Nonsense-mediated mRNA decay in mammalian cells: Splicing-dependent degradation that occurs 5'-to-3' and 3'-to-5' as a consequence of a "pioneer" round of translation

Fabrice Lejeune, Shang-Yi Chiu, Aparna Ranganathan, Yoon Ki Kim, Maciej Kmieciak, Qinshan Gao and Lynne E. Maquat

Department of Biochemistry and Biophysics, School of Medicine and Dentistry, 601 Elmwood Avenue, Box 712, University of Rochester, Rochester, New York, USA

Nonsense-mediated mRNA decay (NMD) in mammalian cells is generally a splicing-dependent mechanism by which cells recognize and degrade mRNAs that prematurely terminate translation during a "pioneer" round of translation. The dependence on pre-mRNA splicing reflects the deposition of exon junction complexes (EJCs) on mRNA. These EJCs then recruit the Upf NMD factors. We recently demonstrated that NMD degrades mRNA from both 5' and 3' ends by recruiting decapping and 5'-to-3' exonuclease activities as well as deadenylating and 3'-to-5' exonuclease activities[1]. In short, down-regulating the Dcp2 decapping protein, the PM/Scl100 component of the exosome, or poly(A) ribonuclease (PARN) was found to abrogate both nucleus-associated and cytoplasmic NMD. Consistent with these results, NMD factors Upf1, Upf2 and Upf3X co-immunopurify with the Dcp2 decapping protein, Rat1 and Xrn1 5'-to-3' exonucleases, PM/Scl100 and Rrp41 components of the exosome, and PARN. Additional studies of the enzymology of NMD will be presented.

The bulk of cellular proteins derive from translation of mRNA that is bound at the 5' cap by eukaryotic translation initiation factor (eIF)4E. However, our studies of NMD indicate that mRNA that is bound by cap binding protein (CBP)80, which is a precursor to eIF4E-bound mRNA, can also be translated during what we call a "pioneer" round of translation[2-4]. We find that the pioneer round, which can be assessed by measuring NMD, is not inhibited by 4E-BP1, which is known to inhibit steady-state translation by competing with eIF4G for binding to eIF4E. Therefore, at least in this way, the pioneer round of translation is mechanistically distinct from steady-state translation. eIF4GI, poly(A) binding protein (PABP)1, eIF3, eIF4AI, and eIF2α co-immunopurify with both CBP80 and eIF4E, which suggests that each factor functions in both modes of translation. Consistent with roles for PAPB1 and eIF2α in the pioneer round, Paip2, which is known to destabilize PAPB1 binding to poly(A) and inhibit steady-state translation, and inactive eIF2α, which is also known to inhibit steady-state translation, also inhibit NMD. Polysome profiles indicate that CBP80-bound mRNAs are translated less efficiently than their eIF4E-bound counterparts.

As noted above, depending on the particular mRNA, NMD can take place in association with nuclei or in the cytoplasm. Differences between the two types of NMD will be discussed.

[1]Lejeune F, Li X, and Maquat LE (2003) Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylating, and exonucleolytic activities. *Mol. Cell* 12:675-87.

[2] Ishigaki Y, Li X, Serin G, and Maquat, LE (2001) Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell* 106:607-617.

[3] Lejeune F, Ishigaki Y, Li X, and Maquat LE (2002) The exon junction complex is detected on CBP80-bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling. *EMBO J.* 21:3536-3545.

[4]Chiu S-Y, Lejeune F, Ranganathan A., and Maquat LE (2004) The pioneer translation initiation complex is functionally distinct from but structurally overlaps with the steady-state translation initiation complex. *Genes & Dev*, in press.