Messenger RNA polyadenylation: A universal modification

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mRNAs in eukaryotic cells, as every freshman biology student of the last 20 or so years has learned, are almost without exception posttranscriptionally modified at their 3' ends by addition of a tract of adenylate residues, the so-called poly(A) tail. In yeasts as well as higher eukaryotes, the poly(A) tail functions in both mRNA turnover and in facilitating translation (reviewed in refs. 1 and 2). Synthesis of the tail involves an endonucleolytic cleavage of the mRNA precursor, which creates the 3' hydroxyl that then functions as primer for the synthesis of poly(A) by poly(A) polymerase (PAP). These two reactions are tightly coupled and require, in addition to PAP, a complex assembly of proteins (reviewed in refs. 3 and 4).

Polyadenylation, like other mRNA processing reactions, such as 5' capping and removal of intervening sequences by splicing, has been considered to be a significant modification only in eukaryotic cells and their viruses. The 3' ends of prokaryotic mRNAs, conventional wisdom has held, are created by transcription termination, with perhaps limited exonucleolytic trimming occurring in a few cases. Furthermore, because translation begins on nascent chains, and because most mRNAs turn over quite rapidly, it seemed that there was little need, or time, for polyadenylation. This view has persisted despite some clear hints to the contrary. For example, an enzyme activity from Escherichia coli capable of synthesizing poly(A) onto an RNA primer was described over 30 years ago (5), only shortly after the description of such an activity in mammalian sources (6). There have also been many reports during the last 20 years documenting the presence of 3' adenylate tracts on bacterial RNAs (e.g., refs. 7 and 8 and references therein). However, the estimated short length (10 to at most 25 bases, compared with 75 in yeast and 200 in mammals) and paucity [only a few percent of mRNAs were estimated to contain poly(A)] of bacterial poly(A) sequences fueled the belief that bacterial polyadenylation was probably just an aberration with little functional consequence.

Proponents of the view that polyadenylation is in fact a significant modification with an important function in bacterial mRNA metabolism can find considerable support in a paper in the current issue of the Proceedings. O'Hara et al. (9) have exploited the recent identification of the gene encoding PAP (10) to investigate in more detail the role of mRNA polyadenylation in bacteria. Their results allow two important conclusions. First, a mutation that inactivates PAP can have significant effects on the stabilities of each of three different mRNAs. This was seen most clearly in a strain containing multiple mutations in genes encoding some of the RNases responsible for mRNA degradation, where inactivation of PAP was found to significantly increase the half-lives of the mRNAs examined. Second, careful biochemical analysis of the poly(A) tails in these cells revealed that they were longer and more abundant than previously thought, with lengths of 50 or more bases readily detected in the RNase mutant strain. Strains lacking PAP had little if any poly(A), indicating that this enzyme is indeed responsible for most if not all poly(A) synthesis in E. coli.

The results of O'Hara et al. strengthen the view that mRNA polyadenylation is a universal and functionally significant process. But there are clearly important differences between polyadenylation in prokaryotes and eukaryotes. First, in eukaryotes (i.e., yeast) the gene encoding PAP is essential for viability (11), while in E. coli it is dispensable [although null mutations do significantly reduce growth (12)]. Second, in eukaryotes the absence of a poly(A) tail has been associated with mRNA instability (e.g., ref. 13), while in E. coli it appears to lead to stabilization, at least of the transcripts tested. However, this difference may not be as striking as it might appear. For example, in both yeast (14) and mammals (15), it appears that shortening of the poly(A) tail can be the first step in mRNA degradation. And in E. coli, as pointed out by O'Hara et al., there may well be some (perhaps many) mRNAs that are stabilized by poly(A).

There are also of course mechanistic differences in polyadenylation between prokaryotes and eukaryotes. In bacteria, it seems that PAP synthesizes poly(A) onto the 3' end created by transcription termination, or perhaps to the end created by limited exonucleolytic trimming (e.g., ref. 8). Thus there is no evidence for a complex mRNA processing machinery such as exists in eukaryotes, and PAP is likely nonspecific with respect to the primer sequence used for poly(A) synthesis. Indeed, there is evidence that a small non-mRNA species can be polyadenylated in E. coli (16), and O'Hara et al. raise the possibility that some fraction of tRNA might be polyadenylated. Purified eukaryotic PAPs also have the ability to synthesize poly(A) in vitro with no specificity for the primer, but this seems not to be physiologically important: PAP appears to work in vivo only following endonucleolytic cleavage at specific sites. Finally, the primary sequence of E. coli PAP is somewhat puzzling. Not only does it lack recognizable motifs, but it shares no similarity with other PAPs. Indeed, the sequences of mammalian (17, 18), yeast (11), and vaccinia virus (19) PAPs have been determined, and with the exception of the yeast and mammalian enzymes, none shares significant homology with the others. While future studies may reveal structural similarities not apparent from the primary sequence, it seems that nature has come up with three different ways of designing enzymes that have very similar, if not identical, catalytic properties.

It is thus now clear that the posttranscriptional addition of poly(A) to mRNA is a process conserved from bacteria to man. In eukaryotes, poly(A) addition is apparently always coupled to the endonucleolytic cleavage that creates the actual mRNA 3' end. Thus, in vitro assays have revealed mechanistic similarities in yeast and human cell extracts (reviewed in refs. 3 and 4). However, a confusing aspect of the yeast system has been the apparent lack of cis-acting signals in the pre-mRNA. In metazoan systems, virtually all pre-mRNAs contain the well-known hexanucleotide AAUAAA, or a variant thereof, just 5' to the site of cleavage, as well as, in many cases, a much less conserved G+U-rich sequence downstream of the cleavage site (see ref. 20 for review). In contrast, in yeast the hexanucleotide is frequently not present or is dispensable when it is present, and indeed no convincing consensus poly(A) signal sequence has emerged. This has raised the possibility that the trans-acting factors for poly(A)-site recognition may be considerably diverged between yeast and metazoans.

In keeping with our theme of the universality of polyadenylation, I conclude by describing recent experiments which have revealed that key proteins involved in
poly(A)-site recognition in human cell extracts correspond to proteins previously identified by genetic methods in Drosophila and Saccharomyces cerevisiae. Cleavage stimulation factor (CstF) is a heterotrimERIC protein (21) that plays an important role in poly(A)-site specification, in part by binding to the downstream sequence element (22, 23). cDNAs encoding one CstF subunit, called 77K, were recently isolated (24) and the protein was found to be homologous to that encoded by the essential suppressor-of-forked gene in Drosophila (25) and by the also essential RNA14 gene in yeast (26). RNA14 was also shown to interact genetically with RNA15 (26). Interestingly, the product of RNA15 shares significant similarity with another subunit of CstF, called 64K, which is in fact the RNA-binding subunit (27). It is satisfying that the yeast and Drosophila proteins were previously implicated in mRNA metabolism, and indeed the RNA14 and RNA15 proteins have now been shown to be subunits of a factor (CF1) known to play an important role in yeast polyadenylation in vitro (28). Thus, just as poly(A) addition and at least some aspects of poly(A) tail function are conserved from bacteria to man, at least some of the proteins involved in mRNA 3'end formation are conserved throughout eukaryotes.