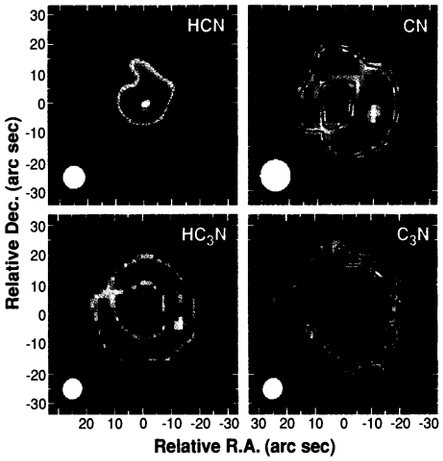

Carbon contours. Microwave emission images of the molecule-rich circumstellar shell surrounding the carbon star IRC+10216. Shown are contour maps of the distribution of four carbon-containing molecules (HCN, CN, HC_3N, and C_3N). White circles indicate the resolution of the telescope for each emission wavelength. (Dec. = declination, R.A. = right ascension) [Images obtained using the Berkeley-Illinois-Maryland-Association Millimeter Wavelength Array at Hat Creek, California (*16*)]

Hence, the importance of neutral-neutral reactions in interstellar and circumstellar chemistry remains an open question.

Kaiser and co-workers (*2*) present compelling evidence that neutral-neutral reactions are, indeed, important in certain astronomical environments. In the reaction that they have studied, C_3H is produced by a route involving neutral species. But ion-molecule routes have also been postulated as sources of C_3H.

A unique aspect of both routes is that the C_3H can be produced in either a linear (*l*-C_3H) or a cyclic (*c*-C_3H) form. The astronomical observation of both forms of C_3H is not by itself sufficient to discriminate between the two reactions. However, the abundance ratio *l*-C_3H/*c*-C_3H should be a signature of the reaction pathway, because the two structures are not isoenergetic. Kaiser *et al.* used electronic structure calculations to show that the neutral production of *l*-C_3H is exothermic by 1.5 kJ/mol, whereas the production of *c*-C_3H is exothermic by 8.6 kJ/mol. Thus, the abundance ratio should be strongly dependent on the physical environment in which the isomers are formed. In fact, abundance ratios for these isomers have been measured in two very different astrophysical environments: the cold, dark molecular cloud TMC-1 (average temperature < 10 K) and the circumstellar shell of the carbon star IRC+10216 (temperatures up to 4000 K) (see figure). Through the use of a crossed molecular beam apparatus, Kaiser and co-workers were able to approximate conditions in these two environments by controlling the collision energy between C and HCCH. They detected both the linear and cyclic reaction products and thus determined the temperature dependence of the reaction product branching ratio. Their observations explain the different *l*-C_3H/*c*-C_3H ratios observed in TMC-1 and IRC+10216 and thus provide strong evidence that neutral-neutral reactions do indeed play an important role in both interstellar and circumstellar environments.

The facile nature of the carbon addition reactions studied by Kaiser and co-workers may also have implications for terrestrial processes involving the (high-temperature) condensation of pure carbon vapor (*14*). Such processes, which can lead to the formation of fullerenes and carbon-rich soot, are remarkably fast. Small carbon clusters occur predominantly at high temperatures as linear chain structures with very low-frequency bending vibrations. This preferred form means that $C_n + C_m$ collision complexes have a high density of nondissociative vibrational states in which excess energy may be partitioned, thus extending the lifetime of such complexes (*15*). Nevertheless, the chemical route to forming the first cluster with such low-frequency vibrations, C_3, is not well understood. The formation of C_3 is probably the rate-determining step in carbon condensation, because (low-pressure) carbon vapor is known to consist of carbon atoms and dimers. Carbon atom insertion into C_2, followed by radiative or collisionally induced stabilization, may be the process that "kick-starts" high-temperature carbon condensation in both terrestrial and extraterrestrial environments.

References

1. D. M. Rank *et al.*, *Science* **174**, 1083 (1971).
2. R. I. Kaiser *et al.*, *ibid.* **274**, 1508 (1996).
3. E. Herbst and W. Klemperer, *Astrophys. J.* **185**, 505 (1973).
4. D. Buhl and L. E. Snyder, *Nature* **228**, 267 (1970).
5. S. Green, J. A. Montgomery, P. Thaddeus, *Astrophys. J.* **193**, L89 (1974).
6. R. C. Woods, T. A. Dixon, R. J. Saykally, P. G. Szanto, *Phys. Rev. Lett.* **35**, 1269 (1975).
7. L. W. Avery *et al.*, *Astrophys. J.* **205**, L173 (1975).
8. P. F. Bernath *et al.*, *Science* **244**, 562 (1989).
9. A. Van Orden *et al.*, *Proc. of the 1994 Kuyper Airborne Astronomy Symposium* (NASA Ames Research Center, Moffett Field, CA, 1994), p. 53.
10. W. A. Schutte, A. Tielens, L. Allamandola, *Astrophys. J.* **415**, 397 (1993).
11. I. R. Sims and I. W. M. Smith, *Annu. Rev. Phys. Chem.* **46**, 109 (1995).
12. N. Haider and D. Husain, *Z. Phys. Chem.* **176**, 133 (1992).
13. Q. Liao and E. Herbst, *Astrophys. J.* **444**, 695 (1995).
14. M. Frenklach, in *Carbon in the Galaxy: Studies from Earth and Space*, (NASA Conf. Publ. 3061, NASA, Washington, DC, 1987).
15. J. R. Heath, in *Fullerenes: Synthesis, Properties and Chemistry of Large Carbon Clusters*, G. S. Hammond and V. J. Kuck, Eds. (ACS Symp. Ser. 481, American Chemical Society, Washington, DC, 1991), pp. 1–23.
16. J. Bieging and M. Tafalla, *Ap. J.* **105**, 576 (1993); A. Dayal and J. Bieging, *ibid.* **439**, 996 (1995).

MOLECULAR BIOLOGY

The End of the Message—Another Link Between Yeast and Mammals

James L. Manley and Yoshio Takagaki

The synthesis of messenger RNA (mRNA) from its gene requires transcription, removal of intervening sequences (splicing), and polyadenylation—a process in which an endonucleolytic RNA cleavage is coupled with synthesis of polyadenosine [poly(A)] on the newly formed 3′ end. For transcription and splicing, the DNA or RNA sequences and many of the protein factors that participate are well conserved from yeast to humans. Until recently, the situation for polyadenylation appeared different. Although the reaction mechanism is similar in yeast and humans, the sequence signals in yeast mRNA have been poorly defined and were apparently unrelated to those in mammalian mRNA precursors. This observation led to the view that the machinery that specifies the mRNA 3′ end is distinct in yeast and mammals. Recent studies, three of which are described starting on page 1511 of this issue (*1–3*), however, provide evidence that this is not the case: The protein factors that recognize the poly(A) site on precursor mRNA are conserved between yeast and mammals.

The sequences that define the poly(A) site in mammals contain certain consensus elements (*4*) (Fig. 1). Foremost among these is the nearly ubiquitous and essential AAUAAA hexanucleotide, found almost invariably 10 to 30 bases upstream of the site of polyadenylation. Indeed, this motif is

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unusually constant, showing less variability than, for example, the TATA motif in RNA polymerase II promoters. A G-U–rich element lies just downstream of the site of cleavage and is important for efficient processing. However, there is no simple consensus, and some transcripts lack a recognizable G-U–rich sequence. The sequence immediately 5′ to the site of RNA cleavage is frequently CA (although this is not essential), and a number of RNAs have sequence elements situated 5′ of AAUAAA. However, these upstream elements do not constitute a consensus (except in cytoplasmic polyadenylation, which is distinct from the nuclear reaction discussed here) and have been detected principally but not exclusively in viral transcripts. Well-characterized protein factors interact with these signals (5) (Fig. 2). The multisubunit cleavage and polyadenylation specificity factor (CPSF) recognizes AAUAAA, whereas the heterotrimeric cleavage stimulation factor (CstF) binds the G-U–rich element. Importantly, neither factor binds RNA avidly by itself, but cooperative interactions between the two result in stable complex formation, which likely defines the site of polyadenylation. Poly(A) polymerase (PAP) and two less well-characterized cleavage factors appear to complete the processing complex.

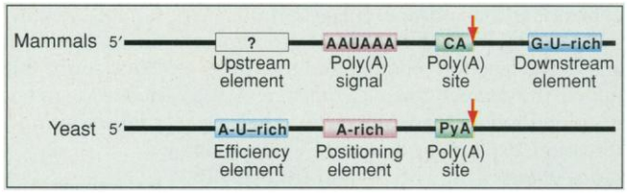

Fig. 1. Elements required for mammalian and yeast polyadenylation. Arrows, site of RNA cleavage; ?, poorly defined elements.

Initial examination of the 3′-end sequences of yeast genes failed to identify strong candidates for polyadenylation signals, and mutations in potential signal sequences frequently did not have significant effects on 3′-end formation (6). However, careful examination of a large number of sequences and mutational analyses has revealed consensus motifs that are both similar to and different from mammalian signals (6) (Fig. 1). These include an A-rich region, which can be AAUAAA and is called the positioning element. This element may be related to the mammalian AAUAAA, although the consensus is much less strict and the sequence is significantly more tolerant to mutation. Although there is no apparent downstream element, there is frequently an A-U–rich upstream efficiency element, which may correspond to the mammalian G-U–rich region. Perhaps it is located upstream instead of downstream because genes are closer in yeast than in mammals.

There are then considerable differences between yeast and mammalian poly(A) signal sequences, although with a little imagination similarities can also be detected. But what about the factors that recognize these sites? The unexpected answer is that there is more similarity among the protein factors than among the RNA signals! The 77-kD subunit of human CstF (7) shares extensive similarity with both the product of a classical modifier gene in *Drosophila*, *suppressor-of-forked* (8), and a yeast protein, Rna14 (9). This similarity added significance to a high but more limited homology between the RNA binding domain of the 64-kD subunit of CstF and a related region of the Rna15 protein, previously linked genetically to Rna14 (9). Rna14 and Rna15 function in polyadenylation in vitro (*10*), constituting at least part of cleavage factor I (CFI) (*11*). Perhaps CFI binds the A-U–rich element and functions in a manner analogous to CstF (Fig. 2).

The three reports in this issue provide evidence that subunits of CPSF are also conserved in yeast. Using elegant genetic (*1*) and biochemical (*2*) approaches, the authors identify a yeast protein, Brr5/Ysh1 (or vice versa), that is the homolog of CPSF-73. CPSF-73 also shares a lower but significant similarity with CPSF-100 (*2*), suggesting that the genes encoding these proteins may have evolved from a common ancestor. Although the precise role of CPSF-73 in polyadenylation is currently unknown, AAUAAA binding appears to be mediated by the largest CPSF subunit, CPSF-160 (*12*). Given the lack of an essential AAUAAA sequence, the finding that yeast contains a functional homolog of CPSF-160, CFT1, was unexpected (*3*). CFT1 and Brr5/Ysh1 may be part of a complex that functions in recognition of the A-rich element (Fig. 2). Brr5/Ysh1 is part of an activity called polyadenylation factor 1 (PF1) (*2*), whereas CFT1 may be a component of a separable factor, cleavage factor II (CFII) (*3*). It seems unlikely that these two proteins would be part of a single factor (CPSF) in mammals but components of distinct factors in yeast. This discrepancy likely reflects differences in biochemical fractionation protocols, and Brr5/Ysh1 and CFT1 are probably components of a single complex in vivo. In any event, it now appears that the basal factors required for poly(A) site specification are conserved from yeast to humans. An interesting question is how two degenerate RNA elements (the A-rich and A-U–rich motifs) (Fig. 1) bring about efficient and precise mRNA 3′-end formation. At least part of the answer will likely involve cooperative interactions among the factors that recognize these elements, analogous to the interactions between CPSF and CstF in mammals.

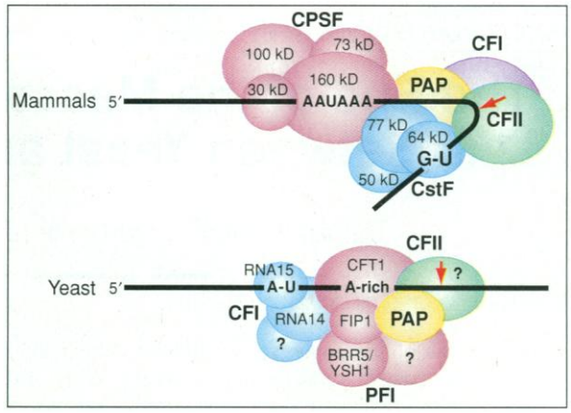

Fig. 2. Mammalian and yeast polyadenylation factors. Arrows, site of RNA cleavage; ?, polypeptides not described in yeast but present in the corresponding mammalian factors.

Polyadenylation now joins the list of cellular processes in which key factors are conserved from yeast to mammals. But like these other processes, there are also differences, many of which undoubtedly reflect the more complex regulatory requirements inherent to multicellular organisms. A good example is provided by PAP. The yeast and mammalian enzymes are homologous throughout the NH_2-terminal catalytic domain (*13*). Although the yeast enzyme is truncated after this region, mammalian PAP contains a long COOH-terminal domain, with different isoforms produced by alternative splicing, that can negatively regulate PAP activity in response to different signals (*14*). But regardless of such differences, comparisons between organisms will continue to provide a wealth of information about how cells work.

References

1. G. Chanfreau, S. M. Noble, C. Guthrie, *Science* **274**, 1511 (1996).
2. A. Jenny *et al.*, *ibid*, p. 1514.
3. G. Stumpf and H. Domdey, *ibid*, p. 1517.
4. Reviewed in N. J. Proudfoot, *Cell* **64**, 671 (1991).
5. Reviewed in J. L. Manley, *Curr. Opin. Genet. Dev.* **5**, 222 (1995); W. Keller, *Cell* **81**, 829 (1995).
6. Z. Guo and F. Sherman, *Trends Biochem. Sci.*, in press.
7. Y. Takagaki and J. L. Manley, *Nature* **372**, 471 (1994).
8. A. Mitchelson *et al.*, *Genes Dev.* **7**, 241 (1993).
9. L. Minvielle-Sebastia *et al.*, *Mol. Cell. Biol.* **11**, 3075 (1991).
10. L. Minvielle-Sebastia *et al.*, *Science* **266**, 1702 (1994).
11. J. Chen and C. Moore, *Mol. Cell. Biol.* **12**, 3470 (1992).
12. K. G. K. Murthy and J. L. Manley, *Genes Dev.* **9**, 2672 (1995).
13. T. Raabe *et al.*, *Nature* **353**, 229 (1991); E. Wahle *et al.*, *EMBO J.* **10**, 4251 (1991); J. Lingner *et al.*, *Nature* **354**, 496 (1991).
14. S. Gunderson *et al.*, *Cell* **76**, 531 (1994); D. F. Colgan *et al.*, *Nature* **384**, 282 (1996).