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Gene expression networks: competing mRNA decay pathways in mammalian cells

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Abstract

Nonsense-mediated mRNA decay and Staufen1-mediated mRNA decay are mechanistically related pathways that serve distinct purposes. In the present article, we give an overview of each pathway. We describe how a factor that is common to both pathways results in their competition. We also explain how competition between the two pathways contributes to the differentiation of C2C12 myoblasts to multinucleated myotubes.

Keywords

eukaryotic initiation factor 4E (eIF4E); exon junction complex (EJC); homoeostatic control of genes; myogenesis; nonsense-mediated mRNA decay (NMD); Staufen1

Introduction

mRNA decay has long been appreciated to constitute a step that is critical for the proper regulation of gene expression (for recent reviews, see [1,2]). In the present paper, we review what is known about the functional significance of two related mRNA decay pathways: NMD (nonsense-mediated mRNA decay) and SMD [STAU1 (Staufen1)-mediated mRNA decay]. We present evidence for the mechanistic convergence of the two pathways in a way that results in their competition. We also illustrate how competition feeds into the complex network of post-transcriptional regulatory steps that leads to skeletal-muscle myoblast maintenance in an undifferentiated state or differentiation to multinucleated myotubes.

NMD

NMD provides a means by which eukaryotic cells control the quality of gene expression: NMD generally eliminates mRNAs that prematurely terminate translation because they harbour either a frameshift or a nonsense mutation (see, e.g., [3–14]). NMD apparently evolved to protect cells from routine inaccuracies in gene expression that result in the production of truncated proteins, which have the potential to acquire dominant-negative or gain-of-function activities that could be deleterious to cells. For example, approximately one-third of alternatively spliced transcripts are NMD targets [15], which findings indicate are largely generated by mistakes made during pre-mRNA splicing [16]. Therefore NMD provides an important mechanism whereby cells ensure the quality of mRNA function and, as a consequence, the quality of gene expression. NMD also eliminates genomic noise, such as non-functional transcripts that have assimilated transposons or retroviral sequences [17].

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Additionally, NMD serves regulatory functions by targeting physiological transcripts that harbour, e.g. an upstream open translational reading frame [ORF (open reading frame)] or an intron within their 3′-UTR (3′-untranslated region) [17–19]. NMD further functions in the homoeostatic control of genes that encode serine-arginine-rich proteins and heterogeneous nuclear RNP (ribonucleoprotein) splicing factors [20–28]. Thus NMD degrades both abnormal and normal transcripts, the latter to establish appropriate levels of gene expression.

The importance of NMD in mammals has been inferred from a number of studies. For example, mouse embryos that lack the NMD factor UPF1 are resorbed shortly after implantation, and mouse blastocysts isolated 3.5 days post-coitum that lack UPF1 undergo apoptosis after a brief growth period [29]. As another example, haemopoietic stem and progenitor cells that lack the NMD factor UPF2 fail to proliferate in part because of the up-regulation of a battery of transcripts that include RNAs deriving from processed pseudogenes, non-productive DNA rearrangements and alternative splicing [18]. However, UPF1, which is a member of the RNA helicase superfamily 1 that manifests RNA-dependent ATP hydrolytic and 5′–3′ ATP-dependent unwinding activities *in vitro* [30–32], and UPF2, the activity of which is less well characterized, function in pathways other than NMD [6]. For example, both UPF1 and UPF2 regulate the binding of telomeric repeat-containing RNA to telomeres and are enriched at telomeres compared with Alu sequences [33]. Furthermore, amino acids of UPF1 that associate with UPF2 during NMD can alternatively bind to STAU1 during SMD so that inhibiting the efficiency of NMD augments the efficiency of SMD ([34], see below). Therefore, although NMD is unquestionably important for cellular growth and maintenance, it has not yet been possible to tease apart critical contributions of NMD from critical contributions of other UPF factor-dependent pathways.

As a rule, NMD in mammalian cells degrades newly synthesized mRNAs during a `pioneer' round of translation. This round of translation utilizes mRNA that is associated with the mostly nuclear yet shuttling CBP (cap-binding protein) heterodimer CBP80–CBP20: studies demonstrating that translational repression is critical for NMD suggest that the pioneer round involves the loading of not only the ribosome that recognizes the nonsense codon but also at least one 43S pre-initiation complex that can be targeted for repression during NMD (Figure 1) [35–42]. Provided the mRNA derives from pre-mRNA splicing, the pioneer translation–initiation complex would also contain a post-splicing EJC (exon junction complex) situated upstream of each exon–exon junction [5,43,44]. The pioneer round precedes subsequent rounds of `steady-state' translation. Steady-state rounds utilize mRNA that is the remodelled product of CBP80–CBP20-bound mRNA so as to contain eIF (eukaryotic initiation factor) 4E at the cap, and they do not detectably support NMD in mammalian cells. The immunity of eIF4E-bound mRNA to NMD can be explained not only because of the lack of CBP80–CBP20 but also because of the absence of detectable EJCs [35–37,40,45,46], which are removed by the pioneer round of translation ([47,48]; H. Sato and L.E. Maquat, unpublished work).

According to the current model (Figure 1), NMD depends on joining of the PI3K (phosphatidylinositol 3-kinase)-related protein kinase SMG1 and UPF1 with the two translation termination factors eRF1 and eRF3 to form the SURF complex at the site of translation termination [39,49]. If an EJC exists sufficiently downstream of the termination event, then SURF-derived SMG1 and UPF1 are thought to bind to that EJC, possibly via SURF, forming a bridge between the termination codon and EJC, so as to trigger UPF1 phosphorylation. UPF1 phosphorylation then results in translational repression by augmenting UPF1 binding to the eIF3 constituent of a 43S pre-initiation complex that is poised at the mRNA translation initiation codon in a way that inhibits 60S ribosomal subunit

joining [38]. UPF1 phosphorylation also activates the recruitment of ribonucleolytic activities [38] that degrade NMD targets from either end, beginning with decapping or deadenylation [50–54]. Recently, NMD has been shown to additionally involve some degree of SMG6-mediated endonucleolytic cleavage [55–57]. Furthermore, NMD can target mRNAs that occur in the absence of splicing occurring downstream of a PTC provided that splicing occurred upstream of the PTC, presumably reflecting the need for a post-splicing EJC situated 5′ to the PTC [8,45].

SMD

SMD, unlike NMD, targets both CBP80–CBP20-bound and eIF4E-bound mRNAs that harbour an SBS (STAU1-binding site) sufficiently downstream of their normal termination codon (Figure 2) and it occurs independently of splicing [36,58]. During SMD, when translation terminates sufficiently upstream of an SBS, UPF1 binding to the SBS via the dsRNA (double-stranded RNA)-binding protein STAU1 is thought to trigger mRNA decay [58,59]. Thus a STAU1-associated SBS functions during SMD analogously to how an EJC-associated exon–exon junction functions during NMD: translation–termination sufficiently upstream of, respectively, SBS-bound STAU1 or the EJC triggers mRNA decay. Since UPF1 binds directly to STAU1, SMD, unlike NMD, does not require EJC constituents (including UPF2, UPF3 or UPF3X) or CBP80–CBP20 [36,58]. Steps in the SMD pathway after UPF1 binds to an SBS via STAU1 may be similar to those in the NMD pathway after UPF1 binds to an EJC via UPF2.

In contrast with NMD, which serves largely as a quality-control mechanism, SMD provides a means to conditionally down-regulate the expression of genes encoding mRNAs that contain an SBS in their 3′-UTRs. Conditionally regulated pathways that modulate mRNA half-life would be expected to target eIF4E-bound mRNA, which constitutes the bulk of cellular mRNA. While SMD targets were initially identified by microarray analyses of transcripts that bind STAU1 [58], once it was realized that STAU1 binding to an mRNA can elicit the decay of that mRNA, it became apparent that a more sensitive approach to identify SMD targets would use microarrays to assay for mRNAs that are up-regulated upon STAU1 depletion. In three independently performed experiments, conservatively 1.1% of the 11569 HeLa-cell transcripts that were analysed were found to be up-regulated upon STAU1 down-regulation, and a number of these have been proven to be SMD targets based on studies that demonstrate STAU1 binding to their 3′-UTRs in a way that shortens mRNA half-life [59]. To date, the SBS of human ARF1 (ADP-ribosylation factor 1) mRNA is the best-characterized SBS, consisting of a 19-bp stem-loop structure in which base-pairing appears to be more critical for STAU1 binding than the precise constitution of the base-paired region [59]. Nevertheless, the exact nature of this and other SBSs has yet to be defined.

As for NMD, it is difficult to tease apart the importance of SMD to mammalian-cell metabolism since, as noted, the efficiency of SMD is influenced by the efficiency of NMD. Furthermore, STAU1 functions not only in SMD but also in other capacities. As examples, STAU1 is involved in dendritic RNP localization that when inhibited correlates with impaired dendritic outgrowth and spine formation [60]. Additionally, STAU1 that has been engineered to bind a 5′-UTR can facilitate mRNA translation [61]. Thus, although STAU1 has been demonstrated to have an essential role in embryonic stem cell differentiation [62], it is unclear how SMD contributes to this essential role.

Evidence for competition between NMD and SMD

Remarkably, the function of UPF1 in both SMD and NMD results in competition between the two pathways (Figure 3) [34]. STAU1- and UPF2-binding sites within UPF1 not only overlap but are also mutually exclusive so that the IP (immunoprecipitation) of STAU1

precludes the detectable co-IP of UPF2 and vice versa. Furthermore, down-regulating the cellular abundance of STAU1, which inhibits SMD, increases the efficiency of NMD, whereas down-regulating the cellular abundance of UPF2, which inhibits NMD, increases the efficiency of SMD. Competition under physiological conditions is exemplified during the differentiation of mouse C2C12 myoblasts to myotubes, which is accompanied by a smaller decrease in the level of STAU1 relative to UPF1 than the level of UPF2 relative to the level of UPF1: during differentiation, the efficiency of SMD increases while the efficiency of NMD decreases, consistent with the finding that more STAU1 but less UPF2 binds UPF1 in differentiated myotubes compared with undifferentiated myoblasts. The functional significance of the remarkable balance between SMD and of NMD becomes apparent with the finding that PAX3 mRNA, whose decay promotes myogenesis [63], is an SMD target, and myogenin mRNA, which encodes a protein that is required for myogenesis [64], is an NMD target.

To further complicate the picture, myogenesis is also accompanied by an increase in the cellular level of UPF3X [34]. This results in an increase in the efficiency of an alternative NMD pathway that, unlike `classical' NMD is largely insensitive to UPF2 down-regulation [25,65]. As a consequence, myogenesis brings about a decreased level of SC1.7 and SC1.6 mRNAs, which are two well-characterized targets of alternative NMD that encode splicing variants of the SC35 splicing factor [34].

Summary

Future studies of SMD and classical and alternative NMD pathways during myogenesis and other cellular processes are certain to elucidate additional targets of post-transcriptional regulation that contribute, along with mechanisms of transcriptional control, to the complex and interdependent network of regulatory events that are required for the maintenance of or progression to distinct cell types.

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Abbreviations used

CBP	cap-binding protein
eIF	eukaryotic initiation factor
EJC	exon junction complex
IP	immunoprecipitation
NMD	nonsense-mediated mRNA decay
PI3K	phosphoinositide 3-kinase
SMD	STAU1 (Staufen1)-mediated mRNA decay
SBS	STAU1-binding site
RNP	ribonucleoprotein
3′-UTR	3′-untranslated region

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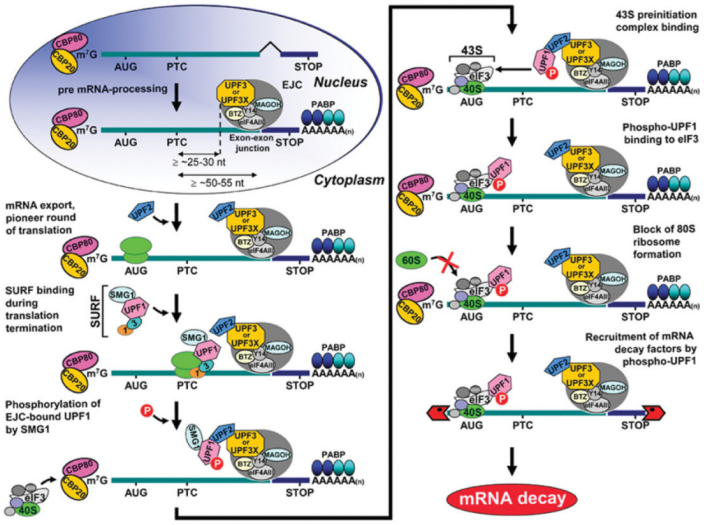

Figure 1. Model for NMD

In mammals, newly synthesized CPB80–CBP20-bound 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 (mago-nashi homologue), Barentz (BTZ) and many other proteins. The UPF3 (also called UPF3a) or UPF3X (also called UPF3b) NMD factor is mostly nuclear but shuttles to the cytoplasm and is thought to join EJCs in the nucleus so as to be exported with mRNA to the cytoplasm. In the cytoplasm, UPF3 or UPF3X recruits UPF2. The translation of CBP80–CBP20-bound mRNA constitutes the pioneer round. Translation termination during the pioneer round at a premature termination codon (PTC) that is situated ≥50–55 nt upstream of an exon-exon junction (i.e. ≥25–30 nt upstream of an EJC) involves the SURF complex, which consists of the PI3K-related protein kinase that phosphorylates UPF1, SMG1, together with UPF1, eRF1 and eRF3. As a consequence, NMD generally occurs. During the process, UPF1 together with SMG1 is thought to bind EJC-associated UPF2 in a way that is promoted by CBP80. UPF1 binding to the EJC results in UPF1 phosphorylation. Phospho-UPF1 triggers NMD by promoting translational repression of the NMD target. Translational repression involves the binding of phospho-UPF1 to eIF3 within the 43S pre-initiation complex that is poised at the AUG translation initiation codon so as to prevent 60S ribosomal subunit joining. Phospho-UPF1 also promotes NMD by recruiting mRNA degradative activities. Not shown are SMG5, SMG6 and SMG7, which activate UPF1 dephosphorylation and thus recycling. SMG6 appears to additionally function as an endonuclease. Very recently, roles for SMG8 and SMG9 as SMG1-interacting proteins have been defined [49]. Notably, mammalian-cell NMD can also target mRNAs that have not undergone splicing downstream of a PTC, in a mechanism that has been called failsafe NMD or EJC-dependent NMD, provided that they have undergone a splicing event upstream of the PTC [5,8]. Nucleolytic activities are indicated by the red irregular hexagons. PABP, poly(A)-binding protein, where darker shapes specify the largely nuclear PABPN1 and lighter shapes denote the largely cytoplasmic PABPC1; AUG, translation initiation codon; STOP, normal termination codon; 1, eRF1; 3, eRF3.

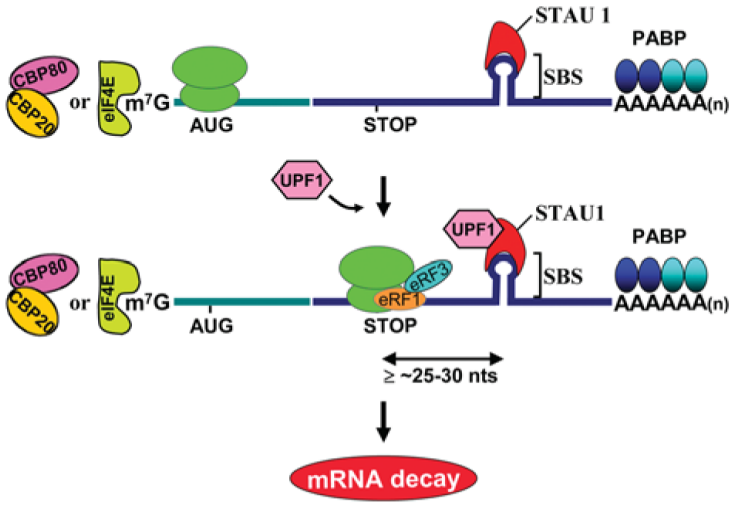

Figure 2. Model for SMD

In mammals, SMD targets both newly synthesized CBP80–CBP20-bound mRNAs and the corresponding steady-state eIF4E-bound mRNAs provided they contain an SBS ≥25–30 nt downstream of the normal termination codon. According to current thinking, when translation terminates sufficiently upstream of SBS-bound STAU1, UPF1 binds STAU1. UPF1 binding then triggers mRNA decay, presumably analogously to how UPF1 binding to an EJC during NMD triggers mRNA decay. AUG, translation initiation codon; STOP, normal translation termination codon; PABP, PABPN1 and/or PABPC1 depending on the cap-binding complex.

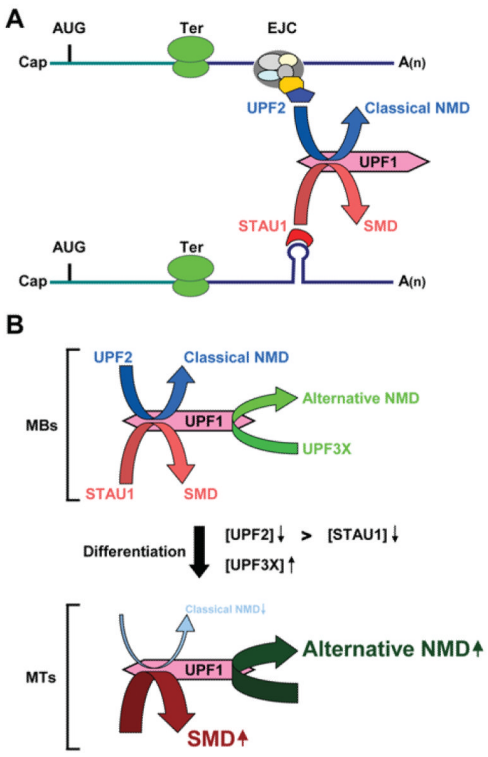

Figure 3. Model for competition between SMD and NMD: competition contributes to the differentiation of C2C12 myoblasts to myotubes

(**A**) UPF2, which is an EJC constituent and functions in the classical NMD pathway, and STAU1, which is an RNA-binding protein that functions in the SMD pathway, compete for binding to UPF1 and thus the recruitment of UPF1 to mRNA. UPF1 functions in both pathways to elicit mRNA decay when translation terminates sufficiently upstream of an EJC, in the case of NMD, or STAU1 that is associated with an SBS, in the case of SMD. Ter can be either a premature or a normal termination codon.

(**B**) As a consequence of C2C12-cell differentiation from myoblasts (MBs) to myotubes (MTs), the efficiency of SMD increases, the efficiency of classical, i.e. UPF2-dependent, NMD decreases, and the efficiency of an alternative NMD pathway that relies on UPF3X, but not appreciably on UPF2, increases. During myogenesis, a larger decrease in the abundance of UPF2, which drops to almost undetectable levels relative to STAU1, permits STAU1 to out-compete UPF2 for binding to UPF1, and a ~4-fold increase in the level of UPF3X supports an increase in the efficiency of the alternative NMD pathway.