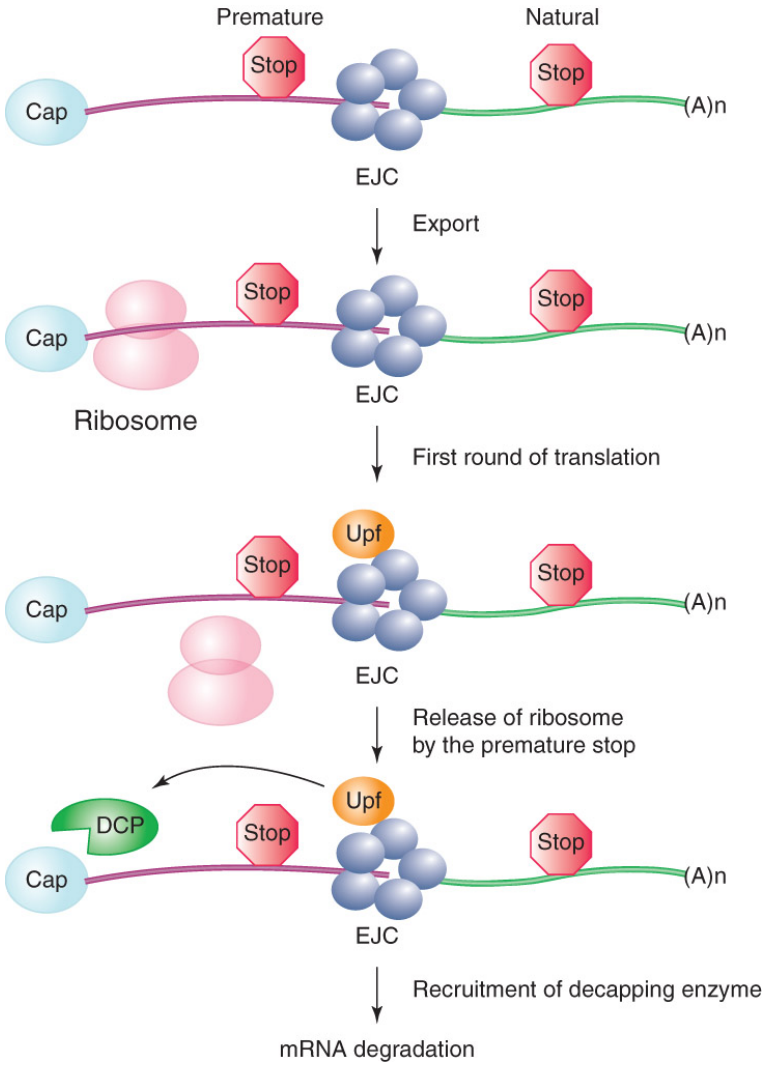
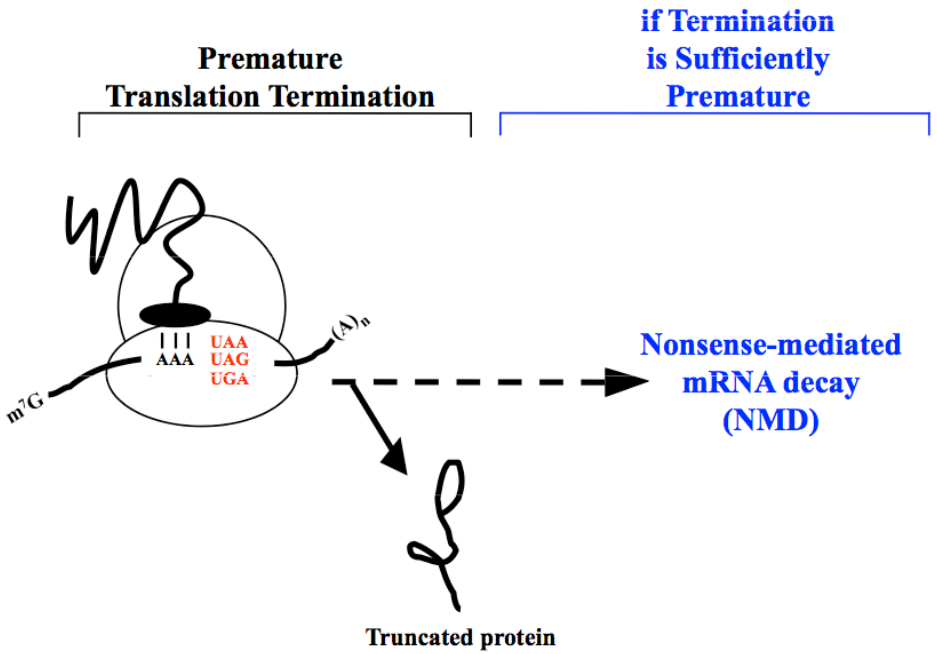
Splicing Is Temporally and Functionally Coupled with Multiple Steps in Gene Expression



- Splicing in the nucleus can influence mRNA translation in the cytoplasm.
- **nonsense-mediated mRNA decay (NMD) –** A pathway that degrades an mRNA that has a nonsense mutation prior to the last exon.

The Exon Junction Complex complex couples splicing with NMD

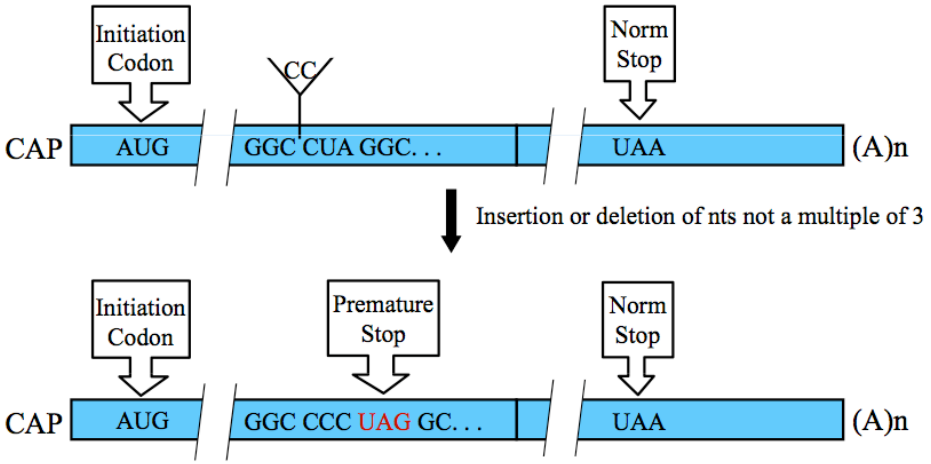
Nonsense-mediated Decay

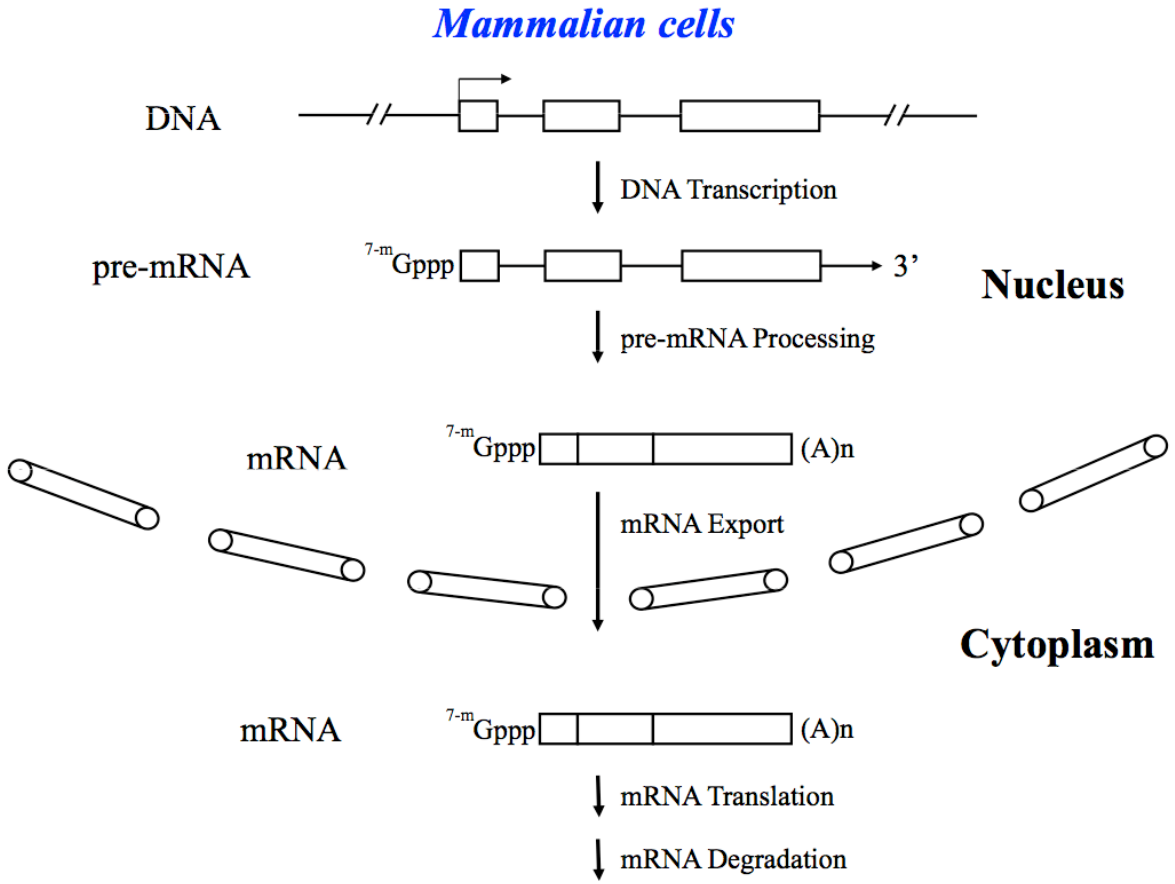


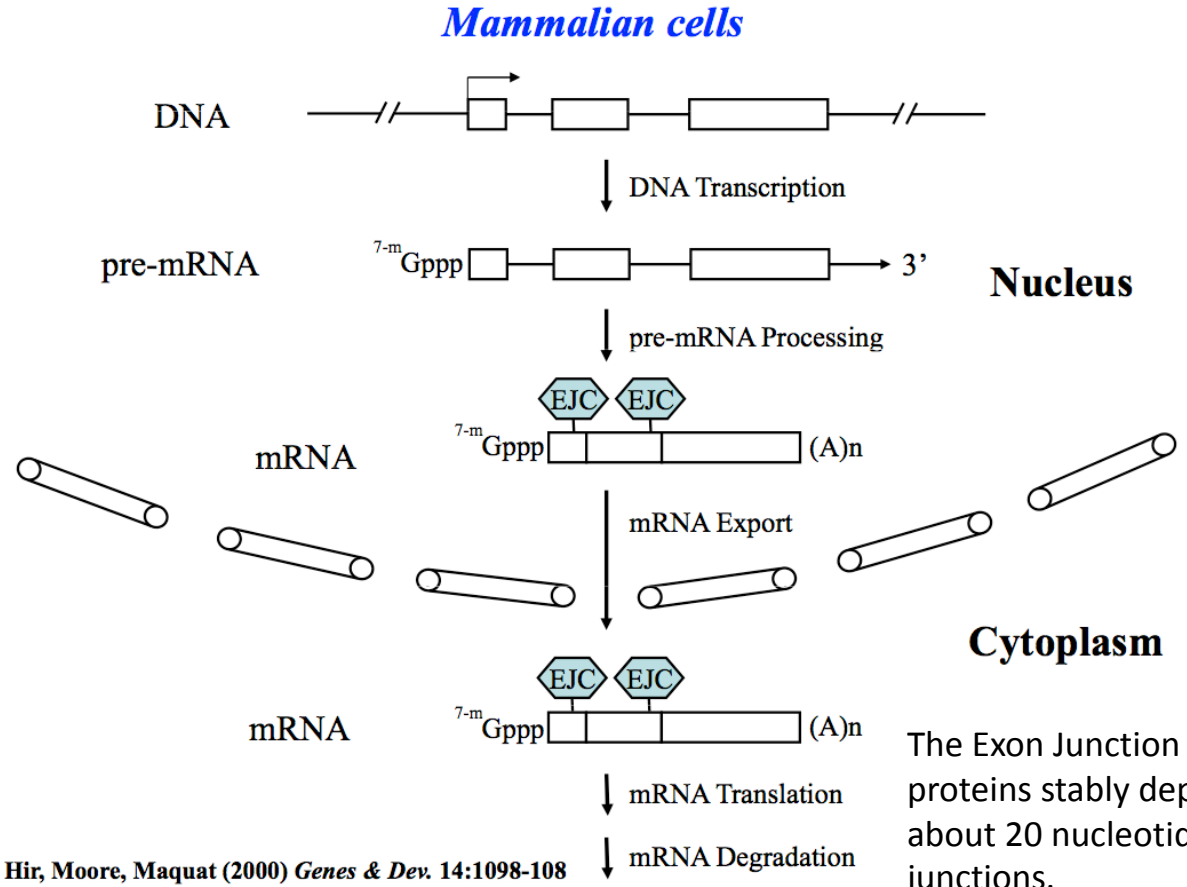
NMD is a translation-dependent degradation pathway specifically targeting mRNAs wherein the first in frame stop codon is a poor context for translation termination. In mammals the presence of one or more Exon Junction Complexes 50 or more nucleotides downstream of such a stop codon enhance the NMD efficiency.

Nonsense Mutation:

Frameshift Mutation:

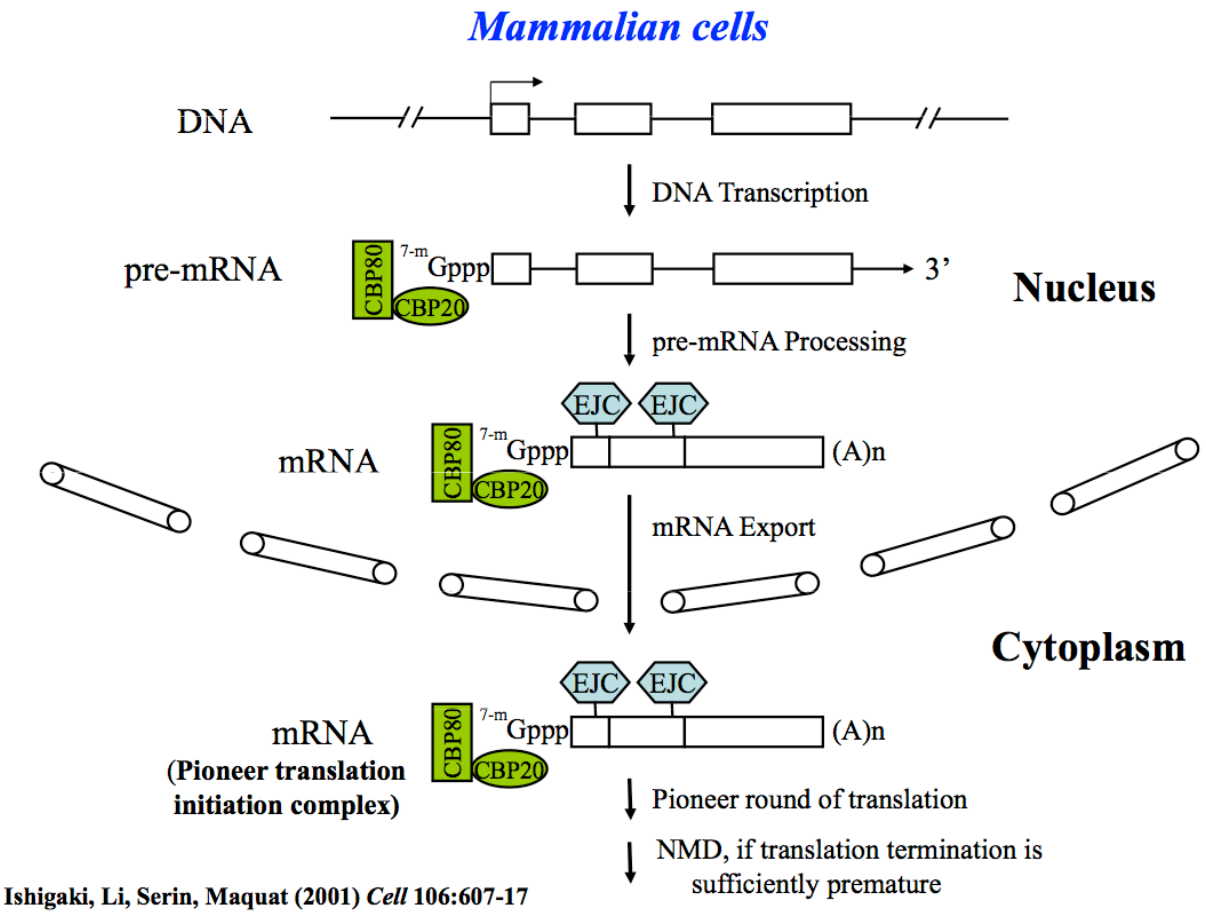



Mammalian cells
DNA
DNA Transcription
pre-mRNA
7-m Gppp
3'
Nucleus
pre-mRNA Processing
mRNA
7-m Gppp
(A)n
mRNA Export
Cytoplasm
mRNA
7-m Gppp
(A)n
mRNA Translation
mRNA Degradation



Le Hir, Moore, Maquat (2000) *Genes & Dev.* 14:1098-108
Le Hir, Izaurralde, Maquat, Moore (2000) *EMBO J.* 9:6860-9

The Exon Junction Complex (EJC) is a set of proteins stably deposited on spliced mRNAs about 20 nucleotides upstream of exon-exon junctions.
The EJC is involved in mRNA export, localization and decay.



Ishigaki, Li, Serin, Maquat (2001) *Cell* 106:607-17

Purpose of NMD

mRNA quality control or mRNA surveillance mechanism

- NMD functions to down regulate abnormal transcripts that are a consequence of abnormalities in gene expression because the resulting protein while often unstable can function in dominant-negative or other ways that are deleterious to the cell.

- It makes sense for cells to eliminate abnormal potentially harmful transcripts early in their biogenesis. To do so. mammalian cells utilize nonsense codon recognition during a pioneer round of translation to elicit NMD

NMD targets alternatively spliced transcripts

- It regulates 35% of alternatively splice transcripts

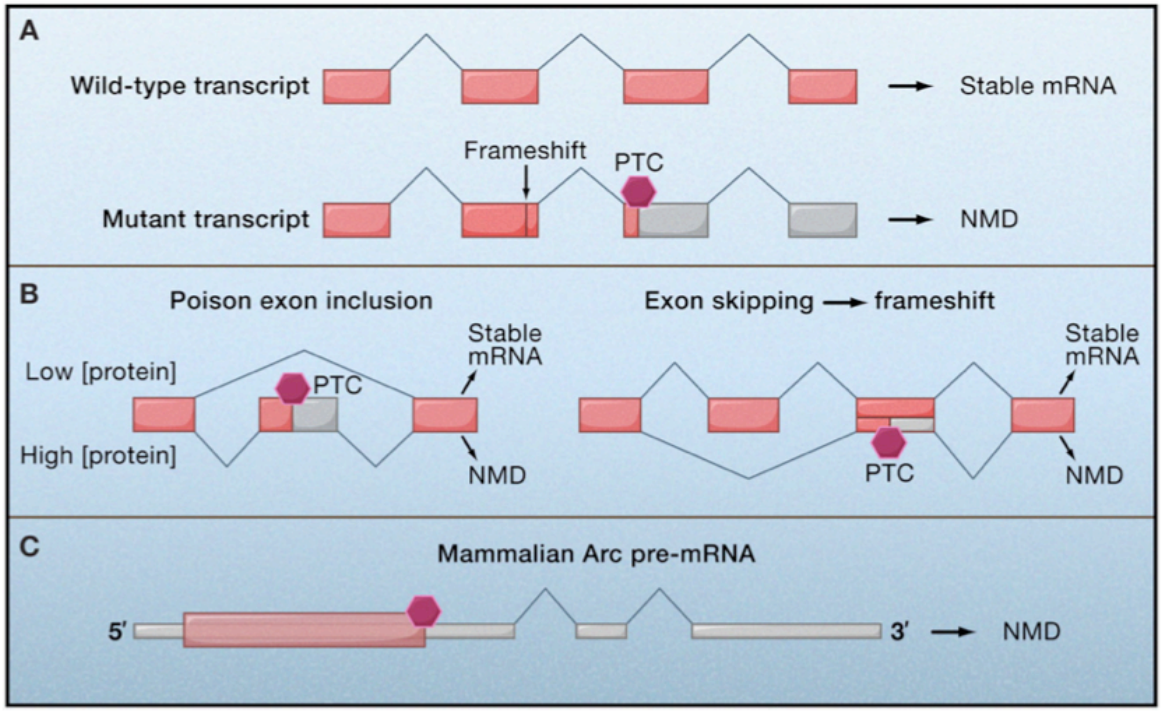


Figure 6. Splicing Patterns of Various NMD Substrates

(A) One function of nonsense mediated decay (NMD) is to eliminate mutant mRNAs containing a truncated open reading frame (red bar).

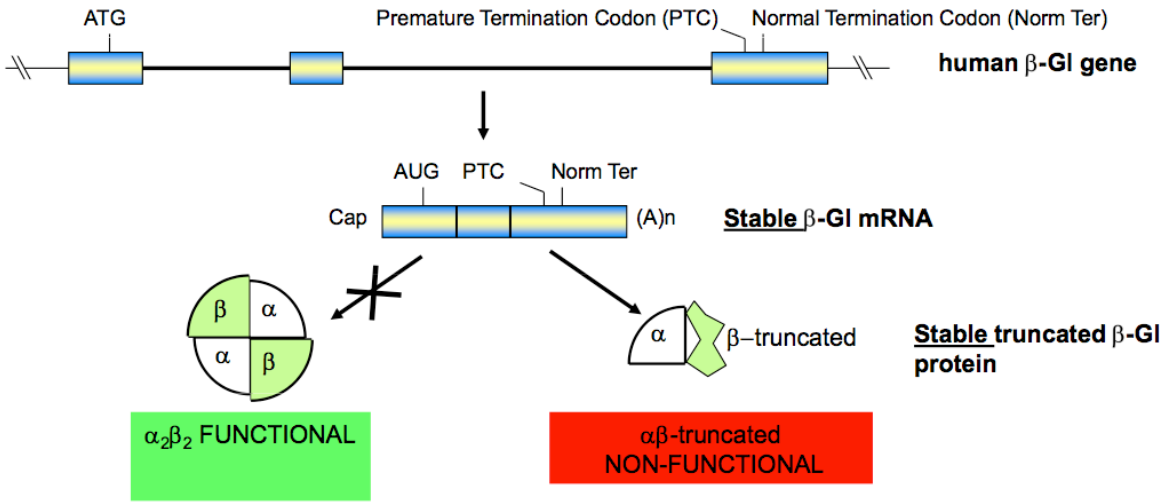
(B) Examples of alternative splicing patterns used to regulate gene expression via NMD. At low concentrations of the encoded protein, the default splicing pattern (top) results in a full-length open reading frame and stable protein expression. However, when the encoded protein concentration becomes too high, it alters splicing of its own message (bottom) to include a PTC. Inclusion of a "poison exon" that introduces a PTC is typical of SR proteins, which are usually splicing activators, whereas exon skipping is typical of heterogeneous ribonucleoproteins (hnRNPs), which tend to act as splicing repressors.

(C) The mammalian *Arc* gene contains two introns downstream of its normal stop codon, making the constitutively spliced mRNA a natural NMD target.

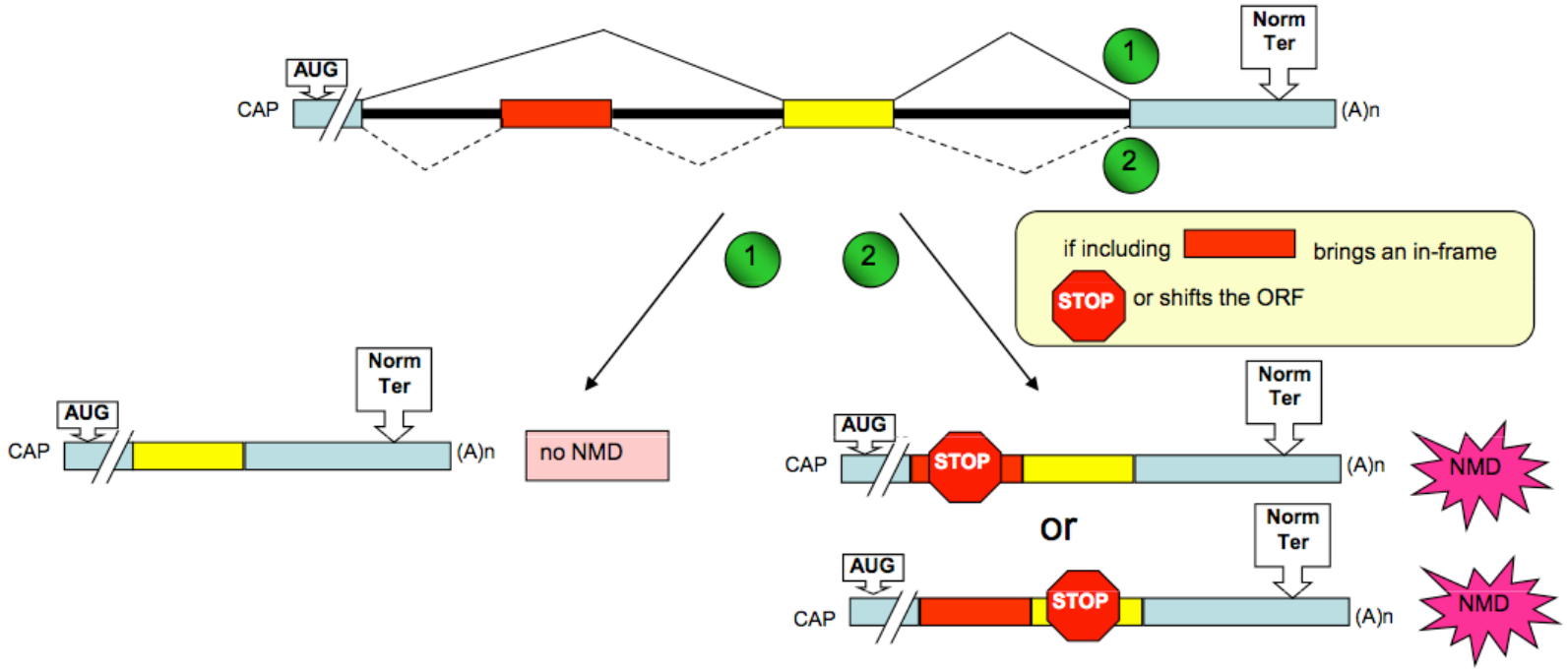
Moore and Proudfoot, Cell 136, 688 2009.

One example of cell toxicity when NMD does not occur

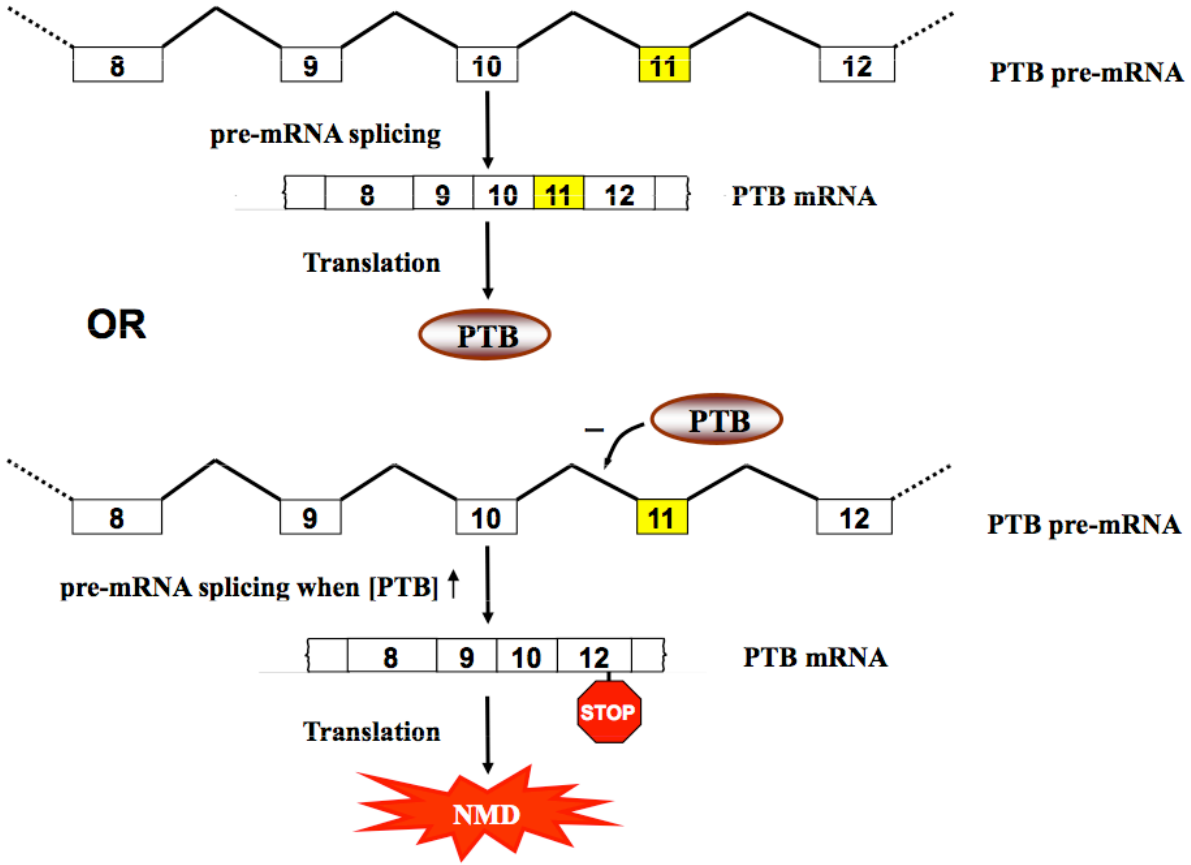
- Nonsense codons within the final exon of the human ß-globin gene, even though they are premature (or PTCs), do not elicit the degradation of ß-globin mRNA and result in a dominantly inherited form of the usually recessive disease thalassemia intermedia.



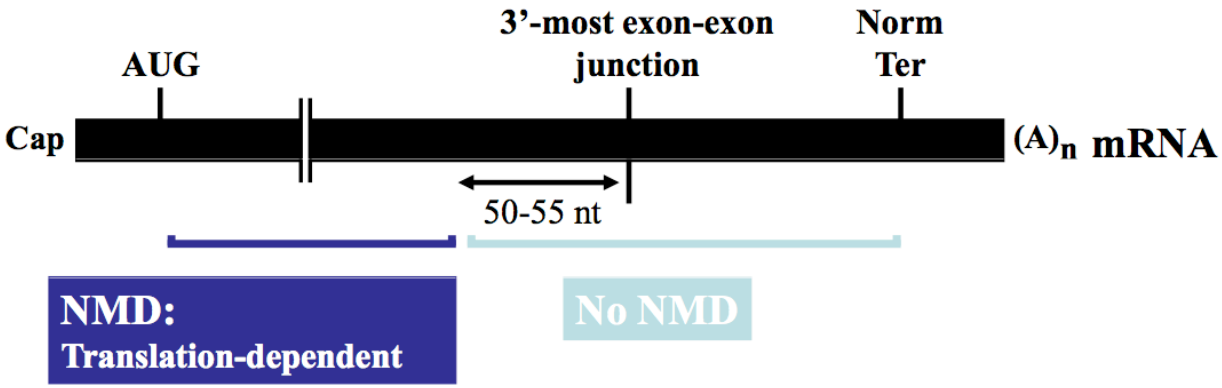
NMD regulates approximately 25-35% of all alternatively spliced human transcripts



Splicing factors can mediate alternative splicing –activated NMD as a means of negative feedback regulation



NMD in mammalian cells



Nagy and Maquat (1998) *Trends Biol. Sci* 23:198-199

Genetic conditions in which NMD can modulate phenotype

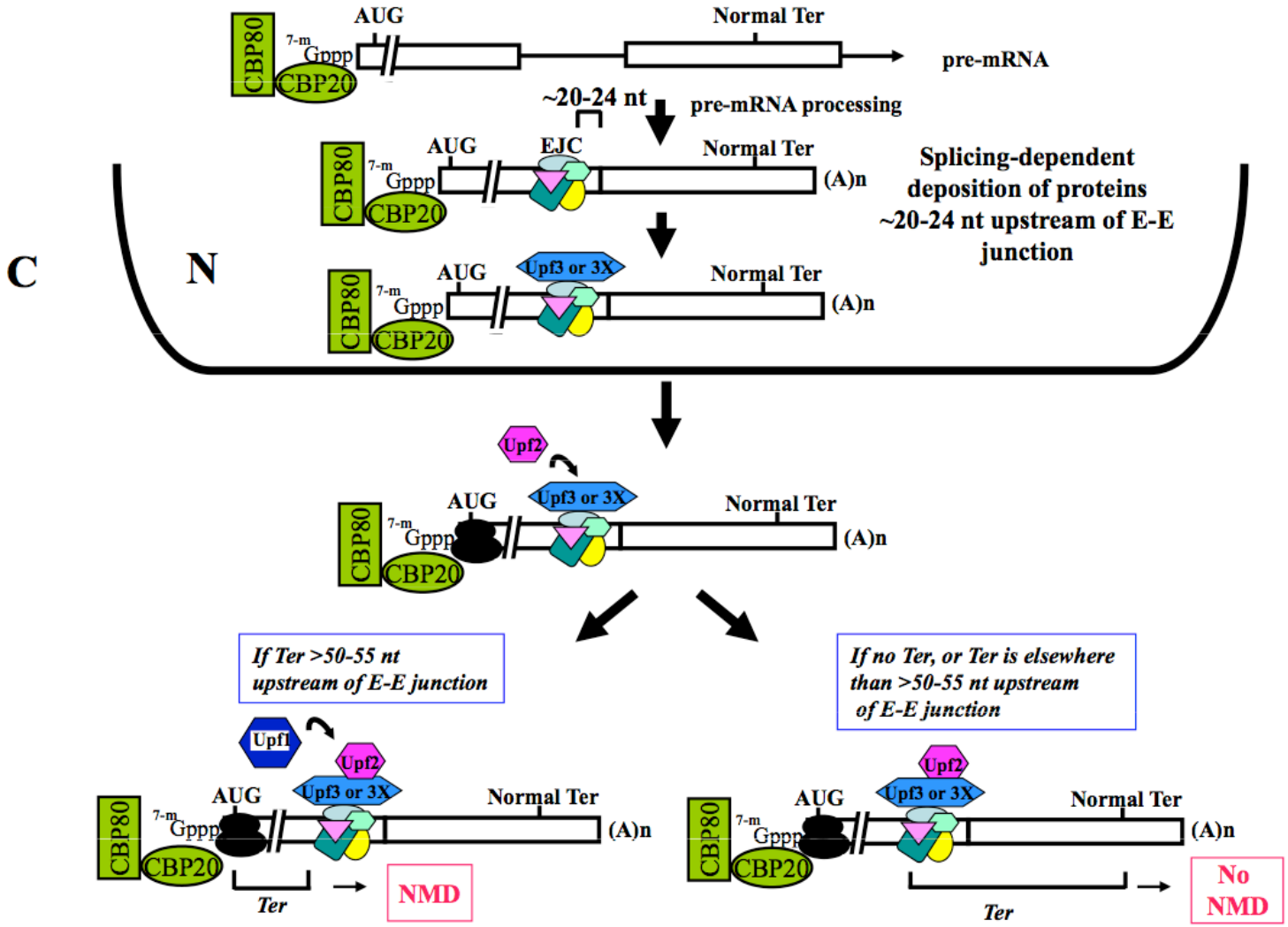
- Even when disease results from NMD-induced protein deficiency, the disease phenotype may be milder than and different from that caused by an expressed truncated protein.

- Therefore, NMD protects many heterozygous carriers of genes with PTCs from manifesting disease phenotypes that would result from expression of truncated proteins.

Gene	Mutation location	Effect of mutation
-globin (*HBB*)	5' to NMD boundary	Recessively inherited -thalassemia major; heterozygotes healthy
	3' to NMD boundary	Dominantly inherited -thalassemia intermedia
Interferon gamma receptor 1	5' to NMD boundary	Recessively inherited susceptibility to mycobacterial infection; heterozygotes healthy
	3' to NMD boundary	Dominantly inherited susceptibility to mycobacterial infection
Receptor tyrosine kinase-like orphan receptor 2 (*ROR2*)	5' to NMD boundary	Recessively inherited Robinow syndrome (orodental abnormalities, hypoplastic genitalia, multiple rib/vertebral anomalies); heterozygotes healthy
	3' to NMD boundary	Dominantly inherited brachydactyly type B (shortening of digits and metacarpals)
Cone-rod homeobox (*CRX*)	5' to NMD boundary	No homozygotes to date; mutation found in unaffected heterozygote
	3' to NMD boundary	Dominantly inherited retinal disease
von Willebrand factor (*VWF*)	5' to NMD boundary	Recessively inherited type 3 von Willebrand disease; heterozygotes healthy
	3' to NMD boundary	Dominantly inherited type 2A disease
Coagulation factor X (*F10*)	5' to NMD boundary	Recessively inherited bleeding tendency; heterozygotes healthy
	3' to NMD boundary	Dominantly inherited bleeding tendency
Rhodopsin (*RHO*)	5' to NMD boundary	Recessively inherited blindness; heterozygotes have abnormalities on retinogram but no clinical disease
	3' to NMD boundary	Dominantly inherited blindness
SRY-Box 10 (*SOX10*)	5' to NMD boundary	Haploinsufficiency leading to congenital neurosensory deafness and colonic agangliosis
	3' to NMD boundary	Dominantly inherited neural developmental defect including neurosensory deafness, colonic agangliosis, peripheral neuropathy and central dysmyelinating leukodystrophy

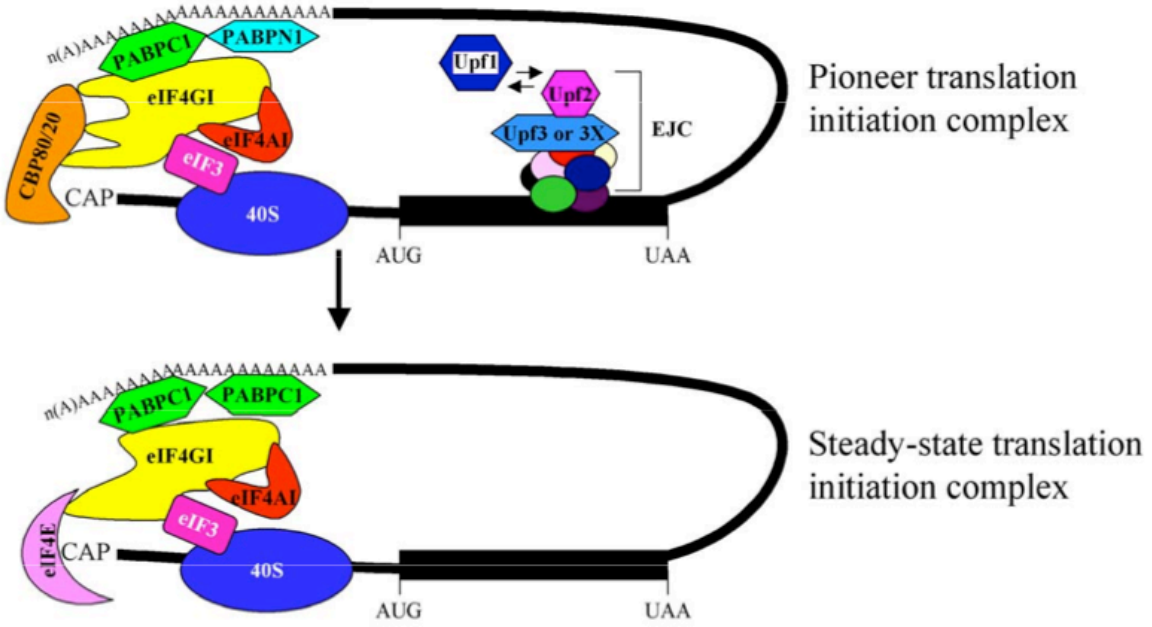
Adapted from Holbrook, Neu-Yilik, Hentze and Kulozik (2004) Nature Genetics 36: 801-8

The pioneer round of translation



UP-Frameshift suppresor proteins (UPF)

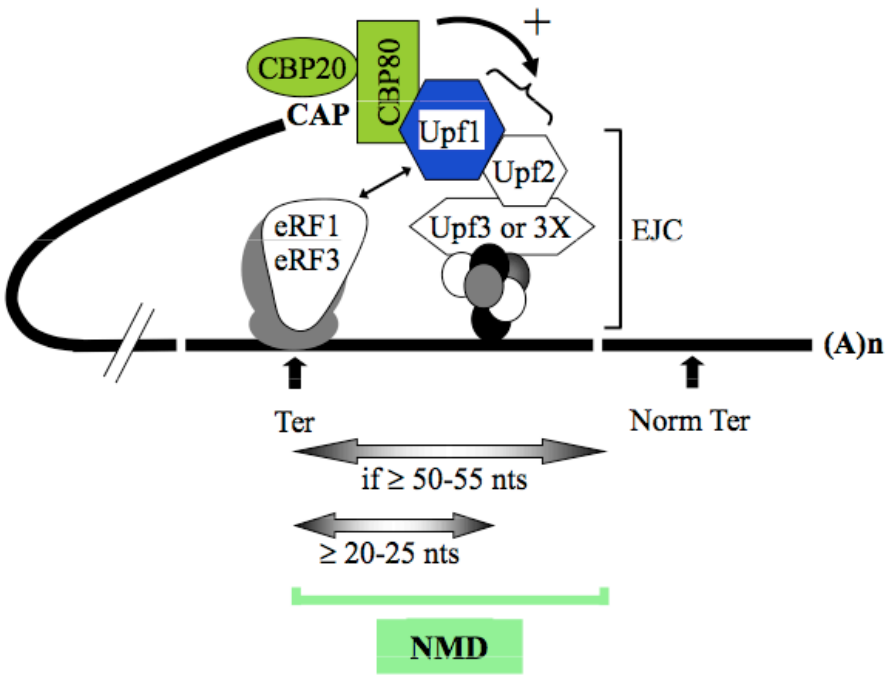
The pioneer translation initiation complex is functionally distinct from but structurally overlaps with the steady-state translation initiation complex



Lejeune, Ranganathan and Maquat (2004)
Nature Struct. Mol. Biol. **10:992-1000**

See also: **Ishigaki, Li, Serin, Maquat (2001)** *Cell* **106:607-617**
Lejeune, Ishigaki, Li, Maquat (2002) *EMBO J.* **21:3536-3545**
Chiu, Ranganathan, Lejeune, Maquat (2004) *Genes Dev.* **18:745-754**

CBP80 promotes the interaction of Upf1 and Upf2



Hosoda, Kim, Lejeune and Maquat (2005) *Nat. Struct. Mol. Biol.* **12:893-901**

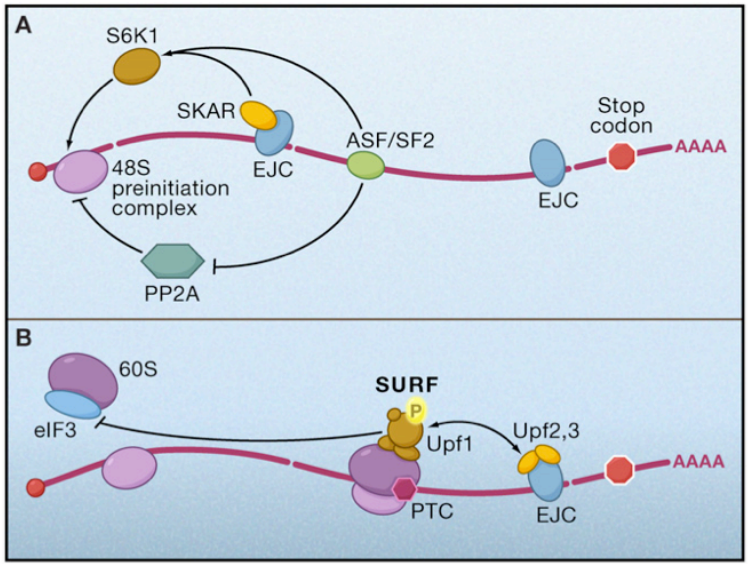


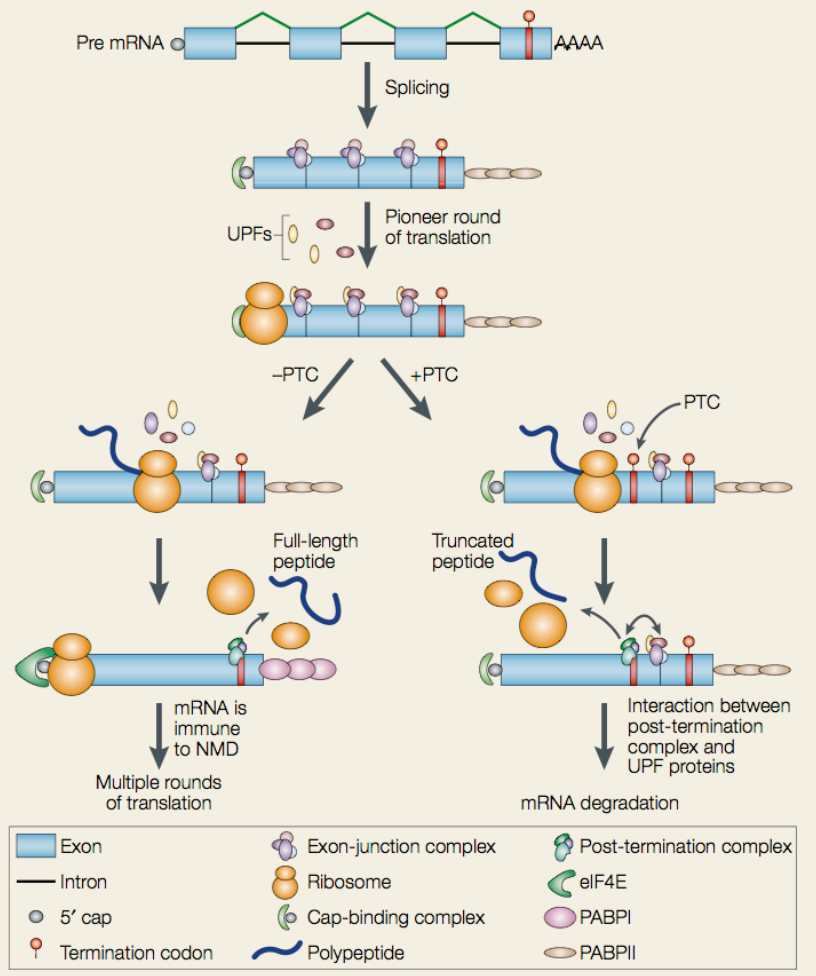
Figure 5. Splicing Factors Regulate Translation Initiation

Effects of splicing-dependent mRNP components on translation initiation

(A) Both the exon junction complex (EJC) and ASF/SF2 bound to spliced mRNA can promote the first or "pioneer" round of translation by recruiting 40S ribosomal protein S6 kinase 1 (S6K1), a component of the TOR signaling cascade. For the EJC pathway, this is accomplished via the EJC-interacting protein SKAR. ASF/SF2 can also promote translation initiation by inhibiting the S6K1 antagonist PP2A phosphatase.

(B) When an EJC is located more than 50 nucleotides downstream of a premature termination codon (PTC), interaction between the EJC and SURF complex causes phosphorylation of Upf1, which then inhibits additional rounds of translation by an interaction with the translation initiation factor eIF3.

Nonsense Mediated Decay Summary



Nonsense-mediated mRNA decay (NMD) is an mRNA surveillance mechanism that has been described from yeast to humans and ensures mRNA quality by selectively targeting mRNAs that harbour premature termination codons (PTCs) for rapid degradation[50,58,108]. PTCs that are introduced as a consequence of DNA rearrangements, frameshifts or nonsense mutations, or are caused by errors during transcription or splicing, can lead to non-functional or deleterious proteins.

PTCs in higher eukaryotes are only recognized as such when they occur upstream of a 'boundary' on the spliced mRNA that is situated ~55 nucleotides before the last exon–exon junction[108]. As summarized in the accompanying figure, the prevalent view of the NMD mechanism is that the splicing process leaves a 'mark' ~20 nucleotides upstream of each exon–exon boundary, in the form of an exon-junction complex (EJC), which in turn provides an anchor for up-frameshift suppressor proteins (UPFs)[108,109]. During the first ('pioneer') round of translation of a normal mRNA, the stop codon is located downstream of the last mark, and all EJCs are displaced by elongating ribosomes[110]. During subsequent rounds of translation, the cap-binding complex is replaced by eIF4E (eukaryotic initiation factor 4E) and PABPII (poly(A)-binding protein II) is replaced by PABPI, new ribosomes no longer encounter EJCs, and the mRNA is immune to NMD. However, when a PTC is present, ribosomes stop and fail to displace the downstream EJCs from the transcript. Interactions between the marking factors and components of the post-termination complex trigger mRNA decay.

Cartegni L, *et al*. Nat Rev Genet. 2002;3:285-98.

NMD readings

- Nonsense-mediated decay approaches the clinic Jill A Holbrook, Gabriele Neu-Yilik Matthias W Hentze& Andreas E Kulozik Nature Genetics 36, 801, 2004.
- Pre-mRNA Processing Reaches Back to Transcription and Ahead to Translation Melissa J. Moore and Nick J. Proudfoot. Cell 136, 688–700, February 20, 2009.
- LISTENING TO SILENCE AND UNDERSTANDING NONSENSE: EXONIC MUTATIONS THAT AFFECT SPLICING. Luca Cartegni,Shern L.Chew and Adrian R.Krainer. Nature Review Genetics , 3, 265, 2002