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Histone 3' ends: essential and regulatory functions

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The five histone proteins are encoded by a multigene family. In many organisms there is a major tandemly repeated gene family with one copy of a gene for each histone in the repeat unit. In mammals and birds there are multiple non-identical copies of the genes for each histone protein. These multiple copies encode the same core histone protein, although each gene has distinctive 5' and 3' untranslated region and flanking sequences. Different genes contribute varying amounts of mRNA to the total pool of histone mRNA (Graves et al., 1985). In mammals the histone genes are found in two large clusters, on chromosome 1 and 6 in humans (Triputti et al., 1986) and 3 and 13 in the mouse (Graves et al., 1985). The histone genes are the one gene family in vertebrates in which different coordinately expressed genes have remained closely linked. Since the biosynthesis of histone mRNAs involves molecules that do not participate in synthesis of other mRNAs, it is likely that this close linkage reflects the localization of histone mRNA biosynthesis at a particular small domain in the nucleus.

Histone genes are unique in that they lack introns. Histone mRNAs are also the only class of mRNAs that are not polyadenylated. Histone mRNAs end in a highly conversed structure, consisting of a potential six base-pair stem and four base loop. The general structure is shown in Figure 1. The sequence of the histone 3' end has been highly conserved in metazoans, being essentially identical in a variety of phyla ranging from cnidaria (Miller et al., 1990), annelids (Sellos et al., 1990), nematodes (Roberts et al., 1989), and Volvox (Miller et al., 1990), to echinoderms, insects, and vertebrates. The features of the stem-loop which have been conserved throughout evolution are the two GC base pairs at the base of the stem, the UA base pair at the top of the stem, and the U in the third position of the loop. The first base in the loop is a U in all species examined except C. elegans, where there is a C in the first base of the loop (Roberts et al., 1989). The 3' end of histone mRNA is involved in basic processes of histone mRNA metabolism, besides playing a major role in both the nucleus and cytoplasm in regulating histone mRNA levels.

Essential nuclear functions: 3' end formation and transcription termination

There is only one processing reaction necessary to form a mature histone mRNA, an endonucleolytic cleavage reaction (Gick et al., 1986) that requires the stem-loop and a purine-rich sequence 9–14 nucleotides 3' of the cleavage site. The purine-rich sequence is recognized by U7 snRNP, which is an obligatory component of the 3' processing machinery. At least three components have been identified: the U7 snRNP, a stem-loop binding factor (Vasserot et al., 1989; Mowry et al., 1989), and a heat-labile factor (Gick et al., 1987). The U7 snRNP contains the U7 snRNA, the core set of Sm proteins, and additional proteins which have not been well characterized. A sequence near the 5' end of U7 snRNA base-pairs with a purine-rich region 3' of the stem-loop (Cotten et al., 1988; Bond et al., 1991). There is apparently only one U7 snRNA in mammals (Gruber et al., 1991), although the sequence of

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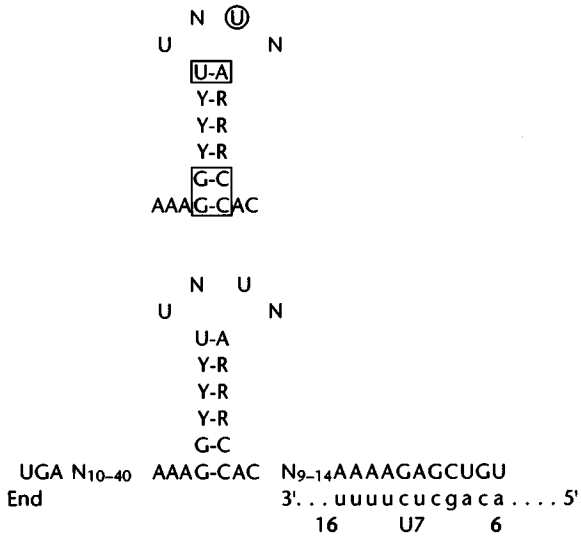

Figure 1. 3′ ends of histone mRNA. At the top is the structure of the 3′ end of histone mRNA. The boxed nucleotides are the ones conserved in evolution. Below is the general structure of the 3′ end of a mammalian histone pre-mRNA. The proposed base-pairing with U7 snRNA is shown.

the purine-rich region varies significantly among different individual histone genes, both in sequence and distance from the stem-loop (Liu et al., 1989). This is in contrast to sea urchins, where U7 snRNA was first described, and where each member of the tandemly repeated histone genes has a nearly identical sequence 3′ of the stem loop, which base-pairs well with U7 snRNA (Strub et al., 1984). It has been definitively shown in both sea urchins and mammals that the base-pairing of the U7 snRNA is critical to its function, since mutants in the purine-rich region can be rescued by compensatory mutations in the U7 snRNA (Strub and Birnstiel, 1986; Bond et al., 1991). In mammals the core AAAGAG sequence seems to be the most critical part of the recognition sequence (Bond et al., 1991). It is not clear, however, how many nucleotides of the U7 snRNA are involved in base-pairing with the histone pre-mRNA, whether the same nucleotides must always be involved in recognition, and whether a minimum number of base-pairs must be formed within a larger region of the U7 snRNA 5′ end.

Biochemical fractionation has revealed two other components required for histone 3′ end formation: a factor which binds the stem-loop and a "heat-labile" factor. In vitro cleavage requires all three components, though the stem-loop binding factor and the heat-labile factor have not as yet been well characterized biochemically.

The 3′ end contributes to the relative level of expression of individual mRNAs

The rate of biosynthesis of a mature mRNA is determined by the rate of transcription and the efficiency of processing, i.e., the percentage of transcripts which ultimately become mature mRNAs. Partly because of the simplicity (one-step) of histone RNA processing, it has become clear that not all transcripts form mature histone mRNAs. Although there are multiple copies of the genes for each histone protein, in mammals each gene does not contribute equally to the pool of histone mRNA. One of the 10–20 histone H3 and H2a genes in the mouse accounts for 35–40% of the histone H3 or H2a mRNA (Graves et al., 1985). Part of this differential expression is due to differences in the promoter, but equally important is a difference in the efficiency of 3′ end formation. Simply exchanging the 3′ region of a highly expressed histone gene with the 3′ region of a poorly expressed histone gene alters the expression of both genes. The highly expressed genes have the best consensus base-pairing with the U7 snRNA, suggesting that this is the key determinant in processing efficiency (Liu et al., 1989). These results imply that most of the transcripts from a poorly expressed histone gene are not processed, but rather must be rapidly degraded in the nucleus, an example of "wastage" of transcripts. Since pre-mRNAs do not accumulate to any significant extent, a histone pre-mRNA which is not rapidly processed must be rapidly degraded. How these RNAs are recognized and rapidly degraded is not known.

A second role of the histone mRNA 3′ end in nuclear RNA metabolism is in termination of transcription. Convincing evidence has been obtained that termination of transcription of pre-mRNAs from most genes requires that the RNA polymerase transcribe a polyadenylation site (reviewed in Proudfoot, 1989). If the polyadenylation site is altered or deleted, the "normal" transcription termination site is ignored. Similarly, an intact histone 3′ processing signal is required for transcription termination in a mouse histone H2a gene (Chodchoy et al., 1991). Whether there is a relationship between the termination of transcription and the "wastage" of

the pre-mRNA is not known, although it is possible that "wastage" occurs if transcription terminates prior to the processing of the histone pre-mRNA. This may account for the low efficiency of processing of histone pre-mRNAs, which show a slower rate of processing.

The final step in mRNA biosynthesis is transport of the mRNA to the cytoplasm. The mechanism whereby a cell selects an RNA for export remains obscure. There is a requirement for a 7-CH_3 guanosine cap, but this cap cannot be sufficient, since it is found on all pre-mRNAs (Hamm and Mattaj, 1990). Recent results from Birnstiel and coworkers demonstrate a critical role of the histone 3′ end in the final step in mRNA biosynthesis, transport of mature histone mRNA to the cytoplasm (Eckner et al., 1991). An intriguing aspect of these results was that an mRNA with a histone 3′ end formed by the "normal" processing mechanism was efficiently transported to the cytoplasm, while the same mRNA formed by a ribozyme-mediated reaction was largely retained in the nucleus. Thus not only the 3′ end sequence but also the proper 3′ end formation mechanism may be necessary for efficient transport of the histone mRNA to the cytoplasm. Interestingly, mRNAs containing the entire histone coding sequence plus the correct 3′ end were transported well, regardless of whether the 3′ end was formed naturally or by a ribozyme-mediated reaction, suggesting that other sequences in the histone mRNA help in targeting the mRNA to the cytoplasm (Eckner et al., 1991).

Nuclear regulatory functions: role of the 3′ processing of histone mRNA in cell cycle regulation

Histone mRNA concentrations are tightly regulated during the cell cycle in mammalian cells, with the concentrations higher in S-phase than in G1-phase. Multiple steps in histone mRNA metabolism are regulated to accomplish this stringent regulation. Schümperli, Birnstiel, and coworkers reported several years ago that a major component of cell-cycle regulation in a temperature-sensitive cell-cycle mutant was due to the 3′ end of histone mRNA (Lüscher et al., 1985). This finding has been extended to cells synchronized by serum starvation (Stauber and Schümperli, 1988) and recently to synchronous CHO cells selected by mitotic shake-off (Harris et al., 1991). The biochemical basis of this regulation is not completely clear. Using an in vitro complementation assay, the heat-labile factor was found to be the missing component in extracts prepared from cells arrested in G1 (Gick et al., 1987; Lüscher and Schümperli, 1987). However, recently studies on the accessibility of the 5′ end of the U7 RNA in the U7 snRNP have shown that the 5′ end of U7 snRNA (which recognizes the purine-rich sequence in histone pre-mRNA) is not accessible in extracts prepared from cells arrested in G1 compared with growing cells, suggesting that the biochemical basis of regulation of 3′ end formation may be due to prevention of the binding of U7 snRNP to the pre-mRNA (Hoffmann and Birnstiel, 1990). The relationship between the alteration in U7 snRNP structure and the heat-labile factor is not clear, although it is possible that the heat-labile factor prevents occlusion of the 5′ end of U7 snRNP. In G1 cells in which the histone genes are transcribed but the transcripts are not processed, the transcripts are present in very low levels, suggesting that the unprocessed transcripts must be rapidly degraded (Stauber and Schümperli, 1988).

Thus the histone 3′ end participates in two different ways in histone mRNA biosynthesis: (1) the intrinsic efficiency of 3′ end formation on each individual histone gene helps to determine its contribution to the histone mRNA pool, and (2) the formation of 3′ ends on all histone mRNAs is regulated during the cell-cycle. The stimulation of 3′ end formation, which occurs shortly before cells enter S-phase, is at least as important as the stimulation of histone gene transcription in causing the increase of histone mRNA levels as cells enter S-phase (Harris et al., 1991). The combination of these two independent regulatory steps results in the large increase in histone mRNA levels as cells progress from G1- to S-phase.

Alternate 3′ ends on some histone mRNAs

Two examples of regulating histone 3′ end formation to alter the expression of specific histone genes have recently been described. The H2a.X histone is synthesized at a high rate during the S-phase but continues to be synthesized in G1. In growing cells the predominant H2a.X mRNA ends at the stem-loop characteristic of other histone mRNAs. However, in non-growing

cells the predominant H2a.X mRNA is polyadenylated at a site 3′ to the stem-loop (Mannironi et al., 1989). A similar result has been found for a mouse histone H1 gene, which forms a long polyadenylated transcript, presumably when cells are not growing, but forms a "normal" histone 3′ end in S-phase cells (Cheng et al., 1989). In the spermatocytes of chickens, the histone mRNAs are polyadenylated. These mRNAs are derived from the same histone genes which are active in somatic cells. In the spermatocytes the histone 3′ ends are not processed; instead, downstream polyadenylation sites are used (Challoner et al., 1989; Kirsh et al., 1989). It seems likely that one component (or all) of the 3′ end processing machinery is missing from chicken spermatocytes, resulting in the production of polyadenylated histone mRNAs.

Cytoplasmic functions of 3′ ends of mRNAs

The poly(A) end at the 3′ end of mRNAs is involved in two important aspects of cytoplasmic mRNA metabolism, both of which are probably mediated by the poly(A) binding protein, a component of the mRNP. The poly(A) tail is involved in determining the stability of the mRNA (reviewed in Bernstein and Ross, 1989). Secondly, and more surprisingly, the poly(A) tail may play a direct role in recruiting mRNAs onto polysomes, i.e., in initiating translation (Sachs and Davis, 1989; Jackson and Standart, 1990). An important translational control mechanism in Xenopus oocyte maturation and presumably also in other embryonic systems is the selective translation of mRNAs, which is mediated largely by changes in polyadenylation of preformed mRNAs (Paris and Richter, 1990; Fox et al., 1989).

The histone 3′ end in the cytoplasm regulates the half-life of histone mRNA

The 3′ end of histone mRNA also plays an important role in the regulation of histone mRNA in the cytoplasm. The half-life of histone mRNA is also tightly regulated with DNA synthesis during the cell cycle. When DNA synthesis is inhibited, cells rapidly degrade their histone mRNA. The 3′ end of the histone mRNA is necessary and sufficient for this response (Pandey and Marzluff, 1987). Interestingly, during the normal cell cycle, rapid degradation of histone mRNA occurs in G2-phase to rapidly reduce histone mRNA levels 30-fold in the 2 hours between the end of S-phase and mitosis (Harris et al., 1991). In G1-phase cells, the mechanisms coupling histone mRNA degradation to DNA synthesis are not present, but apparently are only present after cells have entered S-phase (Harris et al., 1991; Morris et al., 1991).

A protein binds the 3′ end of histone mRNA

The 3′ end of histone mRNA presumably must fulfill the essential functions of the poly(A) tail, and it is probable that these functions are mediated by a protein that binds the histone mRNA 3′ end. Extraction of polyribosomes with 0.5–0.8M salt releases a protein that specifically binds to the stem-loop sequence, and binding is dependent on those features of the stem-loop sequence that have been conserved in evolution (see Fig. 1). A similar stem-loop binding protein can be detected in the nucleus, raising the possibility that the same protein can be bound to the histone mRNA in the nucleus (Pandey et al., 1991). The relationship of the nuclear binding activity to the stem-loop factor required for 3′ end formation is not clear, although it seems likely that the factors are different, since the protein has a preference for the stem-loop sequence at the 3′ end of an RNA (i.e., the mature histone mRNA rather than the histone pre-mRNA). One cannot rule out the possibility, however, that the protein is part of the processing apparatus which remains with the mature RNA after processing.

It seems very likely that the stem-loop binding protein fulfills the essential functions of the poly(A) binding protein on other mRNAs. Clearly the 3′ end of histone mRNA is the major determinant of histone mRNA stability, and we have recently found that the histone 3′ end is also essential for recruiting histone mRNA to polyribosomes (J.-H. Sun, D. R. Pilch, and W. F. Marzluff, unpublished data). Replacing the histone 3′ end with an snRNA 3′ end greatly reduced the translatability of the mRNA in vivo.

Conclusions

In summary, the 3′ end of histone mRNA plays two different roles in histone mRNA metabolism. First, it fulfills the essential functions that the poly(A) tail performs for other mRNAs. These functions, which are just beginning to be appreciated, include recruitment of the mRNA to polyribosomes and stabilization of

the mRNA. The function of the poly(A) tail is mediated by poly(A) binding protein, and it is likely that the protein which binds the 3′ end of histone mRNA mediates similar functions. Secondly, the 3′ end of histone mRNA, conserved in all histone mRNAs regulated coordinately with DNA replication, is a target allowing coordinate regulation of the 50–100 different mRNAs for the five histone proteins. This includes regulation of the 3′ processing reaction in the nucleus and the regulation of histone mRNA half-life in the cytoplasm. The signaling mechanisms which couple these processes to DNA replication are unknown, but they are probably components of cell-cycle regulatory systems, which have multiple functions during the cell cycle.

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