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mRNA DECAY

Cutting in the middle

The eukaryotic exosome is a 3′→5′ exonuclease complex that is responsible for the processing, quality control and turnover of many cellular RNAs. Now, two studies in yeast reveal that the exosome also has endonucleolytic activity. Further, a study in humans shows that endonucleolytic cleavage occurs during nonsense-mediated mRNA decay (NMD).

The exosome consists of nine catalytically inactive subunits and the Dis3 subunit (also known as Rrp44), which has a catalytic RNB exonuclease domain and a conserved amino-terminal PIN domain. Inspired by recent crystal structures of other PIN domains, which revealed similarities to the catalytic core of the endonuclease RNase H, Lebreton *et al.* and Schaeffer *et al.* independently tested whether the PIN domain of Dis3 has nuclease activity. Lebreton *et al.* found that a RNB-domain mutant that lacks exonucleolytic activity degraded RNA substrates *in vitro*; however, this ability was lost when additional mutations in the predicted catalytic centre of the PIN domain were present. Schaeffer *et al.* found that a truncated Dis3 protein had ribonucleolytic activity, which was lost when the PIN domain was mutated. The similarity of degradation profiles of 5′-labelled and 3′-labelled RNA substrates showed that the PIN domain of Dis3 has endonucleolytic activity (exonucleases yield mononucleotides either with 5′- or 3′-labelled substrates, whereas endonucleases yield oligonucleotides with both substrates).

Next, both groups studied the growth phenotypes of wild-type cells, PIN-domain mutants that lacked endonuclease activity and/or mutants that lacked exonuclease activity *in vivo*. Simultaneous inactivation of the endonucleolytic and exonucleolytic activities of the exosome impaired cell growth. In northern blot analyses of total RNA from wild-type and mutant strains, Lebreton *et al.* also showed that the endonucleolytic activity of the PIN domain of Dis3 contributes to the degradation of some natural exosome substrates. These data support the physiological role of the PIN domain.

In human cells, NMD — a surveillance process that eliminates transcripts that cause premature termination of translation — is thought to occur by exonucleolysis. Eberle *et al.* knocked down various exonucleases by RNA interference (RNAi) and assayed the levels of NMD reporter transcripts. Knocking down the predominant 5′→3′ exonuclease XRN1 did not lead to increased levels of full-length premature termination codon (PTC)-containing transcripts, but shorter RNA fragments were generated by endonucleolytic cleavage near the PTC. RNAi-mediated knockdown of SMG6, a NMD factor that contains a PIN domain, abolished endonucleolytic cleavage of NMD reporter transcripts. This defect was rescued by the expression of wild-type SMG6 but not of PIN-domain mutants. Furthermore, the bacterially expressed PIN domain of SMG6 was able to cut circular RNA *in vitro*. These findings point to SMG6 as the endonuclease involved in human NMD.

Together, these studies show that the mode of action of the eukaryotic exosome in RNA processing and degradation and the mechanism of NMD should be revisited, taking into account the importance of endonucleolytic cleavage.

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ORIGINAL RESEARCH PAPERS Lebreton, A. *et al.* Endonucleolytic RNA cleavage by a eukaryotic exosome. *Nature* **456**, 993–996 | Schaeffer, D. *et al.* The exosome contains domains with specific endonuclease, exoribonuclease and cytoplasm mRNA decay activities. *Nature Struct. Mol. Biol.* 7 Dec 2008 (doi:10.1038/nsmb.1528) | Eberle, A. B. *et al.* SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. *Nature Struct. Mol. Biol.* 7 Dec 2008 (doi:10.1038/nsmb.1530)
FURTHER READING Houseley, J. *et al.* RNA-quality control by the exosome. *Nature Rev. Mol. Cell Biol.* **7**, 529–539 (2006) | Garneau, N. L. *et al.* The highways and byways of mRNA decay. *Nature Rev. Mol. Cell Biol.* **8**, 113–126 (2007)