Detekce mutací v genu CAPN3 – na úrovni mRNA a DNA

PACIENT	MUTACE DETEKOVANÁ NA ALELE 1	MUTACE DETEKOVANÁ NA ALELE 2
1	**245C>T, P82L**	***550delA, T184RfsX36***
2	**245C>T, P82L**	***2314-2317del, D772delK773NfsX3***
3	***550delA, T184RfsX36***	**598-612del, F200_L204del**
4	***550delA, T184RfsX36***	**1468C>T, R490W**

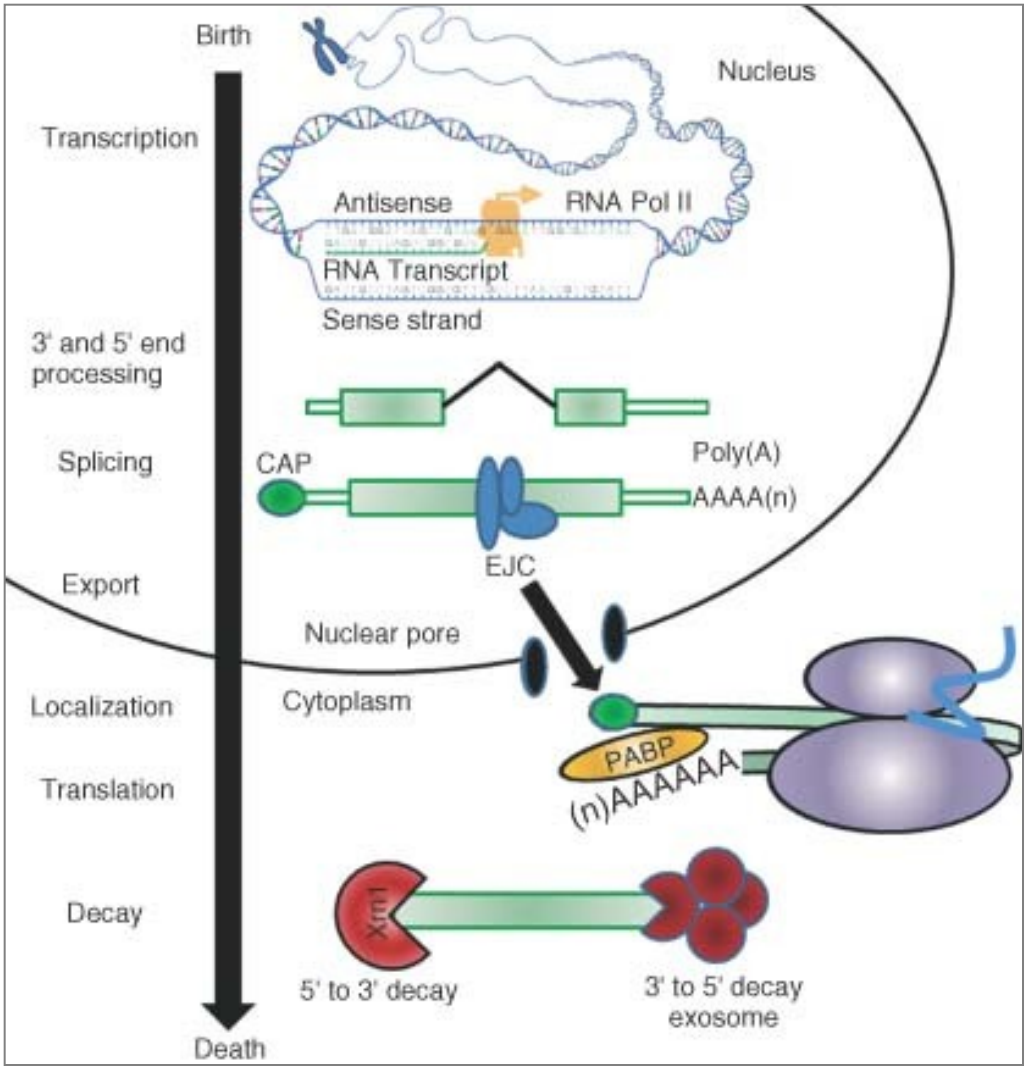
mRNA: homozygotní výskyt *missense* mutace nebo *in-frame* delece

DNA: heterozygotní výskyt *missense* mutace nebo *in-frame* delece + detekce ***frame-shift*** **delece**

→ **degradace mRNA nesoucí *frame-shift* deleci**

mechanismem *nonsense mediated mRNA decay*

• The process of eukaryotic gene expression involves a number of interlinked steps - transcription, capping, polyadenylation, splicing, translation, and mRNA degradation.



• RNA-polymerase mediated transcription.

• Packaging of mRNA into mRNP begins almost immediately with the initiation of transcription, with addition of **the m7GpppG cap**.

• **Intron splicing** from the pre-mRNA can also begin before transcription is complete and results in the deposition of **the exon-junction complex (EJC).**

• Upon transcriptional termination, the 3′ end is processed resulting in the addition of **the poly(A) tail.**

• **Nuclear export** of the mRNA is a regulated process which in metazoans **involves the EJC**.

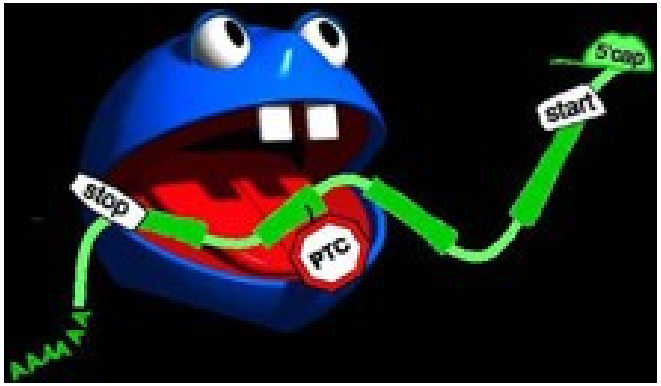
• In the cytoplasm: the mRNA undergoes a **pioneer round of translation which removes many of the proteins bound to the mRNA** in the nucleus and these proteins shuttle back into the nucleus. In mammalian cells, several surveillance mechanisms control the mRNA during the pioneer round of translation. If the surveillance decay mechanisms are not activated, then the message is either translated into protein, stored for later translation, or degraded. Message degradation utilizes both the 5′-to-3′ and 3′-to-5′ exosome-mediated decay pathways.

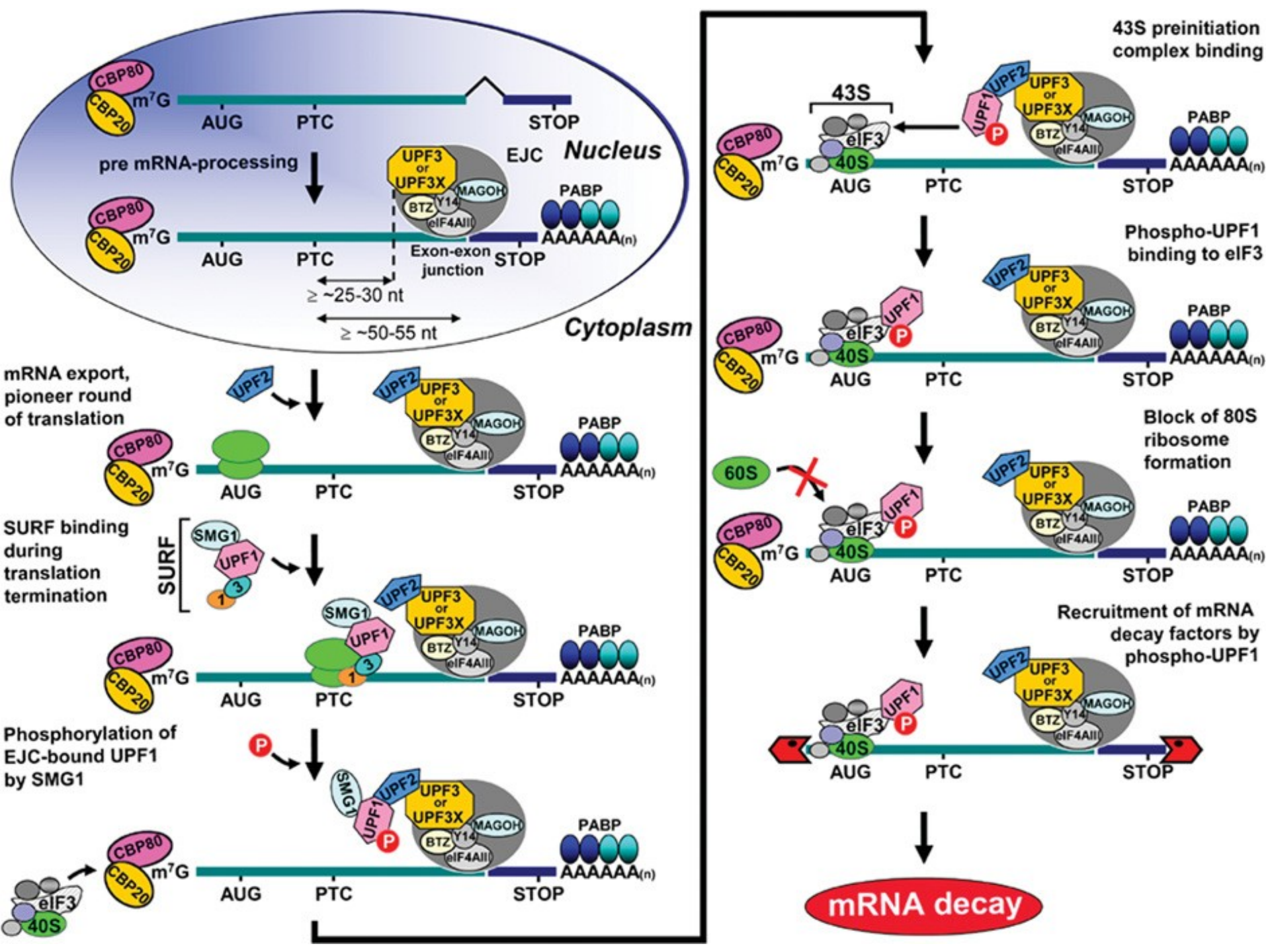
Wiley Interdisciplinary Reviews – RNA, Vol. 1, Is. 1, 2010

• **NMD (nonsense-mediated mRNA decay) is quality control mechanism preventing the production of potentially deleterious C-terminally truncated proteins translated from PTC-containing (protein termination codons) mRNAs.**

• Nonsense mutations and frame-shifting deletions/insertions generate PTCs. **It is estimated that 30% of known disease-associated mutations are due to PTC-containing mRNAs**.

• At the RNA level, errors in transcription and pre-mRNA splicing also generate mRNAs with PTCs that are substrates for NMD. 95% of multiexon human genes are alternatively spliced - **the average number of alternatively spliced mRNA isoforms per gene is approximately 3.5**. Using bioinformatics approaches, it was proposed that about **one-third of the alternatively spliced human mRNAs contain a PTC**.




CBP80
CBP20
m7G
AUG
PTC
STOP
EJC
Nucleus
pre mRNA-processing
UPF3 or UPF3X
MAGOH
Y14
BTZ
eIF4AIII
PABP
AAAAAA(n)
Exon-exon junction
≥ ~25-30 nt
≥ ~50-55 nt
Cytoplasm
mRNA export, pioneer round of translation
UPF2
SURF binding during translation termination
SURF
SMG1
UPF1
Phosphorylation of EJC-bound UPF1 by SMG1
P
eIF3
40S
43S preinitiation complex binding
43S
Phospho-UPF1 binding to eIF3
Block of 80S ribosome formation
60S
Recruitment of mRNA decay factors by phospho-UPF1
mRNA decay

Model for NMD

In mammals, mRNA is targeted for NMD once mRNA has been generated by pre-mRNA processing and exported from the nucleus to the cytoplasm. During pre-mRNA processing, splicing results in the deposition of an EJC of proteins upstream of mRNA exon–exon junctions. EJC components include eIF4AIII, Y14, MAGOH, BTZ and many other proteins.

In the cytoplasm, UPF3 recruits UPF2; the pioneer round of translation; termination of translation during the pioneer round of translation at a premature termination codon (PTC); binding of the SURF complex, phosphorylation of UPF1; 43S preinitiation complex binding to the AUG translation initiation codon so as to prevent 60S ribosomal subunit joining. Phospho-UPF1 also promotes NMD by recruiting mRNA degradative activities.

Notably, mammalian-cell NMD can also target mRNAs that have not undergone splicing downstream of a PTC.

Nucleolytic activities are indicated by the red irregular hexagons. PABP, poly(A)-binding protein, where darkershapes specify the largely nuclear PABPN1 and lighter shapes denote the largely cytoplasmic PABPC1; AUG, translation initiation codon; STOP, normal termination codon.

Biochemical Society Transactions (2009) Volume 37, part 6

NMD ovlivňuje klinickou závažnost některých chorob:

• **NMD zhoršuje klinická projevy** - DMD/BMD - nonsense a frame-shift mutace blízko 3 -konce *DMD* genu mohou mí za následek rozdílné fenotypové projevy v závislosti na NMD.

NMD +

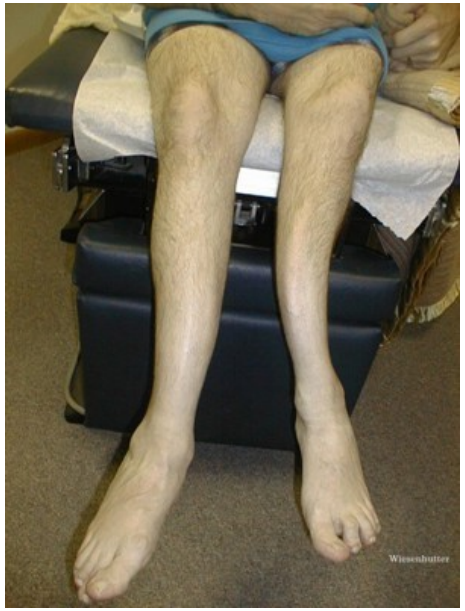
Phenotype	*Localisation (exon)*	*Mutation detected at cDNA level*	*Mutation detected at DNA level*	*Mutation at protein level*	*Immunohistochemical labelling using antibodies DYS1,2,3*
DMD	70	c.10094C>A	Not performed	p.S3365X	Negative
DMD	70	c.10108C>T	Not performed	p.R3370X	Negative
DMD	70	c.10141C>T	Not performed	p.R3381X	Negative
BMD	**74**	**Not performed**	**c.10489delT**	**p.S3497PfsX2**	**Not performed**

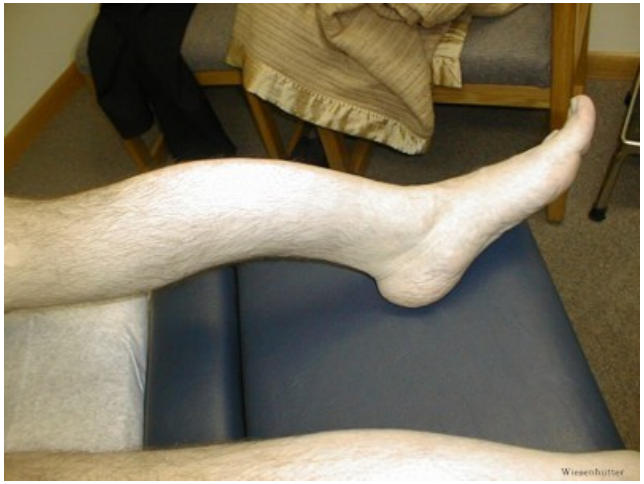
NMD -

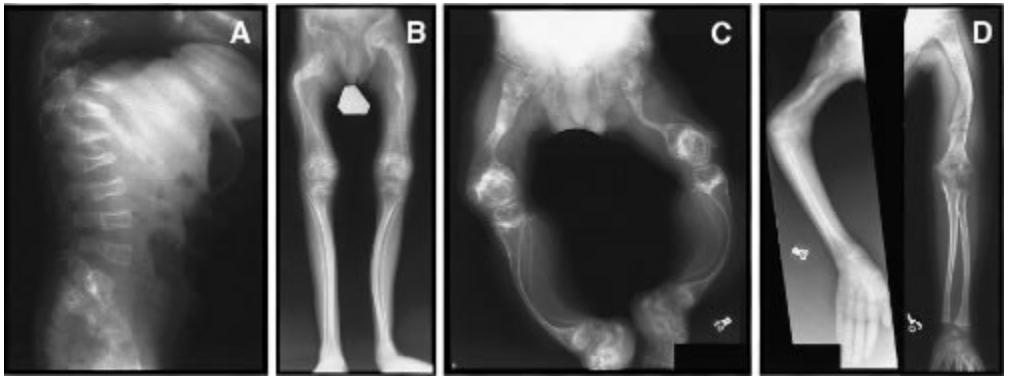
• **NMD zmírňuje klinické projevy** - OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta:

- **Skupina dědičných poruch kolagenu typu I - hlavní strukturní protein kostí a jiných vazivových tkání.**
- **Mutace v genu *COL1A1* a *COL1A2* - náchylnost ke lomivosti kostí, deformitám skeletu (pozoruhodný rozsah klinických příznaků (od letální formy po jen mírné zvýšení frekvence zlomenin).**







• **Kolagen typu I – kolagen typu I je tvořen ze dvou řetězců prokolagenu α1 (gen *COL1A1*) a jednoho prokolagenu α2 (*COL1A2*).**

• **Missense mutace** asociované s genem ***COL1A*** jsou příklady **dominantně-negativních alel** ..... ruší konformaci kolagenových podjednotek a jsou spojeny s těžkými klinickými fenotypy **osteogenesis imperfecta typu II–IV.**

• **PTC mutace** vyvolávající NMD jsou spojeny s mírnějšími klinickými fenotypy **osteogenesis imperfecta typu I.**

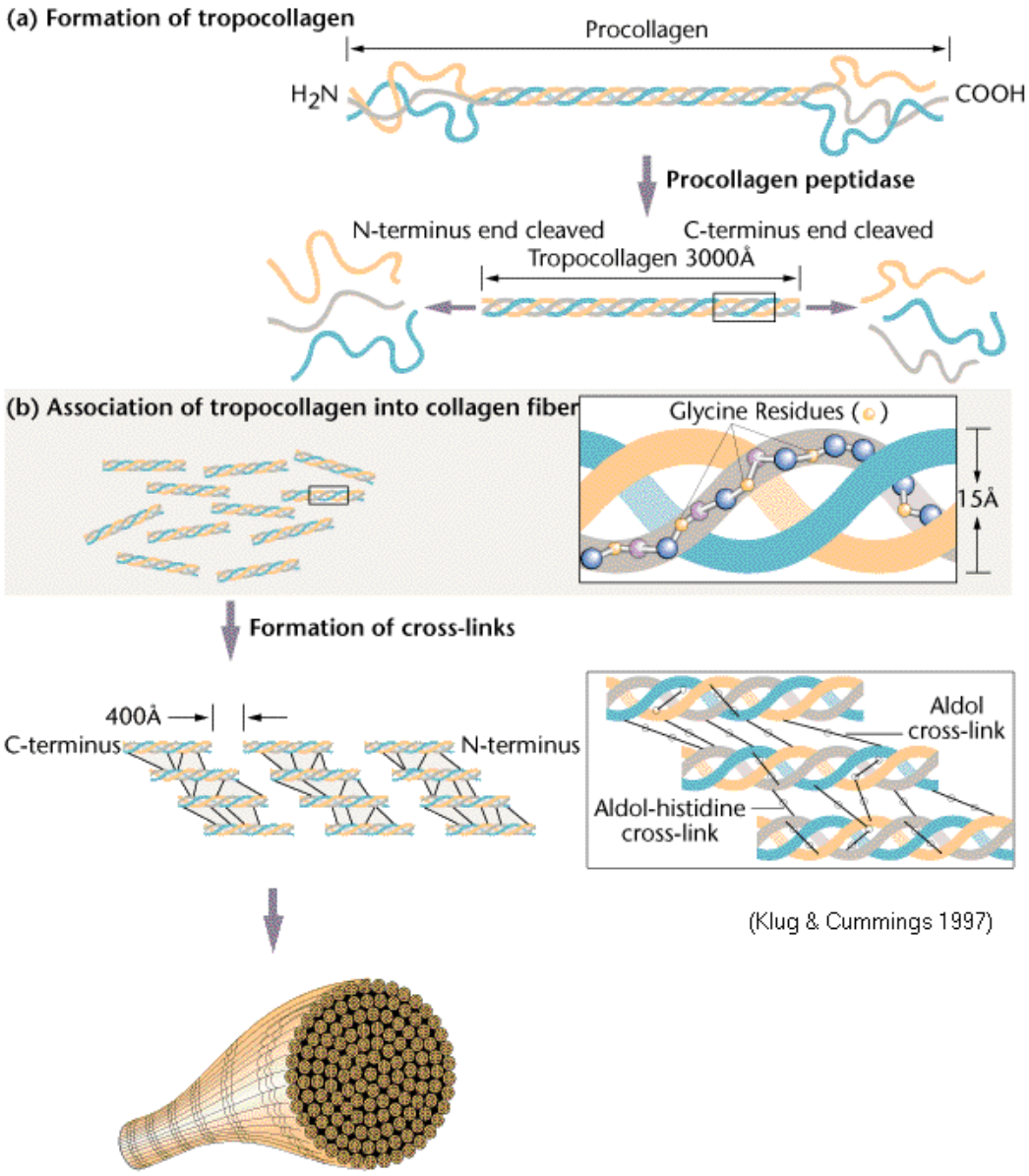


Table. Classification of Osteogenesis Imperfecta - Modified from Sillence

Type	Severity	Clinical features	Growth impairment	Blue sclera	Inheritance	Gene defect
I	Mild	Few fractures, slight bowing	Minimal	Present	AD	Nonsense & frameshift mutations resulting in STOP codons in *COL1A1*
II	Perinatal lethal	Many rib & long bone fractures at birth, severe bowing, respiratory insufficiency	-----	Present	AD, parental mosaicism AR	Glycine substitutions in *COL1A1* or *COL1A2*. Inactivating mutations of *CRTAP*
III	Severe, progressive deforming	Moderate to severe bowing, multiple fractures	Severe	Greyish	AD	Glycine substitutions in *COL1A1* or *COL1A2*
IV	Moderately deforming	Mild to moderate bowing, fractures	Moderate	Greyish or absent	AD	Glycine substitutions in *COL1A1* or *COL1A2*
V	Moderately deforming	Mild to moderate bone fragility, ossification of interosseous membrane of forearm, develop hyperplastic callus at fracture site	Mild to moderate	Absent	AD	Unknown
VI	Moderately to severely deforming	Onset of fractures in infancy, increased osteoid, fish-scale pattern of lamellation	Moderate	Absent or faint	AD	Unknown
VII	Moderately deforming	Fractures present at birth, rhizomelia, limb deformities	Moderate	Absent or faint	AR	Inactivating mutation (duplication) of *CRTAP*

Adapted and modified from Barnes AM, et al (2006) and Rauch F, Glorieux FH (2004).

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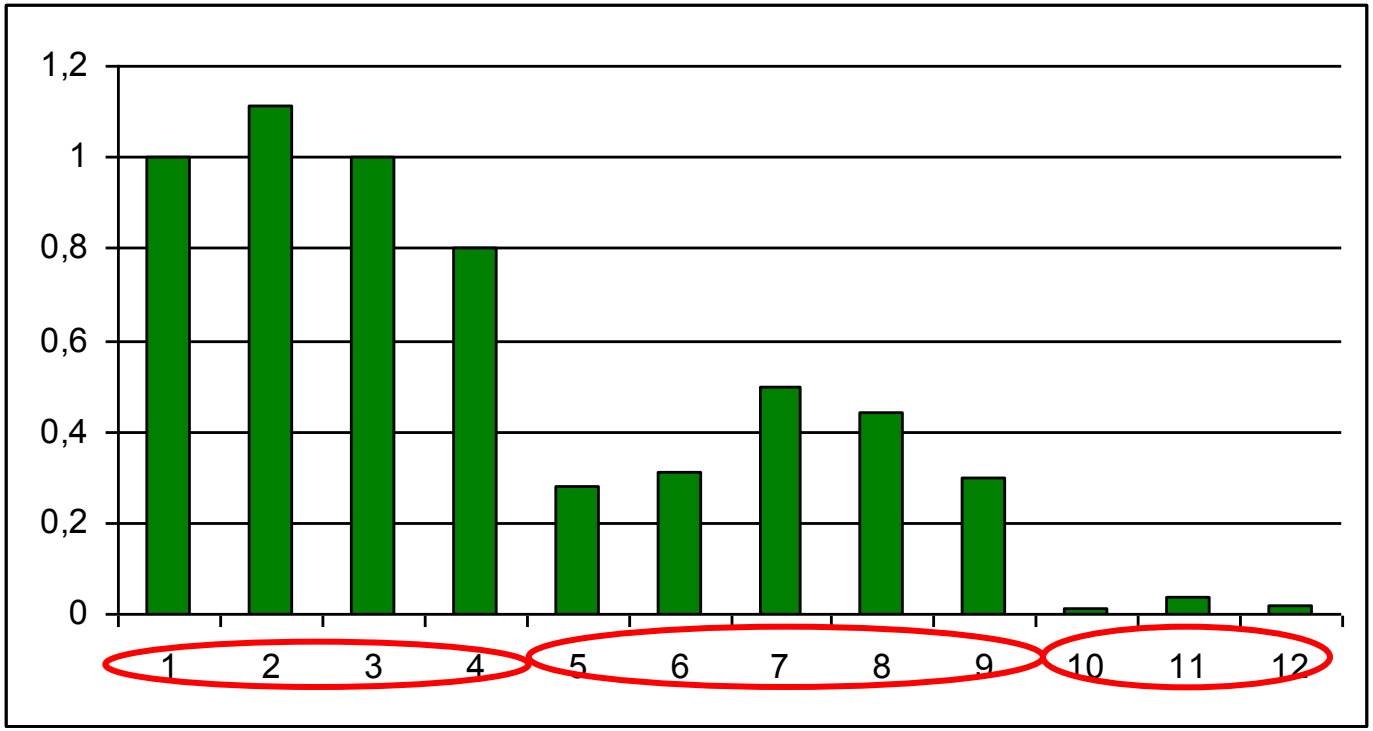
mechanismem *nonsense mediated mRNA decay*

Stanovení relativního množství mRNA genu pro kalpain-3



Pacient 1-4: ***non*-PTC/*non*-PTC**

Pacient: 5-9: ***non*-PTC/PTC**

Pacient 10-12: **PTC/PTC**

Relativní množství mRNA v závislosti na typu mutace:

***non*-PTC/*non*-PTC: 0,97**

***non*-PTC/PTC: 0,37**

PTC/PTC: 0,02