Nonsense-mediated and Staufen1-mediated mRNA decay: Related pathways of post-transcriptional control in mammalian cells

Prof. Lynne E. Maquat

Nonsense-mediated mRNA decay (NMD)

Premature Translation Termination

If Termination is Sufficiently Premature

Truncated protein

Anemias

- β-thalassemia, a deficiency in the β-globin component of hemoglobin
- Triosephosphate isomerase deficiency, a deficiency in the glycolytic enzyme triosephosphate isomerase

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Topics of first half of talk

- Significance of the pioneer translation initiation complex to NMD
  
  NMD not only targets mRNA bound by CBP80-CBP20 and at least one EJC
  but CBP80 actually promotes NMD by stabilizing the interaction of NMD factors at the EJC

- Degradative enzymology of NMD

Purpose of NMD

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

As such, NMD can be viewed as a type of "mRNA quality control" or "mRNA surveillance" mechanism

It makes sense to eliminate abnormal, potentially harmful transcripts early in their biogenesis

To do so, cells utilize nonsense codon recognition during a pioneer round of translation to elicit NMD

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One example of cell toxicity when NMD fails to occur
• Nonsense codons within the final exon of the human β-globin gene, even though they are premature (or PTCs), do not elicit the degradation of β-globin mRNA and result in a dominantly inherited form of the usually recessive disease thalassemia intermedia.

NMD also down-regulates ~35% of all alternatively spliced human transcripts

• Considering that at least 74% of human genes are thought to encode pre-mRNA that harbors at least one alternative exon, the number of protein isoforms regulated by NMD could be large.

• However, it may be that NMD mostly targets alternatively spliced transcripts that are not largely productive.
  • The level of alternatively spliced transcripts that are NMD targets is usually too low to affect cell metabolism.
  • Alternatively spliced transcripts that are NMD targets are rarely subject to tissue-specific regulation.

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Glutathione peroxidase (GPx)1 RNA


Importance of NMD

- Mouse embryos that are inactive in NMD resorb shortly after implantation
- Blastocysts that are isolated 3.5 days post-coitum and that are inactive in NMD undergo apoptosis in culture after a brief growth period


NMD in mammalian cells


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

CBP80 promotes the interaction of Upf1 and Upf2.
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Down-regulating CBP80 inhibits NMD that is elicited by tethering Upf2 or Upf3 but not Upf1 downstream of a termination codon

Since Upf1 is the last of the Upf proteins to function in NMD, and since Upf1 is recruited to mRNA by Upf2, these data suggest that CBP80 may no longer be necessary for NMD once Upf1 interacts with Upf2

Down-regulating CBP80 inhibits the NMD of Gl mRNA

Evidence that CBP80 interacts with Upf1

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Down-regulating CBP80 inhibits the co-immunopurification of Upf1 and Upf2

CBP80 promotes the interaction of Upf1 and Upf2

Degradative enzymology of NMD

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Experimental Results

EJC-independent SMD

EJC-dependent, CBP80-promoted NMD

Human Upf1 interacts with human Staufen1

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Tethering human Staufen1 downstream of a termination codon reduces mRNA abundance

Down-regulating Upf1 but not Upf2 or Upf3X inhibits this reduction in mRNA abundance

Presuming translation upstream of the site of Staufen1 tethering inhibits this reduction in mRNA abundance

These data suggest that Staufen1 binding to an mRNA 3'UTR elicits mRNA decay by recruiting Upf1 independently of Upf2 or Upf3X but dependent on translation terminating normally


Staufen1 binding to the Arf1 3'UTR reduces Arf1 mRNA half-life

Therefore, Arf1 mRNA is targeted for Staufen1-mediated mRNA decay

Results from three independent microarray analyses in which Staufen1 had been downregulated demonstrated that 1.1% of those HeLa-cell transcripts analyzed were upregulated at least 2-fold

Therefore, SMD may be widely used by cells as a means of posttranscriptional control

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CBP80 promotes NMD but not SMD
Tethering Staufen1 downstream of a termination codon reduces mRNA abundance in a way that is not affected by down-regulating CBP80

down-regulating CBP80 does not inhibit the SMD of fos-Fluc mRNA

SMD targets both CBP80-bound mRNA and eIF4E-bound mRNA
4E-BP1, which inhibits the translation of eIF4E-bound mRNA but not CBP80-bound mRNA, inhibits the reduction in mRNA abundance brought about by tethering Staufen1 downstream of a termination codon


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Summary
The double-stranded RNA binding protein Staufen 1 can recruit the Upf1 NMD factor to the 3'UTR to target CBP80-bound but not detectably eIF4E-bound mRNA when translation terminates normally. This pathway is called Staufen1-mediated mRNA decay (SMD). CBP80 does not promote the interaction of Staufen1 and Upf1, and SMD targets both CBP80-bound and eIF4E-bound mRNA.
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