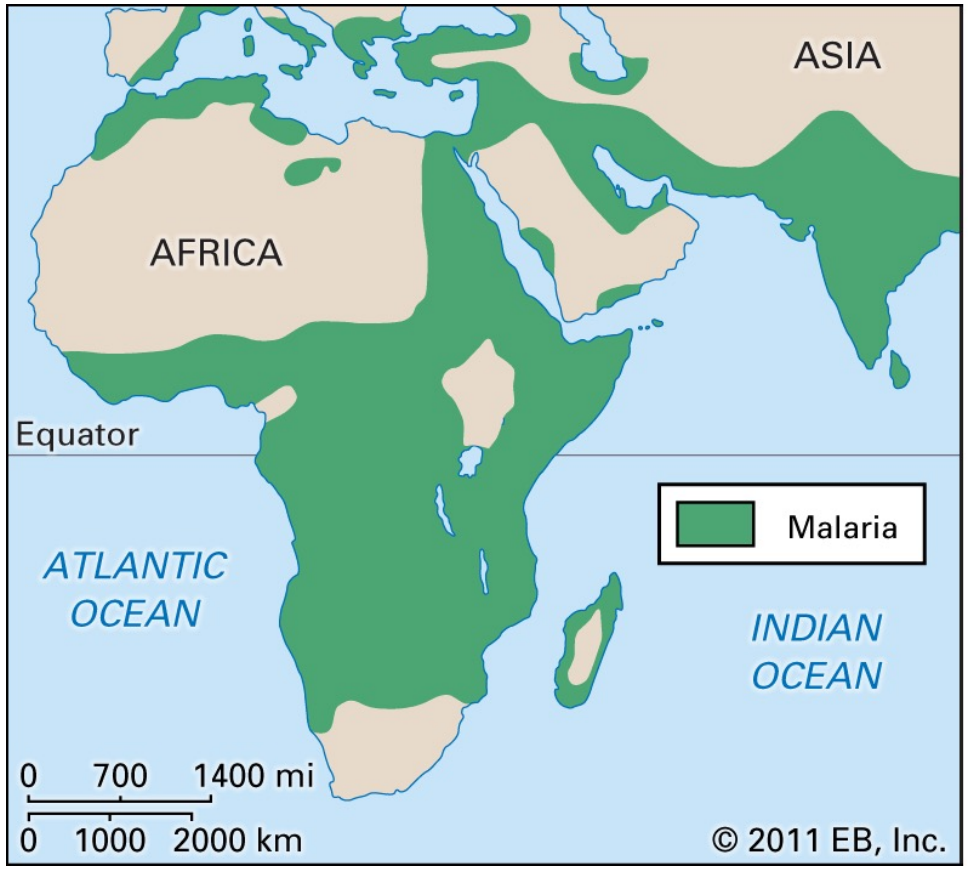
  novel property mutations

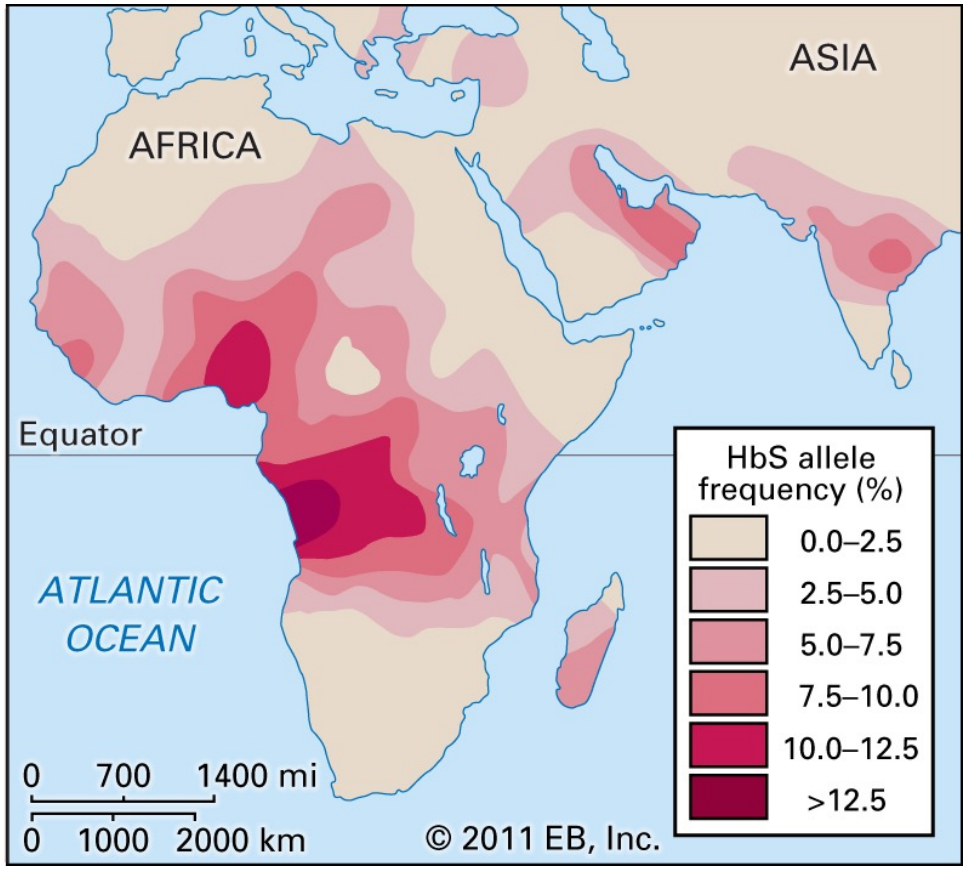
  f.e.: sickle cell globin; HbC

Sickle Cell Disease

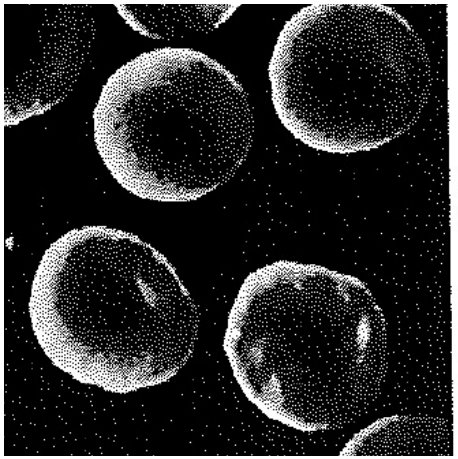
- HbS: first abnormal Hb detected (Glu6Val mutation in β-chain)
- severe **AR** condition
- common in equatorial Africa; 1/600 African Americans is born with the disease

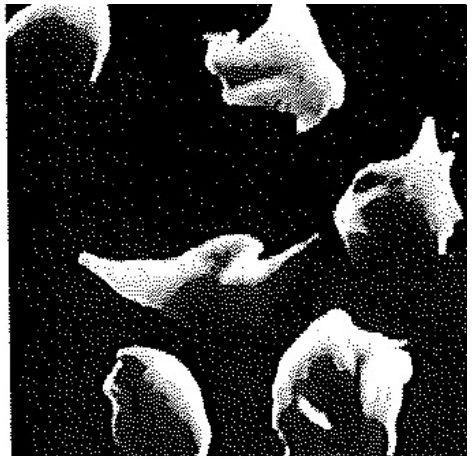
- sickle cell **trait** refers to the heterozygous state
- about 8% of African Americans are heterozygous
- heterozygotes are protected against *malaria*


ASIA
AFRICA
Equator
ATLANTIC OCEAN
INDIAN OCEAN
Malaria
0 700 1400 mi
0 1000 2000 km
© 2011 EB, Inc.


ASIA
AFRICA
Equator
ATLANTIC OCEAN
HbS allele frequency (%)
0.0–2.5
2.5–5.0
5.0–7.5
7.5–10.0
10.0–12.5
>12.5
0 700 1400 mi
0 1000 2000 km
© 2011 EB, Inc.

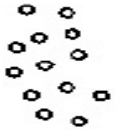
Sickle Cell Disease





Hb S solution

Hb S fiber



Oxy

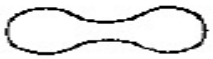
Deoxy

Normal codon — *Sickle cell codon*

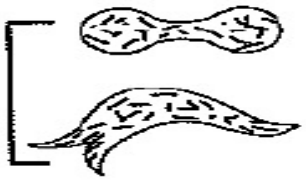
GAG → GTG

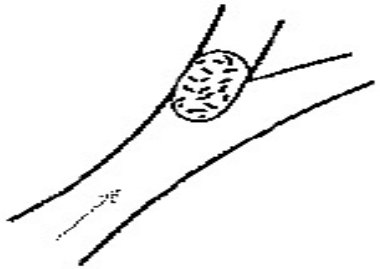
β6 Glu → Val → Hb S

Amino acid substitution



Cell heterogeneity





Vaso-occlusion

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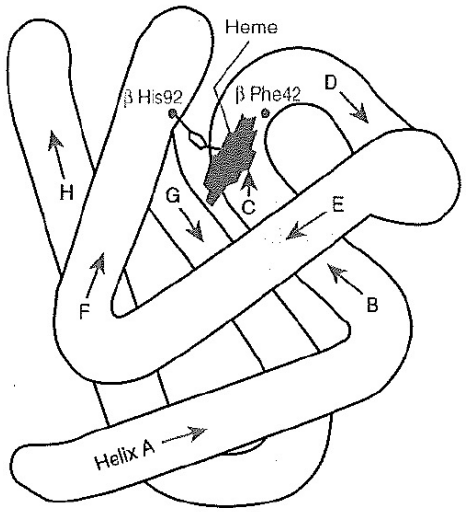
- three classes:
  - mutants that cause hemolytic anemia
  - mutants that alter oxygen transport
  - mutants that reduce the abundance of the globin chain (thalassemias)

Hemoglobin Structural Variants that alter oxygen transport

• Hb Hyde Park (β-chain His92Tyr)
~ normal hemoglobin stability
iron resistant to the enzyme methemoglobin reductase (Fe^{3+} (not able to bind O_2) → Fe^{2+}).
➔ accumulation of methemoglobin → cyanosis (usually asymptomatic)
homozygous state presumably lethal.

• Hb Hammersmith (β chainPhe 42 Ser)
➔ instable Hb, lower O2 affinity
• mutations in α:β interface (Hb Kempsey)
prevent oxigen related movement
➔ locked in high O2 affinity state
➔ Polycythemia



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    mutations in the coding region

    rate of synthesis↓

    severe instability of the chains.

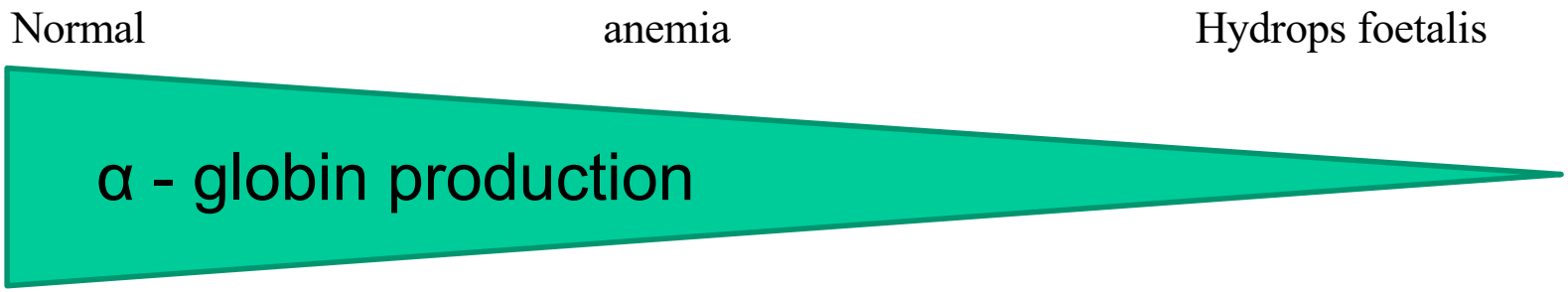
2. HEMOGLOBIN SYNTHESIS DISORDERS (THALASSEMIAS)

- collectively the most common human single-gene disorders!
- carriers: protective against malaria
- > θαλασσα (sea): first discovered in Mediterranean area

- **imbalance in α : β chain ratio**
  - ↓synthesis
  - instability (cfr supra)
- ↑normal chains: damage to the RBCs (hemolytic anemia)
- ↓Hb synthesis → hypochromic, microcytic anemia

- Dd Iron deficiency

THE A - THALASSEMIAS

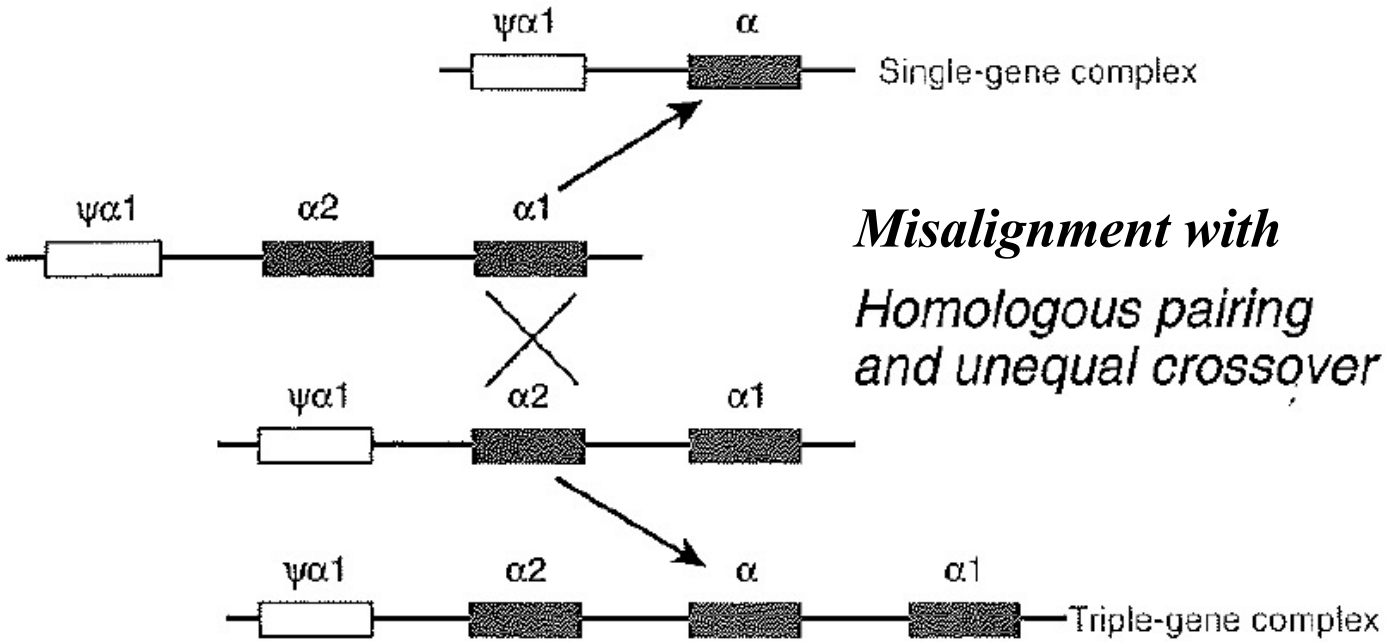


- affect the formation of both **fetal and adult** Hb
- in the absence of α–globins:
  - Hb Bart's: γ_4
  - Hb H: β_4

  } Homotetrameric Hb: ineffective oxygen carriers
  ➔ *Hydrops fetalis*

THE A - THALASSEMIAS

- most commonly due to deletion of the α–globin genes



THE A - THALASSEMIAS

clinical condition	number of functional α–genes	α–globin gene genotype	α–chain production
Normal	4	αα/αα	100%
Silent carrier	3	αα/α–	75%
α–thalassemia trait (mild anemia, microcytosis)	2	α–/α– or αα/– – *	50%
Hb H (β_4) disease (moderately severe hemolytic anemia)	1	α–/– –	25%
Hydrops fetalis (Hb Bart's γ_4)	0	– –/– –	0%

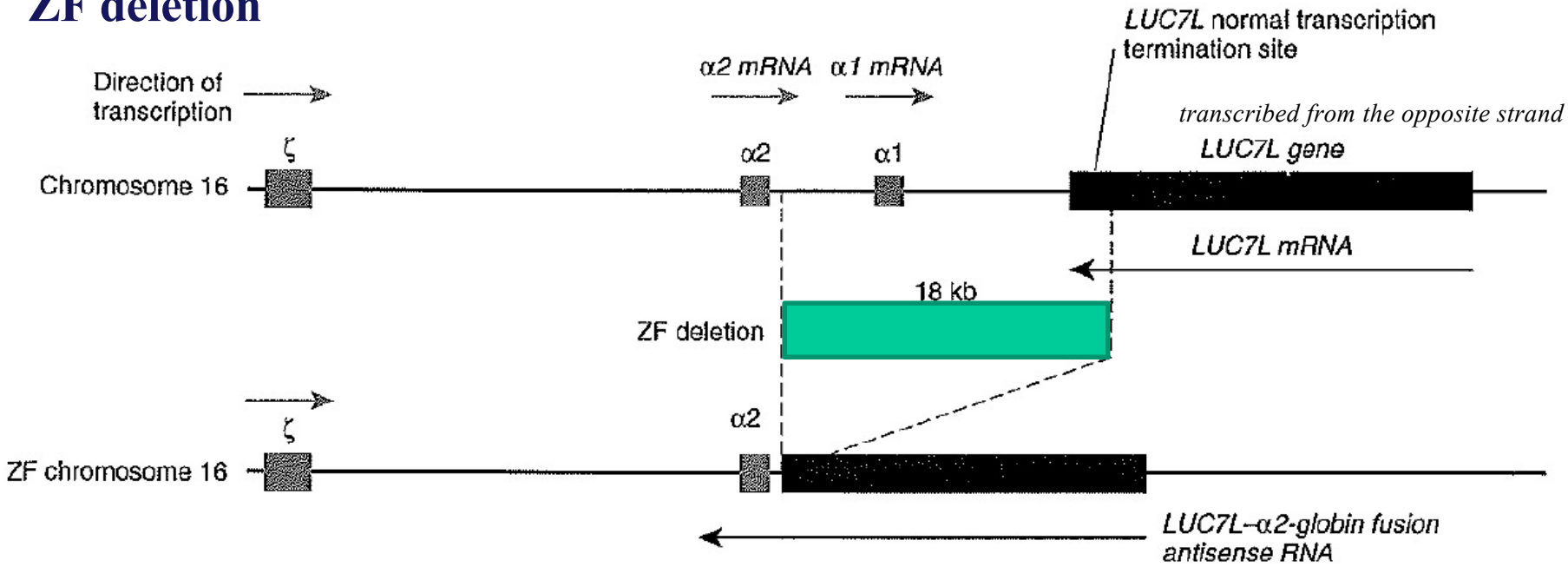
* Carriers frequent in Southeast Asia

THE A - THALASSEMIAS

- Rare forms:
  - form due to the ZF deletion (named after individual ZF)
  - the ATR-X syndrome

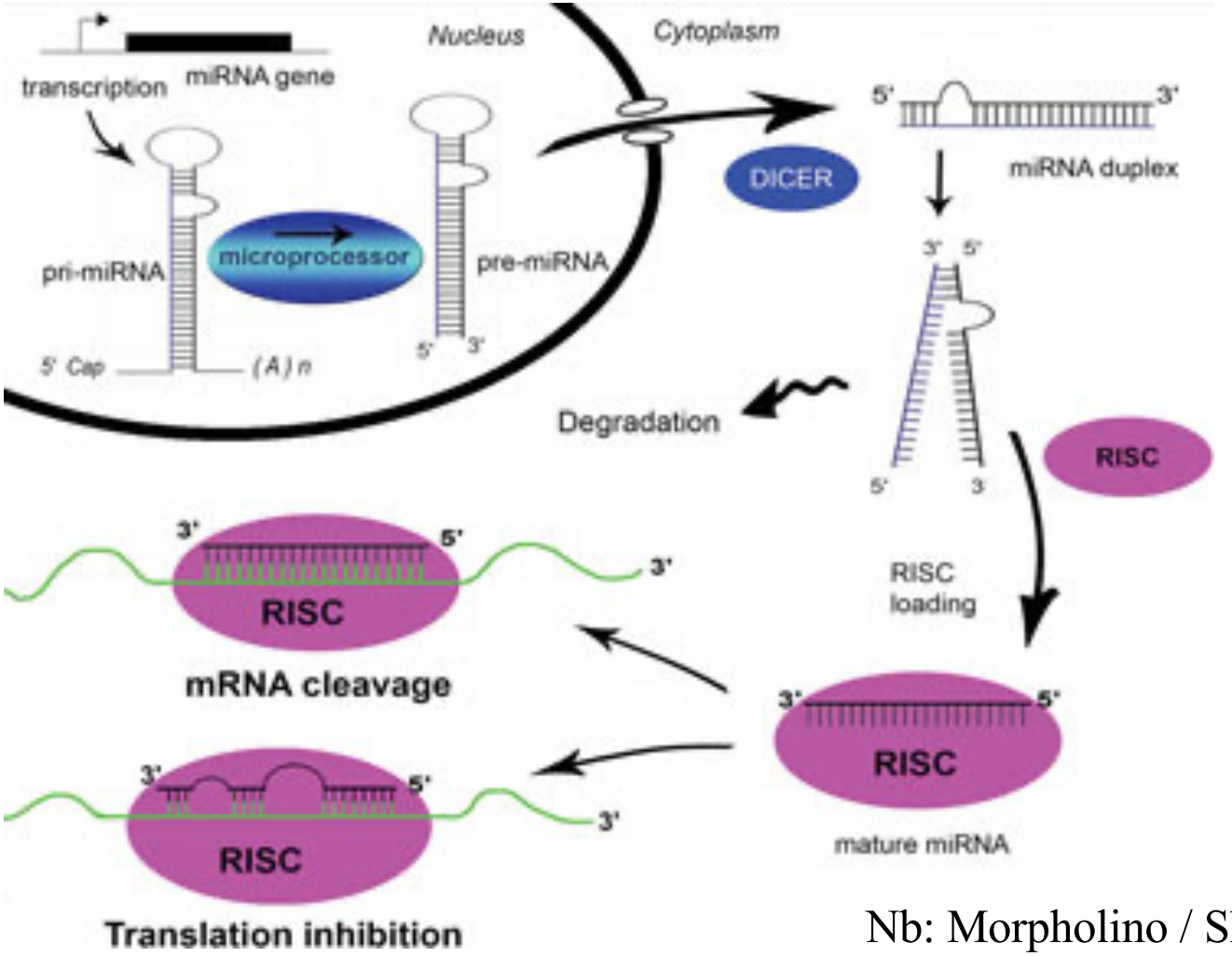
THE A - THALASSEMIAS

ZF deletion



α_2–gene is silenced due to the generation of antisense RNAs from the truncated LUC7L gene

wild-type antisense transcripts do also exist and play a role in regulation of gene expression (f.e. X inactivation, miRNA, lnRNA)!



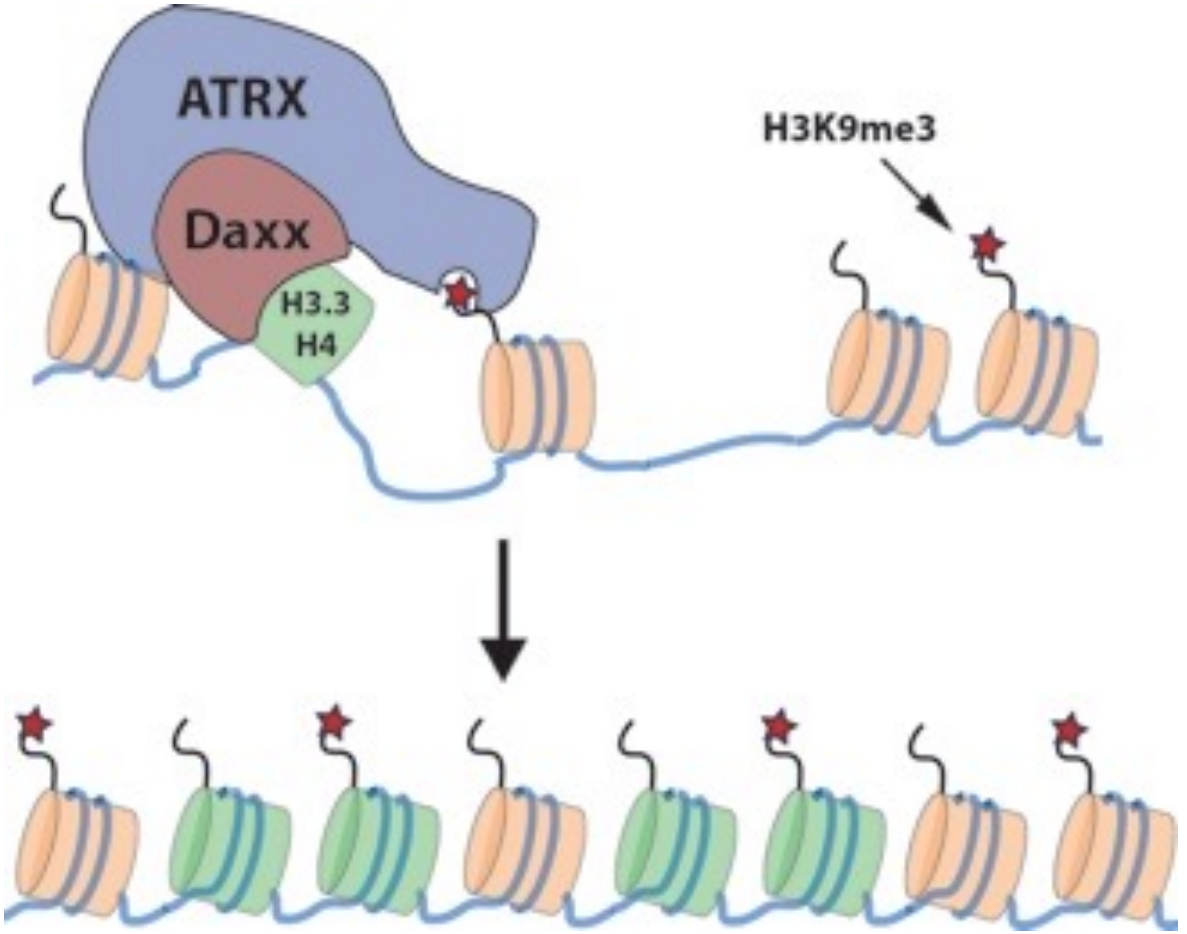
Nb: Morpholino / ShRNA

THE A - THALASSEMIAS

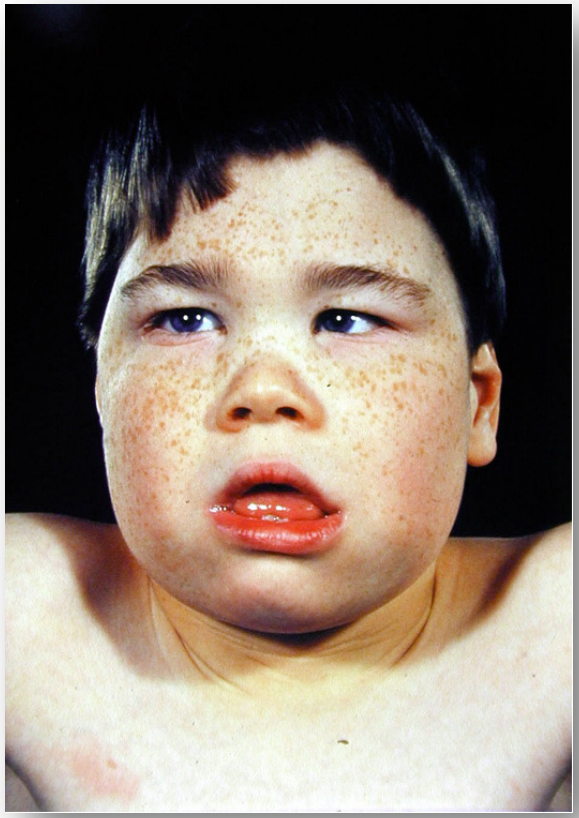
The ATR-X syndrome

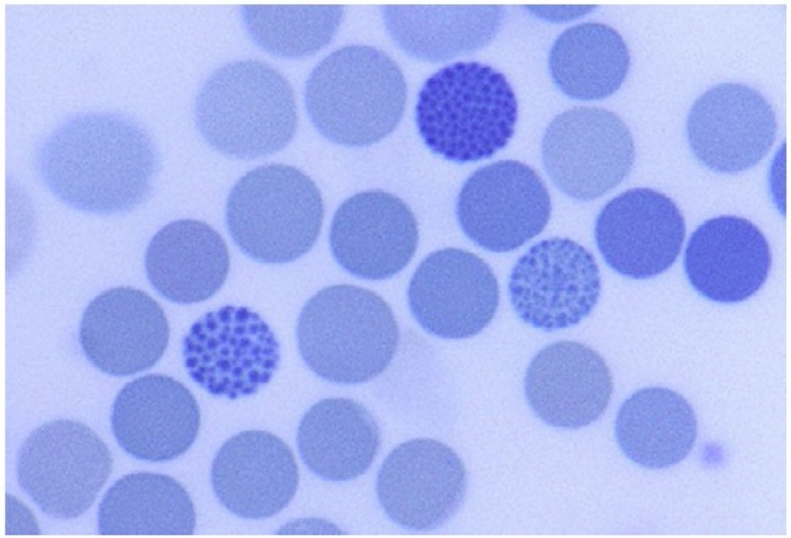
- MR and α–thalassemia
- due to mutations in the X-linked ATRX gene
- encodes a chromatin remodeling protein (methylation)
- activates expression in ***trans***

- partial loss-of-function mutations result in modest reduction of α–globin synthesis
- *somatic* (more severe) mutations in *ATRX* cause the α–thalassemia myelodysplasia syndrome (if germline: hydrops fetalis!)


ATRX
Daxx
H3.3
H4
H3K9me3

The ATR-X syndrome





Erythrocytes after incubation in briljant cresyl blue.
Hb H inclusions :'golf ball'

- Profound MR (X-L)
- MC
- Short stature
- Genital Δ
- (Mild) anemia

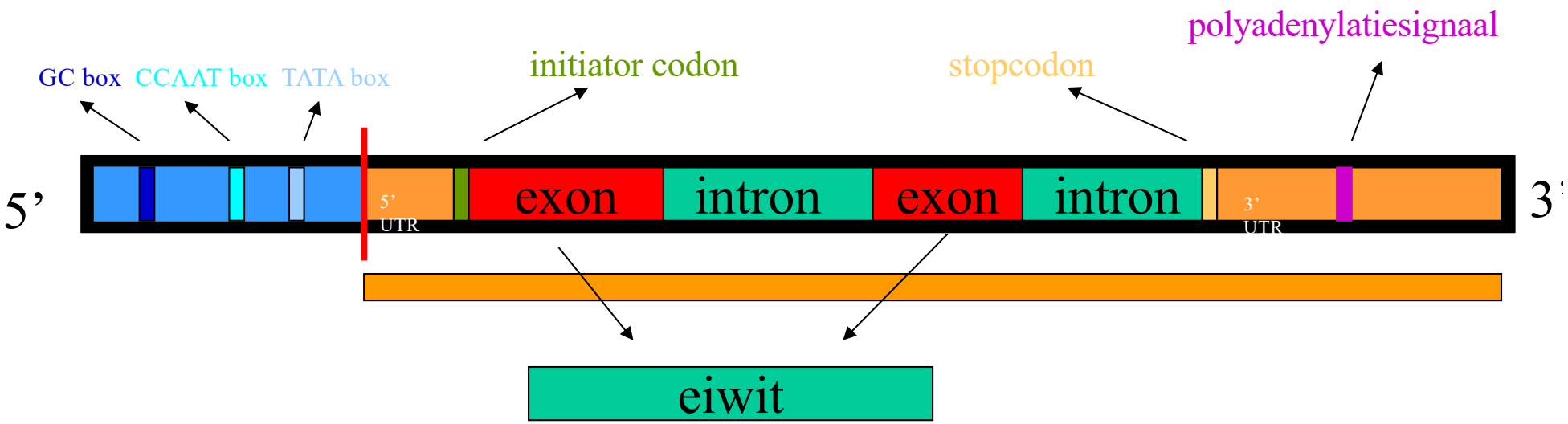
From Gibbons R. Orphanet Journal of Rare Diseases 2006;1:15

THE B - THALASSEMIAS

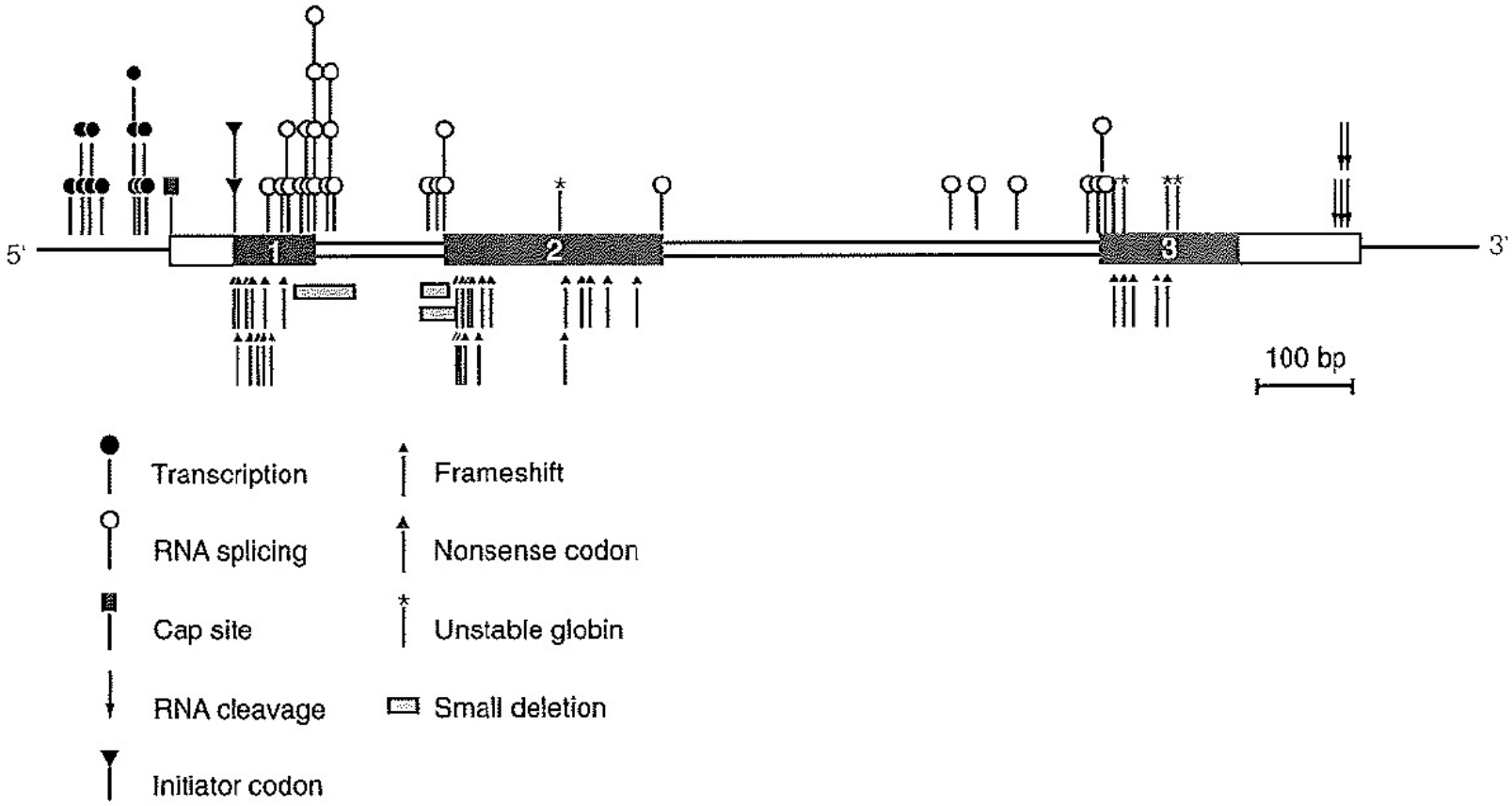
- ↓β–globin production
  - two β–thalassemia alleles: usually thalassemia **major** (severe anemia)
  - one β–thalassemia allele: thalassemia **minor** (mild anemia, no clinic)
- **postnatal**
- precipitation of excess α–chains → hemolysis
- low β-chain production → hypochromic, microcytic anemia
- ↑HbA_2 ($\alpha_2\delta_2$) and ↑ HbF ($\alpha_2\gamma_2$)
- >> single-base pair substitutions (rather than deletions)
- >> compound heterozygous
- simple versus complex β–thalassemia

The Molecular Basis of Simple β-Thalassemia

Type	Example	Phenotype	Affected Population
DELETIONS*			
β-globin gene deletions	619-bp deletion of the 3' end of the gene	β^0	Indian
DEFECTIVE MRNA SYNTHESIS			
>> RNA splicing defects (see Fig. 11-12)	Abnormal acceptor site of intron 1: AG → GG	β^0	Black
Promoter mutants	Mutation in the ATA box −31 −30 −29 −28 −31 −30 −29 −28 A T A A → G T A A	β^+	Japanese
Abnormal RNA cap site	A → C transversion at the mRNA cap site	β^+	Asian
Polyadenylation signal defects	AATAAA → AACAAA	β^+	Black
NONFUNCTIONAL MRNAS			
Nonsense mutations	codon 39 gln → stop CAG → UAG	β^0	Mediterranean (especially Sardinia)
Frameshift mutations	codon 16 (1-bp deletion) *normal* trp gly lys val asn 15 16 17 18 19 UGG GGC AAG GUG AAC UGG GCA AGG UGA *mutant* trp ala arg stop	β^0	Indian
CODING REGION MUTATIONS THAT ALSO ALTER SPLICING*			
Synonymous mutations	codon 24 gly → gly GGU → GGA	β^+	Black

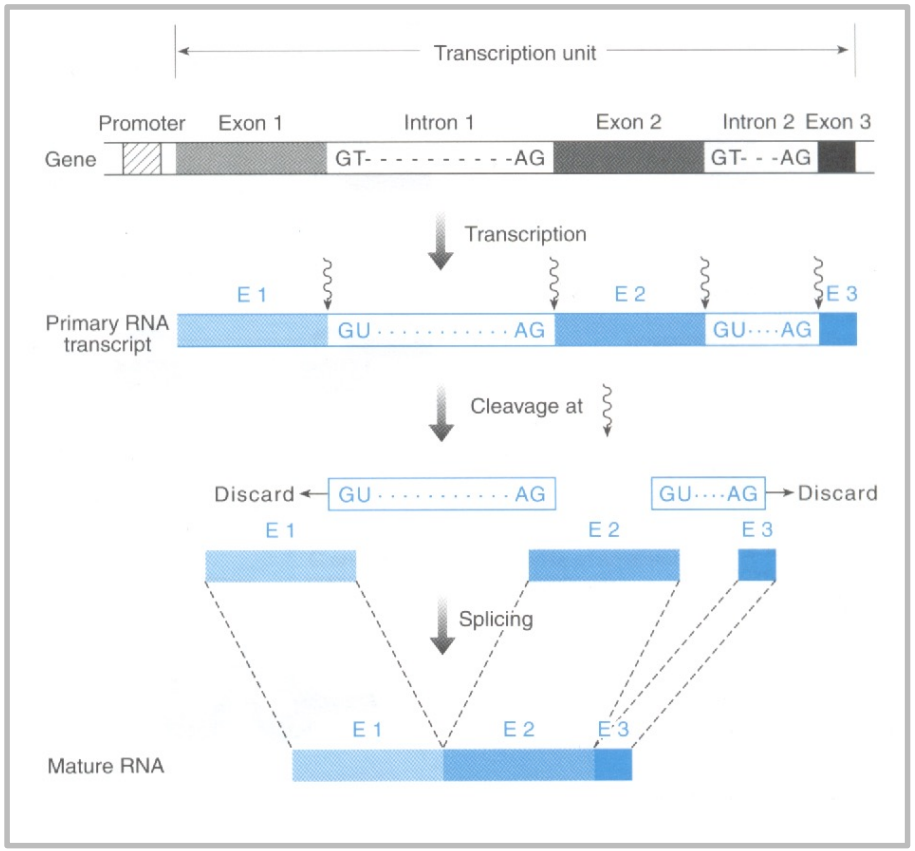


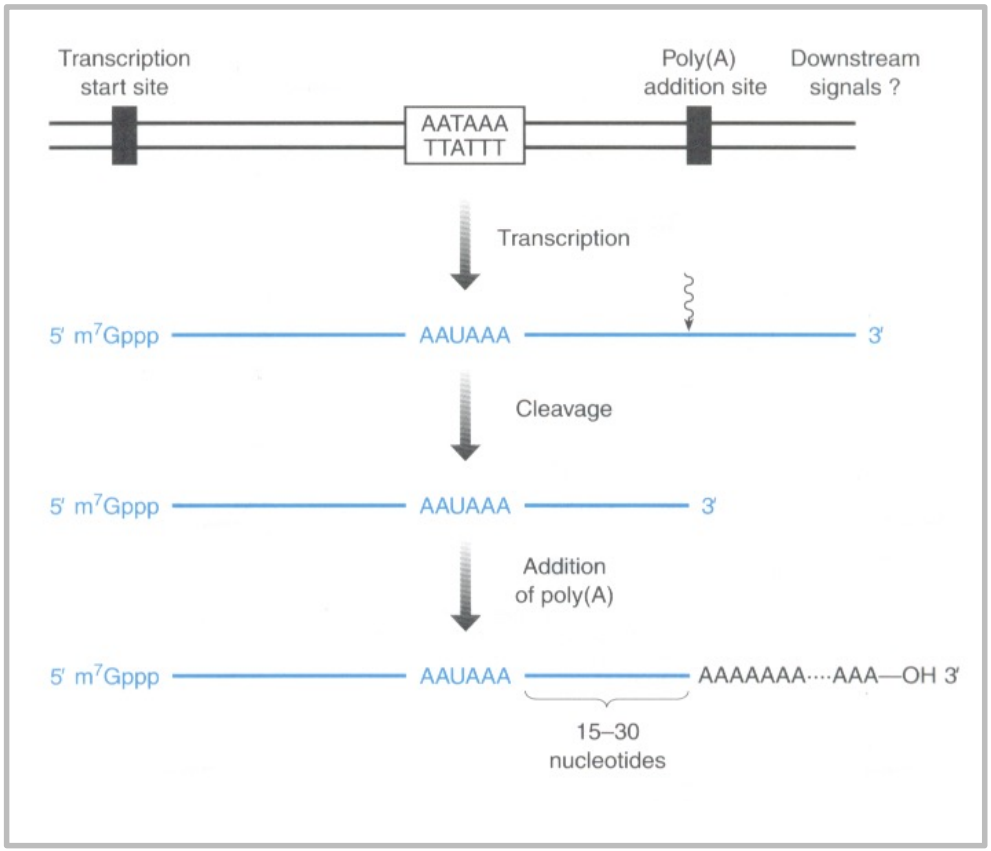
promoter regio: GC box; CCAAT box; TATA box
transcriptie: start (+cap)
translatie: initiator codon; stopcodon
polyadenylatiesignaal



Point mutations that cause β-thalassemia are distributed throughout the gene.
They affect virtually every process required for the production of normal β-globin.

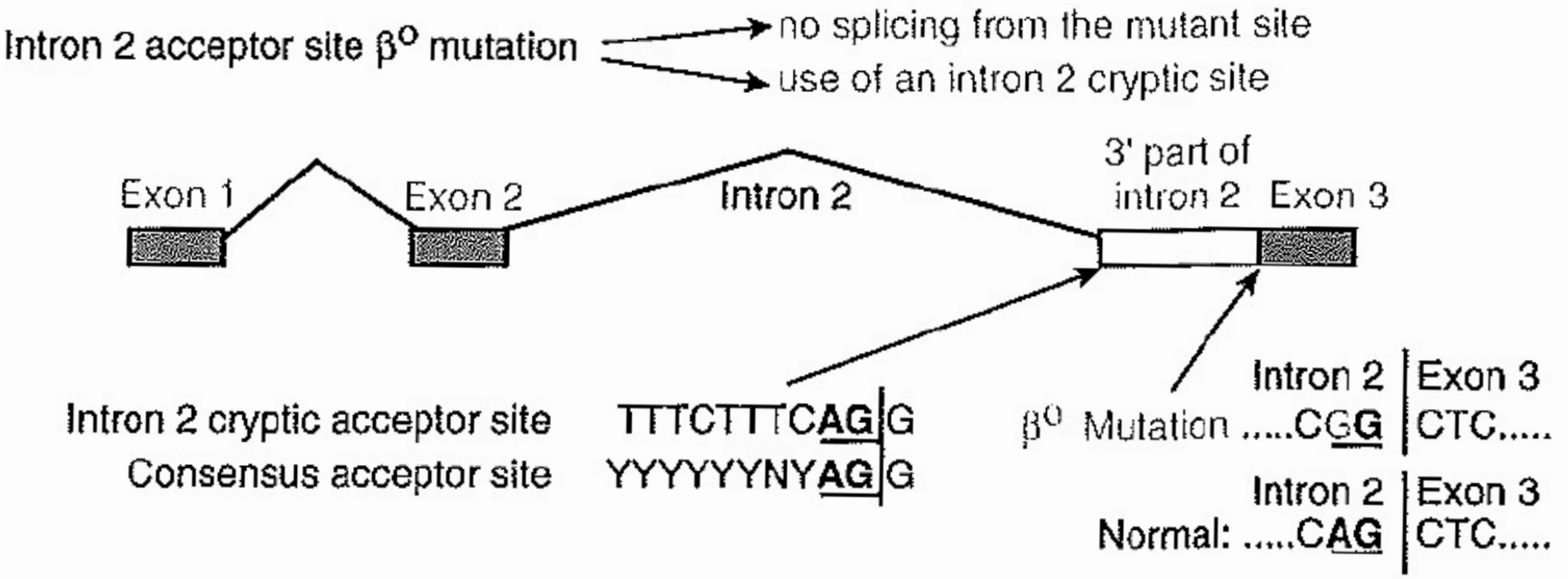
Posttranscriptional modifications of mRNA



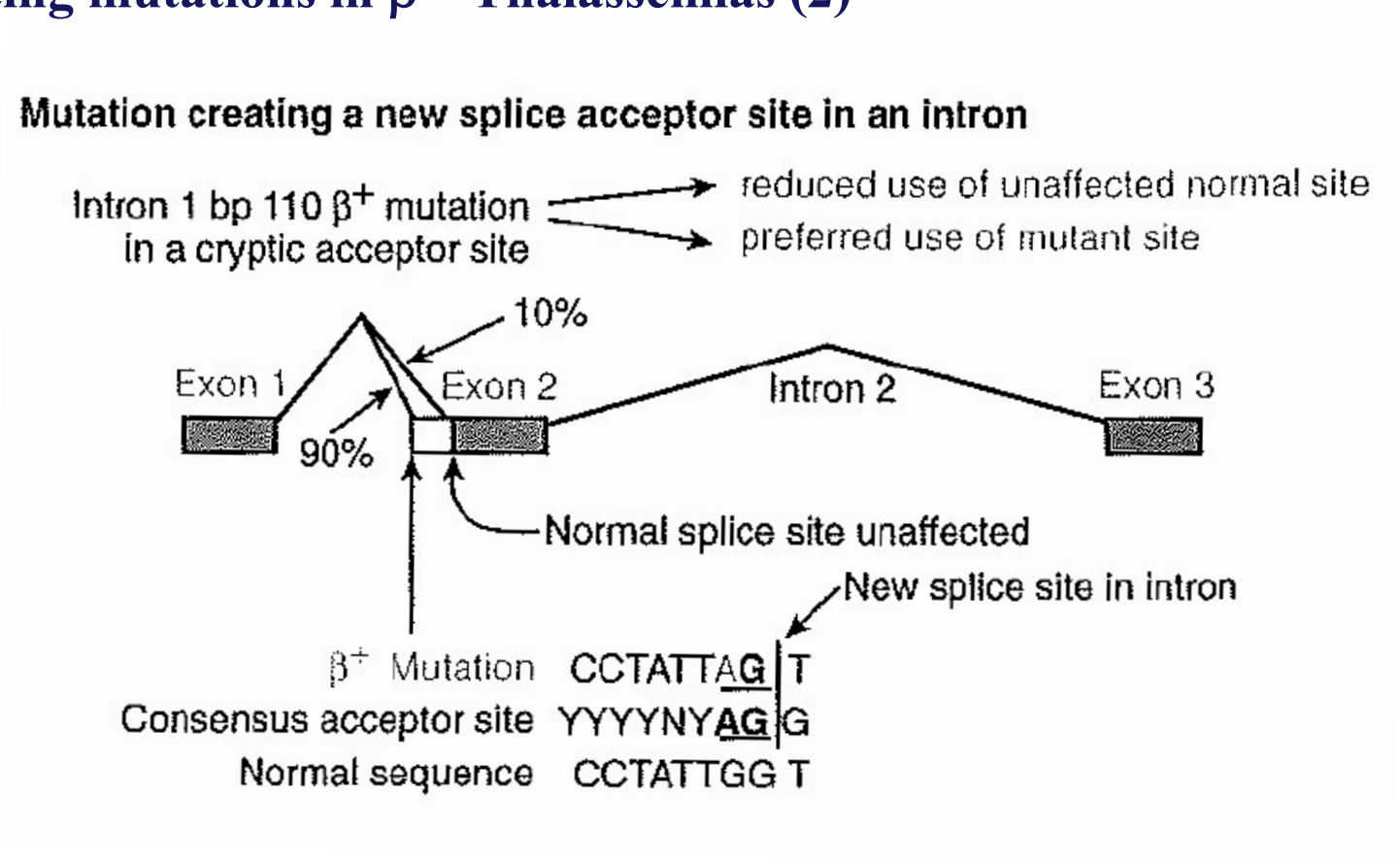


RNA splicing mutations in β – Thalassemias (1)

Mutation destroying a normal splice acceptor site and activating a cryptic site

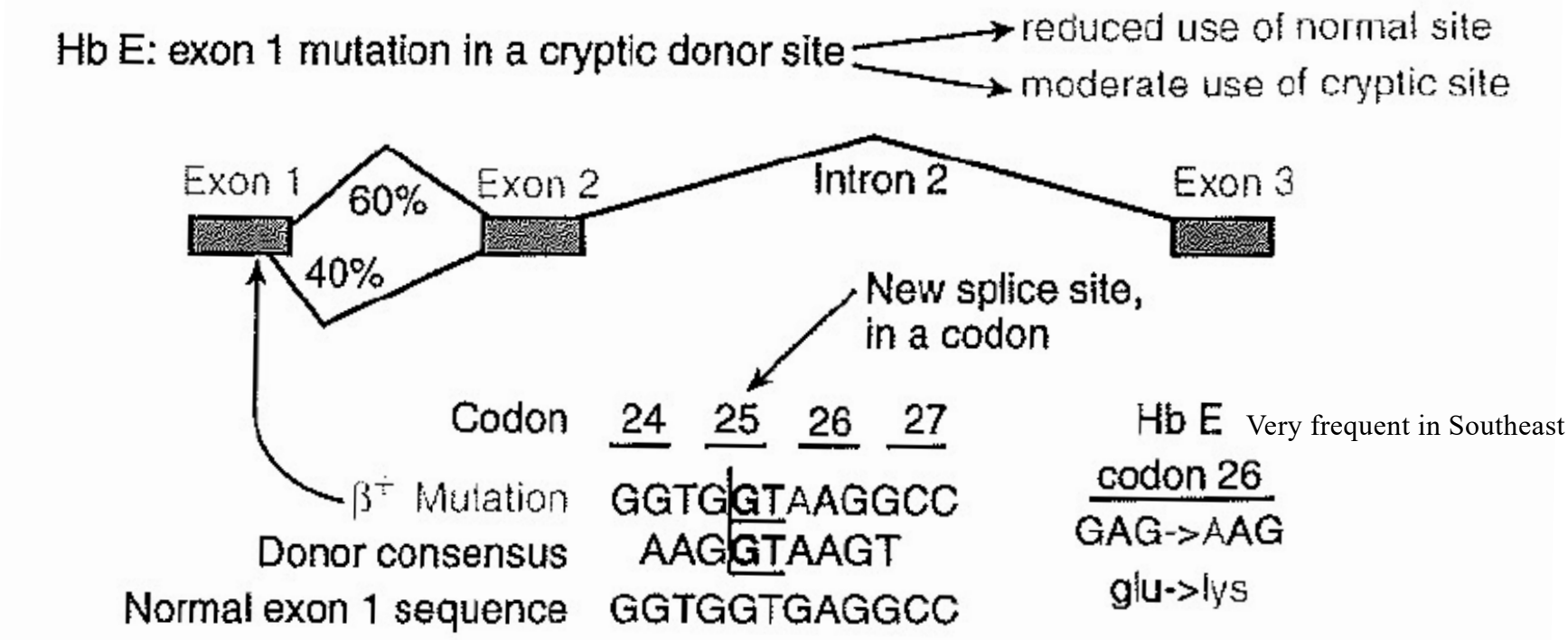


RNA splicing mutations in β – Thalassemias (2)



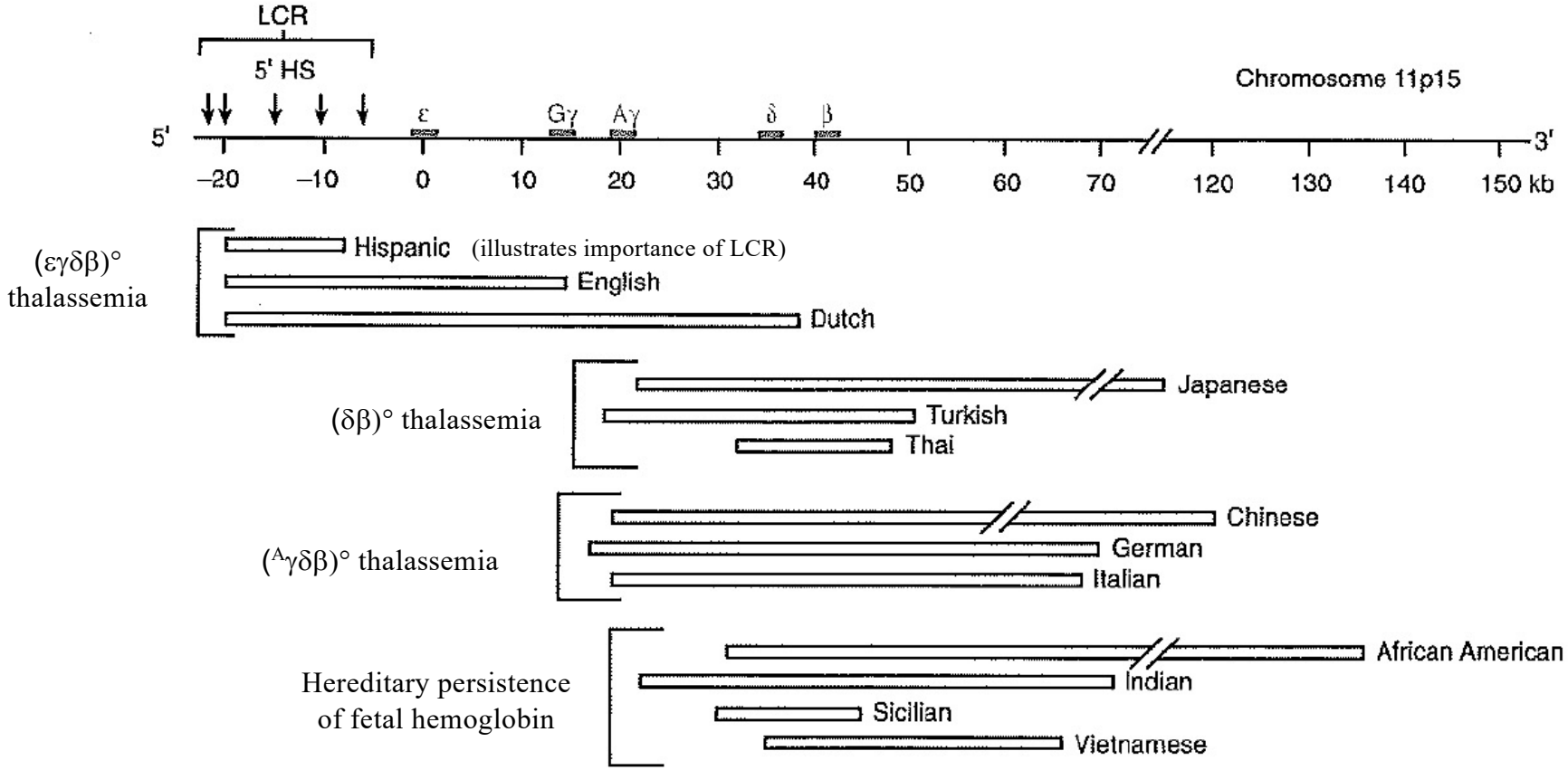
RNA splicing mutations in β – Thalassemias (3)

Mutation enhancing a cryptic splice donor site in an exon



Hb E: example of a single nucleotide substitution that affects both RNA splicing and the coding sequence

Complex β–Thalassemias



3. GLOBIN DEVELOPMENTAL SWITCHING DISORDERS

- hereditary persistence of fetal hemoglobin
- group of clinically benign conditions
- production of higher levels of Hb F than is seen in (δβ)° thalassemia
- they impair the perinatal switch from γ-globin to β-globin synthesis


Thanks for your attention
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