favor the transport of noncondensable gases outside the polar region.

Another aspect of the martian polar night atmosphere that is far from understood is the formation of CO_2 ice clouds and snowfall. Although it is thought that most of the carbonic ice directly condenses on the surface, a fraction should also condense in the atmosphere, strongly influencing the radiative properties of the atmosphere and the martian surface (*3*). Most of these clouds form in the polar night, and thus evidence of their existence has remained theoretical (*4*) or indirect (*3*). It is only with the advent of the Mars Global Surveyor laser altimeter MOLA—which acts as a light detection and ranging (LIDAR) instrument—that a variety of cloud shapes varying over space and time have been observed (*5*). There have been several attempts to model the complex behavior of these clouds, which seem to form in topography-induced updrafts, buoyancy waves in the lee of mountains, or even in exotic convection cells (*4*, *6*, *7*). One difficulty is that, because CO_2 is the major constituent of the atmosphere, the microphysics of martian CO_2 ice clouds is unlike that of any clouds on Earth or on other planets of the Solar System.

At the end of the polar night, condensation stops, but the behavior of CO_2 ice does not become simple (*8*). The sublimation of the frozen atmospheric layer is characterized by spectacular albedo changes (*9*) and explosive gas eruptions that erode the surface year after year (*10*), forming curious dark spots of multiple shapes (*8*) (see the figure). In the southern hemisphere each year, a large part of the cap (the so-called cryptic regions) remains quite transparent and dark and rapidly sublimates (see the figure). In contrast, other areas at the same longitude become very bright and ultimately outlast the summer to form the perennial CO_2 ice cap at the south pole (*9*). This geographical distribution still has not been explained. Furthermore, the existence of the perennial CO_2 ice cap, a relatively thin (*11*) frozen atmospheric reservoir near the south pole, is puzzling. Any changes in its albedo or the evolution of the planet's orbital parameters (which are highly variable) would make the CO_2 ice cap either disappear or grow much bigger within a few years. Somewhere hidden in the alien meteorology that controls the formation of the martian CO_2 ice cap, there must be some stabilizing feedbacks that remain to be discovered.

References

1. A. L. Sprague *et al.*, *Science* **306**, 1364 (2004); published online 7 October 2004 (10.1126/science.1098496).
2. S. Hess, *J. Geophys. Res.* **84**, 2969 (1979).
3. F. Forget *et al.*, *J. Geophys. Res.* **100**, 21219 (1995).
4. F. Forget *et al.*, *Icarus* **131**, 302 (1998).
5. G. H. Pettengill, P. G. Ford, *Geophys. Res. Lett.* **27**, 609 (2000).
6. G. Tobie *et al.*, *Icarus* **164**, 33 (2003).
7. A. Colaprete *et al.*, *J. Geophys. Res.* **108**, 5081 (2003).
8. H. H. Kieffer, *Sixth International Conference on Mars*, Pasadena, California, 20 to 25 July 2003 (LPI, Houston, TX, 2003), p. 3158.
9. H. H. Kieffer *et al.*, *J. Geophys. Res.* **105**, 9653 (2000).
10. S. Piqueux *et al. J. Geophys. Res.* **108**, 3-1 (2003).
11. J. P. Bibring *et al.*, *Nature* **428**, 627 (2004).

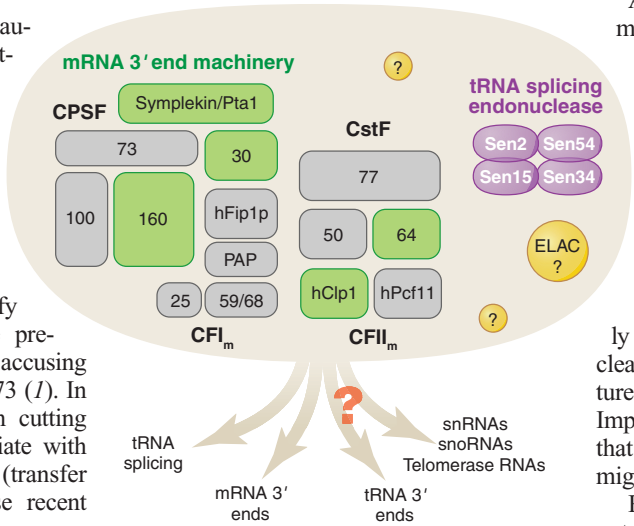
MOLECULAR BIOLOGY

Knives, Accomplices, and RNA

Marvin Wickens and Tania N. Gonzalez

The weapon is missing and the authorities are frustrated. The attacks are simple—a phosphodiester bond is severed cleanly to form the 3′ end of messenger RNA (mRNA), which is then free to receive its tail of polyadenosine [poly(A)]. The attacking gang of proteins is large and well known, but an intensive search has been mounted to identify the culprit that actually cuts the pre-mRNA. A recent study points an accusing finger at one gang member, CPSF73 (*1*). In another clue, proteins involved in cutting pre-mRNAs also physically associate with proteins that cleave pre-tRNAs (transfer RNAs) during splicing (*2*). These recent findings suggest surprising links among proteins that cut different types of RNA.

Primary RNA transcripts are cleaved by endonucleases to generate the 3′ ends of mRNAs, tRNAs, microRNAs, and certain small nuclear and nucleolar RNAs (snRNAs snoRNAs). The formation of mRNA 3′ ends can be reconstituted in the test tube (*3*–*6*), and in mammalian cells at least 14 different proteins are required for this process (see the first figure). The factors CPSF and CstF recognize the critical sequences in pre-mRNAs, whereas other factors are required for the cleavage step and for addition of the poly(A) tail. All of these factors, except the poly(A) polymerase (PAP), are complexes containing multiple proteins. Although 900 kD worth of factors have been isolated, it is unclear whether the enzyme that actually cuts pre-mRNA is among them.



A Swiss Army endoknife? The tRNA splicing endonuclease (purple) and mRNA 3′-end machinery (green and gray) associate with one another. Proteins in purple associate with those in green (*2*); proteins in gray have not been tested. The complex may process other types of RNAs, and contain other endoribonucleases (yellow). [mRNA factors adapted from a figure by W. Keller (Biozentrum, Basel)]

A serendipitous clue to the identity of the mRNA endonuclease comes from studies of tRNA processing. Mutations in the *ELAC2* gene appear to cause susceptibility to prostate cancer (*7*). ELAC2 is an endonuclease that cleaves 3′ extensions from pre-tRNAs (*8*) in mammalian cells, like its close relatives in plants and Archaea (*9*). The ELAC proteins are similar in sequence to the 73-kD subunit of CPSF (CPSF73) (*7*, *10*). CPSF73 is a member of a subfamily of metallo-β-lactamase enzymes that cleave nucleic acids using a distinctive structure that coordinates two zinc ions (*11*). Implicit in these reports (*8*–*11*) is the idea that if ELACs cut pre-tRNAs, then CPSF73 might cut pre-mRNAs.

Ryan *et al.* (*1*) recently showed that the putative active site of CPSF73 is essential for viability of yeast cells. Moreover, mRNA 3′ cleavage in vitro, long thought to be metal independent, is stimulated by zinc, consistent with CPSF73 being the perpetrator (*1*). Yet the case against CPSF73 is open: It is unclear whether mRNA 3′ cleavage is defective in CPSF73 mutants or whether CPSF73 is even a nuclease. Complicating matters further, the *Drosophila* zinc-finger endoribonuclease, Clipper, is related to a different CPSF subunit (*12*, *13*).

The notion that CPSF73 is the enzyme that forms mRNA 3′ ends is seductive, in part because the two steps that form both

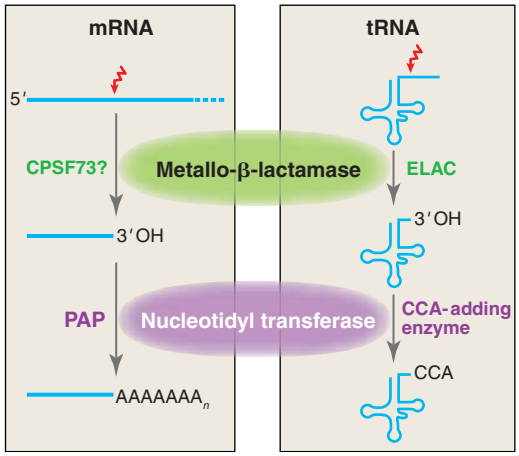
The authors are in the Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA. E-mail: wickens@biochem.wisc.edu

mRNA and tRNA 3′ termini would then be strikingly similar (see the second figure). Pre-mRNAs and pre-tRNAs first are cleaved by related metallo-β-lactamase enzymes, leaving a 3′-hydroxyl group. Then, two related nucleotidyl transferases—CCA-adding enzyme for tRNA, and PAP for mRNA—add nontemplated RNA sequences [CCA and poly(A)] to the new ends (*10*). The presence of tRNA-like structures at the ends of certain viral genomes may be an evolutionary relic of this process (*14*).

In a recent study, Paushkin *et al.* (*2*) reveal a startling collusion among proteins involved in cleaving mRNA and tRNA precursors. The tRNA splicing endonuclease of yeast consists of four subunits: Sen2, Sen34, Sen54, and Sen15 (see the first figure) (*15*). Sen2 cleaves the 5′ splice site, and Sen34 the 3′ splice site of tRNAs. Using sequence comparisons, Paushkin *et al.* (*2*) identified candidate tRNA splicing endonucleases in human cells, then isolated the splicing complexes with tagged proteins. As expected, complexes containing all four subunits were detected and accurately cleaved pre-tRNAs. Unexpectedly, these tRNA splicing complexes also contained factors involved in the formation of mRNA 3′ ends. Among these mRNA 3′ cleavage factors were HsClp1, CstF64, and symplekin. These proteins are required for 3′ end formation in vitro (*16*, *17*), and associate with one another in cell extracts (*18*). Remarkably, yeast mutants with a mutation in the symplekin homolog, PTA1, are defective in tRNA splicing (*19*), providing an independent link between tRNA splicing and mRNA processing.

The association between the tRNA splicing endonuclease and the mRNA 3′-end machinery may be dynamic. Most components of the mRNA machinery may be able to interact with the tRNA endonuclease (see the first figure), although they may not all reside in a single complex. Indeed, the proportion of different mRNA 3′ end factors associated with the tRNA endonuclease varies considerably. It will be critical to determine whether the isolated tRNA endonuclease accurately cleaves pre-mRNAs.

Might the enzyme that cuts tRNAs also produce the 3′ ends of mRNAs? The "forensic" evidence says no. Whereas mRNA 3′ end cleavage leaves a 3′-hydroxyl and 5′-phosphate (*20*, *21*), tRNA splicing endonucleases leave a 5′-hydroxyl and 2′,3′-cyclic phosphate (*15*). If tRNA-specific splicing endonucleases cut mRNA at all, they probably do not produce normal polyadenylated mRNAs. Most likely, the tRNA and mRNA endonucleases carry out separate duties even though they are found together. In human cells where tRNA splicing endonuclease activity has been reduced by RNA interference, uncleaved pre-mRNAs accumulate (*2*). This defect in mRNA 3′ end processing implies a functional link between mRNA and tRNA maturation. This defect may reflect a collapse of the endonuclease complex, loss of mRNA cleavage activity, or indirect effects.



Parallels in mRNA and tRNA 3′ end processing. Two pairs of related enzymes may act consecutively to form the 3′ ends of mRNAs and tRNAs.

Regulated mRNA splicing of Sen2, the tRNA splicing endonuclease subunit, may coordinate the abundance of certain tRNAs and mRNAs. Human Sen2 pre-mRNA is alternatively spliced such that exon 8 is either included (Sen2) or omitted (Sen2Δ8). The Sen2Δ8 protein appears to associate less efficiently with other tRNA endonuclease subunits, and to differ in its association with mRNA 3′ end factors (*2*). Moreover, complexes containing Sen2Δ8 conceivably may act on non-tRNA substrates because they cut pre-tRNAs with altered specificity.

Perhaps multiple endoribonucleases assemble into a Swiss Army endoknife—a complex of distinct blades, each honed to attack different RNAs. The mRNA 3′-end machinery alone may cut many other types of RNA. For example, formation of the 3′ ends of certain snRNAs, snoRNAs, and perhaps yeast telomerase RNA requires certain proteins that also are needed to cut pre-mRNAs (*22*, *23*). ELAC, the enzyme that processes tRNA 3′ ends, might well be in the complex, partnered with the tRNA splicing endonuclease. And what of Drosha, the endonuclease that processes the 3′ ends of microRNAs? Or the activity that forms the ends of nonadenylated histone pre-mRNAs? This hypothetical endonuclease complex may be analogous to the exosome, a complex of 3′-to-5′ exoribonucleases (*24*).

Currently, the only activities known to be associated with one another are the tRNA enodonuclease and several mRNA 3′ end processing factors. Might these interactions be artifactual? Only a small fraction of the mRNA cleavage factors appear to be associated with the tRNA splicing enzymes, and in vitro it appears that tRNA processing enzymes are not required to cleave mRNA 3′ termini. Yet, a direct assessment of tRNA splicing proteins in mRNA cleavage factor preparations seems warranted. Termination of transcription from both mRNA and snoRNA genes requires common proteins, suggesting another link in the biogenesis of different types of RNA (*25*). Moreover, translation, transcription, and mRNA processing factors all form higher order assemblies that are dispensable for minimal reactions in vitro. RNA cleavage factors may have similar complexities to reveal.

The observation that endoribonucleases associate with one another, even transiently, prompts new views of the cellular assaults on RNAs, and a new search for the weapons cache. If diverse endoribonucleases roam the cell together, then sorting out which blade cuts which RNA will require much care, including site-directed interrogation of each suspect. CPSF73 has not yet been convicted of pre-mRNA 3′ cleavage, but is taking the stand even as tRNA splicing endonucleases are revealed as accomplices. These proteins, along with other shadowy members of endoribonuclease complexes, may yet be tried for crimes against other types of RNAs.

References

1. K. Ryan *et al.*, *RNA* **10**, 565 (2004).
2. S. V. Paushkin *et al.*, *Cell* **117**, 311 (2004).
3. D. F. Colgan, J. L. Manley, *Genes Dev.* **11**, 2755 (1997).
4. M. Edmonds, *Prog. Nucleic Acid Res. Mol. Biol.* **71**, 285 (2002).
5. L. Minvielle-Sebastia, W. Keller, *Curr. Opin. Cell Biol.* **11**, 352 (1999).
6. J. Zhao et *al.*, *Microbiol. Mol. Biol. Rev.* **63**, 405 (1999).
7. S. V. Tavtigian *et al.*, *Nature Genet.* **27**, 172 (2001).
8. H. Takaku *et al.*, *Nucleic Acids Res.* **31**, 2272 (2003).
9. S. Schiffer *et al.*, *EMBO J.* **21**, 2769 (2002).
10. L. Aravind, *In Silico Biol.* **1**, 69 (1999).
11. I. Callebaut *et al.*, *Nucleic Acids Res.* **30**, 3592 (2002).
12. C. Bai, P. P. Tolias, *Mol. Cell. Biol.* **16**, 6661 (1996).
13. S. M. Barabino *et al.*, *Genes Dev.* **11**, 1703 (1997).
14. N. Maizels *et al.*, Eds., *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 79–112.
15. J. Abelson *et al.*, *J. Biol. Chem.* **273**, 12685 (1998).
16. H. de Vries *et al.*, *EMBO J.* **19**, 5895 (2000).
17. J. Zhao *et al.*, *Mol. Cell. Biol.* **19**, 7733 (1999).
18. Y. Takagaki, J. L. Manley, *Mol. Cell. Biol.* **20**, 1515 (2000).
19. J. P. O'Connor, C. L. Peebles, *Mol. Cell. Biol.* **12**, 3843 (1992).
20. C. L. Moore *et al.*, *EMBO J.* **5**, 1929 (1986).
21. M. D. Sheets *et al.*, *Mol. Cell. Biol.* **7**, 1518 (1987).
22. S. Dheur *et al.*, *EMBO J.* **22**, 2831 (2003).
23. M. Morlando *et al.*, *Mol. Cell. Biol.* **22**, 1379 (2002).
24. P. Mitchell, D. Tollervey, *Nature Struct. Biol.* **7**, 843 (2000).
25. E. J. Steinmetz, D. A. Brow, *Mol. Cell. Biol.* **23**, 6339 (2003).