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RNA 3′ processing regulates histone mRNA levels in a mammalian cell cycle mutant. A processing factor becomes limiting in G1-arrested cells

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Post-transcriptional regulation of histone gene expression in a mouse mastocytoma cell cycle mutant (21-Tb) depends largely on conserved DNA sequences that are essential for RNA 3′ processing. We have analyzed whether this regulation occurs at the level of RNA 3′ processing. We show, by RNase mapping, that nuclear H4 mRNA precursors, which are hardly detectable in total RNA from exponentially dividing cells, accumulate in G1-arrested cells, i.e. when mature mRNAs are drastically reduced. Furthermore, we show that a heat-labile component of the processing apparatus, recently identified in HeLa cell nuclear extracts, is limiting in extracts from G1-arrested 21-Tb cells. In contrast, this activity is in excess in extracts from exponentially dividing cells, whereas both extracts contain similar amounts of snRNPs of the Sm serotype. These fluctuations in the heat-labile activity may generally contribute to proliferation or cell cycle dependent histone gene regulation.

Key words: cell cycle mutant/histone mRNA/post-transcriptional regulation/RNA 3′ processing/*in vitro* system

Introduction

The notion that RNA processing may be involved in gene regulation is not new. For instance, it was shown that alternative poly(A) site selection and splicing are key steps in the switch from a single early adenovirus L1 transcript to three different RNA species late in infection (Akusjärvi and Persson, 1981; Nevins and Wilson, 1981). A further prominent example is the change in expression from the membrane-bound to the secreted form of immunoglobulins during differentiation of B cells into plasma cells, which seems to result from a switch between two alternative poly(A) sites (Blattner and Tucker, 1984). Our interest in RNA processing comes from our studies on the post-transcriptional regulation of histone gene expression in the mammalian cell cycle (Lüscher *et al.*, 1985; Stauber *et al.*, 1986).

The histone genes of mammals, most of which are expressed in a replication-dependent manner, represent a moderately repeated gene family. The genes coding for the five types of histone proteins are expressed in stoichiometric proportions and in parallel with the DNA synthetic activity of the cell (reviewed in Stein *et al.*, 1984; Schümperli, 1986). This cell cycle-dependent regulation is achieved by both transcriptional and post-transcriptional mechanisms (DeLisle *et al.*, 1983; Heintz *et al.*, 1983; Plumb *et al.*, 1983; Sittman *et al.*, 1983; Graves and Marzluff, 1984). The transcriptional regulation, as revealed by both *in vivo* and *in vitro* studies, seems to be driven by specific transcription factors that interact with multiple upstream elements of the histone gene promoters (Artishevsky *et al.*, 1985; Hanly *et al.*, 1985; Dailey *et al.*, 1986; Osley *et al.*, 1986; Sive *et al.*, 1986). Concerning the post-transcriptional regulation, it is not yet clear whether it acts at the level of histone mRNA stability or at some earlier step, possibly even prior to the appearance of newly synthesized mRNAs in the cytoplasm (see Discussion).

We have been studying histone gene regulation in 21-Tb cells, a temperature-sensitive mouse mastocytoma cell cycle mutant that can be blocked specifically in G1 phase (Zimmermann *et al.*, 1983). In these cells, the levels of H4 mRNA fluctuate over about two orders of magnitude (Lüscher *et al.*, 1985). Considering that H4 gene transcription varies only over a 3- to 4-fold range (Lüscher *et al.*, 1985), post-transcriptional events are most important in these cells. By DNA transformation experiments, we found that a 3′-terminal mouse H4 gene fragment, when fused to the SV40 early promoter and introduced into 21-Tb cells, produced a fusion RNA that was regulated like endogenous H4 mRNAs during a temperature-shift experiment (Lüscher *et al.*, 1985). In a mutational analysis, we then demonstrated that the minimal sequences required for this regulation essentially coincided with the histone mRNA 3′ processing signal (Stauber *et al.*, 1986). These results indicated that the observed regulation was either a function of RNA processing itself or of some later step involving the conserved 3′-terminal sequence element of mature histone mRNA.

The replication-dependent histone genes have their own, unique processing mechanism for the generation of mRNA 3′ ends (reviewed in Birnstiel *et al.*, 1985). It is controlled by a highly conserved stem-loop structure at the 3′ end of the mRNA and by a second conserved sequence in the 3′ spacer (Birchmeier *et al.*, 1982, 1983; Georgiev *et al.* 1985; Stauber *et al.*, 1986). Moreover, faithful *in vitro* processing of histone mRNA precursors by components present in nuclear extracts from HeLa cells could be specifically inhibited by human systemic lupus erythematosus antisera of the Sm-serotype, indicating the involvement of snRNPs (Gick *et al.*, 1986). For the sea urchin, a specific type of snRNP is required for histone mRNA processing; it contains U7 snRNA, a member of the U RNA family (Galli *et al.*, 1983; Strub *et al.*, 1984; Strub and Birnstiel, 1986). Recently a second, heat-labile component, which is distinct from Sm-type snRNPs, was identified in HeLa cells and was shown to be also essential for the endonucleolytic cleavage reaction *in vitro*: heat-inactivated and snRNP-depleted nuclear extracts complemented each other to full activity (O.Gick and M.L.Birnstiel, in preparation).

Our present investigation addresses the question whether changes in RNA 3′ processing are responsible for the post-transcriptional regulation of histone gene expression in the 21-Tb cell cycle mutant. We have detected a genuine, nuclear H4 mRNA precursor and found that it accumulated in G1-arrested 21-Tb cells when mature histone mRNA levels were minimal. A subsequent analysis of the *in vitro* processing efficiencies in nuclear extracts from 21-Tb cells revealed that extracts from arrested cells were only poorly active in contrast to extracts from exponentially dividing cells. This deficiency could be traced back to an almost complete lack of the heat-labile activity in extracts

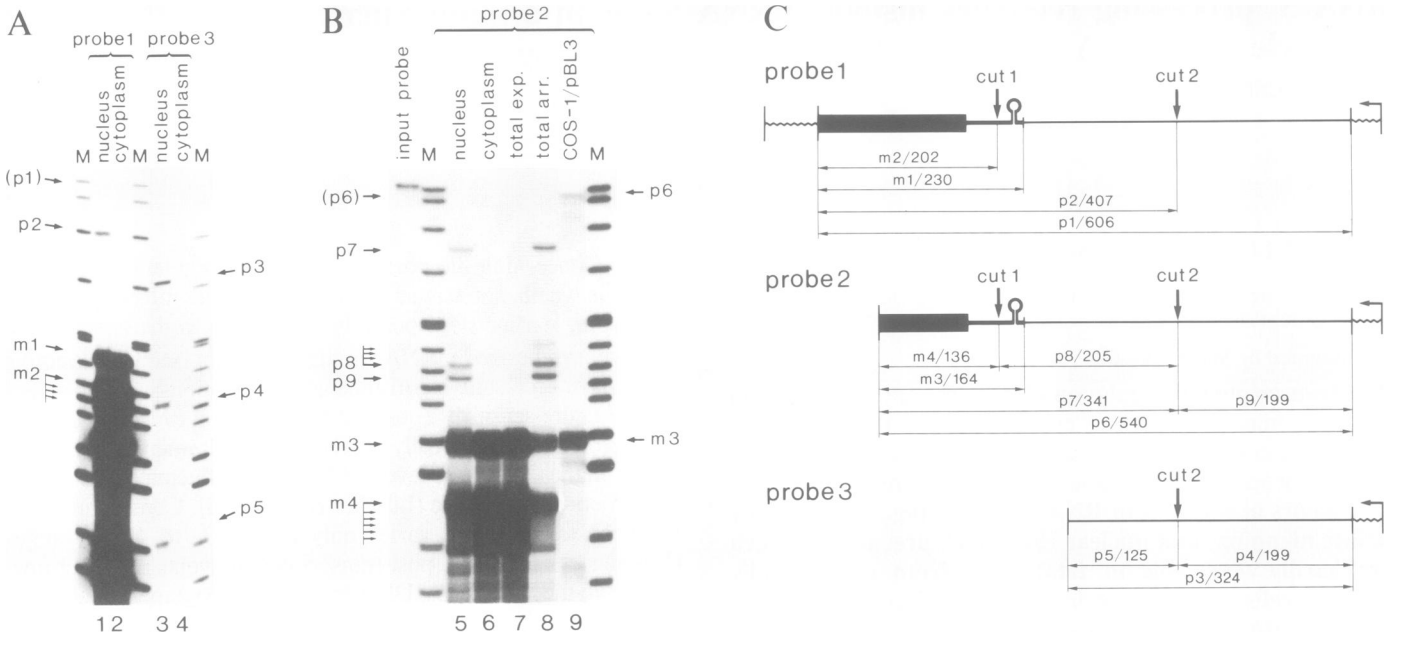

Fig. 1. RNA mapping of exponentially dividing and G1-arrested 21-Tb cells and structure of the RNA probes. (**A**) Nuclear and cytoplasmic RNA from 21-Tb cells were mapped with probe 1 (**lanes 1** and **2**) and probe 3 (**lanes 3** and **4**). Protected bands are indicated schematically in Figure 1C and described in the text. The structure of the probes is shown in Figure 1C. M: end-labelled DNA size markers (*Hpa*II digest of pBR322). (**B**) Nuclear and cytoplasmic RNA (**lanes 5** and **6**) from exponentially dividing 21-Tb cells and total RNA from exponentially dividing (**lane 7**) and G1-arrested 21-Tb cells (**lane 8**) were mapped with probe 2 (see Figure 1C). **Lane 9** shows a mapping with probe 2 using nuclear RNA from COS-1 cells which had been transfected with pBL3, a histone H4 recombinant (Lüscher *et al.*, 1985). (**C**) Structures of the three RNA probes of the mouse histone H4 gene (see also Materials and methods). Black bars: protein-coding body of the H4 gene; thick line with stem-loop structure: 3′ trailer of H4 mRNA with mature 3′ end: thin line: 3′ spacer sequences; and wavy line: plasmid vector sequences. Arrows within the RNA trailer (cut 1) and in the spacer region (cut 2) indicate the positions where the RNA:RNA hybrids were cut during RNase treatment (see text). Arrows at the right edge of the probes indicate the direction of SP6 transcription. The protected fragments and their calculated lengths are also indicated. Nomenclature: m: bands specific for mature H4 mRNA; p: precursor-specific bands.

from G1-arrested cells. The relevance of these findings for histone gene regulation in the cell cycle and in response to changes in cell proliferation will be discussed.

Results

Mapping of a genuine histone H4 mRNA precursor

To establish conditions for the characterization of histone mRNA precursors, we transfected a mouse H4 recombinant (pBL3; Lüscher *et al.*, 1985) into monkey COS-1 cells. Nuclear RNA isolated from these cells was analyzed by a RNase protection experiment using a homogeneously labelled RNA probe derived from a 3′-terminal *Hpa*II fragment of the H4 gene (probe 2; Figure 1B). Protected bands corresponding to mature H4 mRNA (m3) and to RNA extending through the entire length of the hybridization probe (p6) were obtained. The full-length protected band p6 potentially corresponded to a nuclear H4 mRNA precursor.

Using the same assay conditions to characterize endogenous H4 mRNA precursors in mouse 21-Tb cells, we obtained several additional RNase-protected bands. These were apparently due to sequence polymorphisms in the 3′ trailer and spacer region between the cloned H4 gene and its endogenous homologue in 21-Tb cells. For instance, the shorter hybridization probe 3, which contains only 3′ spacer sequences, produced, in addition to the expected full-length protected band p3, two shorter bands, p4 and p5 (Figure 1A) whose sizes added up to the size of p3. Using the mapping data shown in Figure 1A and 1B, we could unambiguously identify each of the protected RNA bands as indicated in the graph in Figure 1C. There appeared to be two sequence polymorphic sites at which the hybrids were cleaved, cut 1 in the untranslated trailer (in fact a series of successive cuts in a region that contains 14 consecutive C residues), and cut 2 about 177 nucleotides downstream from the mature H4 mRNA 3′ end. The fact that RNase cleavage at these sites was somewhat variable, depending upon the temperatures used for hybridization and RNase digestion (data not shown), further supported the notion that they were caused by sequence polymorphisms. The bands corresponding to mature H4 mRNA were designated as m1−m4, whereas the bands extending into the 3′ spacer region which corresponded to the potential H4 mRNA precursors were designated as p1−p9. However, strictly speaking, the bands m2 and m4 are a combination of signals from both the mature and precursor RNAs, but since the mature RNA is the predominant species, the small contribution made by the precursor RNA can be neglected.

The notion that the bands containing 3′ spacer sequences indeed corresponded to H4 mRNA precursors was corroborated by the fact that they could only be detected in nuclear but not in cytoplasmic RNA. In contrast, the bands corresponding to the mature RNA species were very abundant in both nuclear and cytoplasmic RNA preparations.

Histone H4 pre-mRNA and mature mRNA levels in 21-Tb cells change inversely in response to a block in G1 phase

Being able to detect and quantitate an endogenous precursor of H4 histone mRNA, we analyzed whether its concentration in 21-Tb cells changed in response to a G1-specific arrest of cell proliferation. For this purpose, we mapped equal amounts of total 21-Tb RNA from exponentially dividing and G1-arrested cells

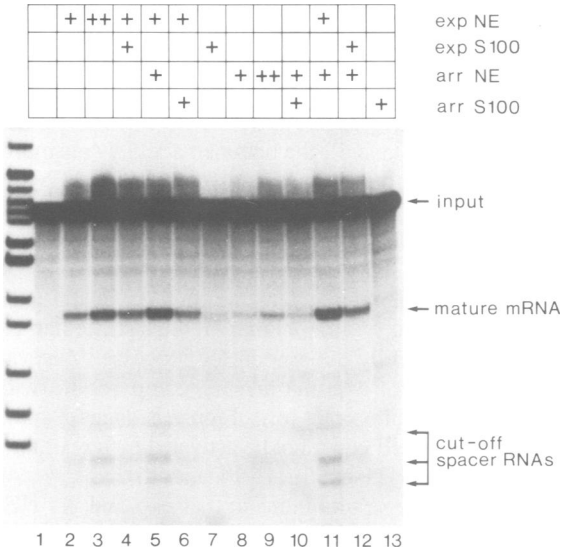

Fig. 2. *In vitro* processing of preformed precursor RNAs. Mixing experiments using extracts from exponentially dividing and G1-arrested cells. Every cross in the table stands for 5 μl of the particular extract(s). Abbreviations are: exp NE, nuclear extract from exponentially dividing cells; exp S100, post-nuclear supernatant of the same preparation; arr NE, nuclear extract from G1-arrested cells; arr S100, post-nuclear supernatant of the same preparation.

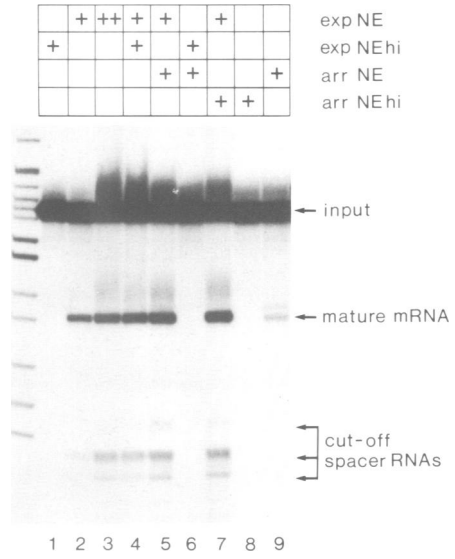

Fig. 3. *In vitro* processing of preformed precursor RNAs. Mixing experiments using heat-inactivated and untreated extracts. Abbreviations are as indicated in the legend to Figure 2. hi stands for the corresponding heat-inactivated extracts.

using probe 2 (Figure 1C) which detects the mature RNA and its precursor simultaneously. As expected, total RNA from exponentially dividing cells revealed intense bands for the mature RNA (m3 and m4, Figure 1B, lane 7). However, the precursor-specific bands (p6−9) were hardly detectable, if at all. In contrast, the same bands were easily detected and had accumulated ~20− to 30-fold in cells arrested in the G1 phase of the cell cycle (Figure 1B, lane 8). At the same time, the intensities of the bands representing the mature RNA were reduced at least 15-fold (but probably more, as these bands had been exposed beyond the linear range). The inverse behavior of RNase-protected bands specific for the mature RNA and for the longer spacer transcripts again suggested a direct precursor−product relationship between them. The fact that their total intensities did not add up to the same amount in exponentially dividing and G1-arrested cells may be explained if the half life of the precursor RNA is shorter than that of the mature H4 mRNAs. It appears from these results that, in response to the G1-specific block, the conversion of precursor to mature H4 histone RNA is slowed down, possibly by a deficiency in 3′ processing of histone pre-mRNA in G1-arrested cells. Another, less likely explanation would be that the different intensities of the RNase-protected bands are caused by simultaneously altered half-lives of both precursor and mature RNA.

H4 RNA 3′ processing in nuclear extracts from G1-arrested cells is inefficient but can be complemented by extracts from exponentially dividing cells

The availability of an *in vitro* system to study histone mRNA 3′ processing (Gick *et al.*, 1986) allowed us to confirm our *in vivo* data by corresponding experiments *in vitro*. We prepared nuclear extracts and S100 supernatants from both exponentially dividing and G1-arrested 21-Tb cells (see Materials and methods). When SP6-generated and capped histone H4 pre-mRNAs derived from plasmid SP65-H4-119/70 (Gick *et al.*, 1986) were incubated in nuclear extracts from exponentially dividing cells (exp NE), the expected bands characteristic for the mature RNA and for the cut-off spacer pieces were generated (Figure 3 in Gick *et al.*, 1986; and Figure 2 of this paper, lane 2). In contrast, nuclear extracts from G1-arrested cells (arr NE) produced only minimal amounts of the same products (Figure 2, lane 8). These and all subsequent results were verified using several independently prepared extracts. The amount of mature RNA produced was linearly dependent on the amount of nuclear extract present in the reaction (Figure 2, lanes 2, 3 and lanes 8, 9). The S100 supernatants of both extracts did not show any significant processing activity (lanes 7 and 13) and, when they were mixed with the corresponding NE, the total activity was increased minimally in the case of exp NE (lane 4) but not at all in the case of arr NE (lane 10). This indicates that, during the preparation of the exp NE, a fraction of an important component had leaked out from the nucleus into the S100 fraction. When exp NE was mixed with an equal volume of arr NE, the observed activity was surprisingly much higher than the sum of the individual activities of the two extracts (lanes 5 or 11). Apparently, the arr NE was activated by some component of the exp NE. This component was limiting the processing efficiency in the arr NE but, in turn, must have been present in excess in the exp NE. A very similar but less pronounced activation was obtained by mixing the arr NE with the exp S100 (lane 12), suggesting that a fraction of the activating component had also leaked out from the nuclei during the preparation of the exp NE.

G1 phase nuclear extracts are deficient in a heat-labile component of the processing system

It was shown very recently that the 3′ processing of histone pre-mRNAs in nuclear extracts from HeLa cells could be abolished either by depletion with human systemic lupus erythematosus antisera of the Sm-serotype (Gick *et al.*, 1986) or by mild heat treatment. However, the two depleted extracts were able to complement each other to full activity, indicating that at least two separate components are essential for the processing reaction (O.Gick and M.L.Birnstiel, in preparation). We therefore analyzed the effects of heat inactivation on our extracts from

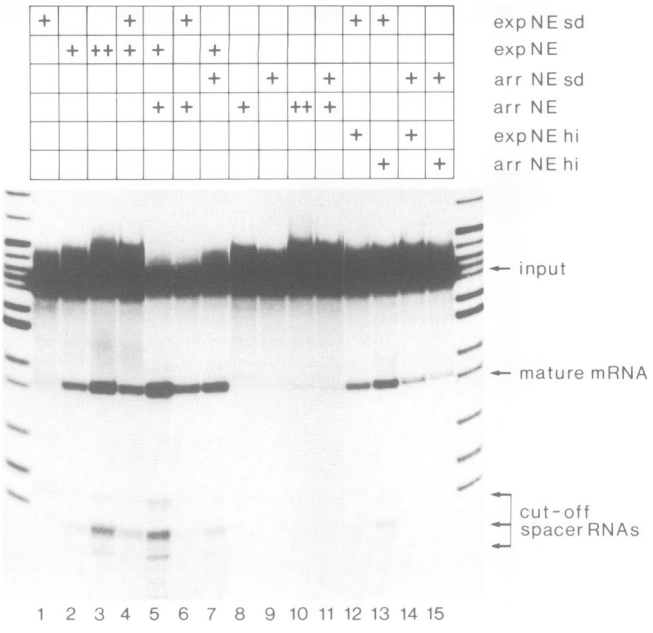

Fig. 4. *In vitro* processing of preformed precursor RNAs. Mixing experiments using snRNP-depleted, heat-inactivated and untreated extracts. Abbreviations are as indicated in the legends to Figures 2 and 3. sd stands for the corresponding snRNP-depleted extracts. The reactions in **lanes 1–4** and **8–11** are from one experiment and those in **lanes 5–7** and **12–15** are from another experiment performed under the same conditions. Identical reactions carried out in both experiments were used to calibrate the photographic exposures, so that the intensities of the bands corresponding to the mature mRNA can be compared directly.

21-Tb cells. A heat-inactivated exp NE could be efficiently reactivated by an untreated exp NE (Figure 3, lanes 1 to 4). By this criterion, the heat-inactivated extract was similar to an arr NE (lane 5). This similarity was further supported by the finding that a heat-inactivated exp NE was no longer able to activate an arr NE (Figure 3, lane 6). In contrast, even a heat-inactivated arr NE could still be activated by an active exp NE (lane 7). The apparent deficiency of heat-labile activity in G1-arrested nuclear extracts was not due to direct heat-inactivation by the temperature shift protocol, because the temperature inactivation curves of processing extracts from 21-Tb cells and from the parental cell line K21 were indistinguishable (data not shown) and because both K21 cells and spontaneous revertants of 21-Tb cells have been shown to proliferate even better at 39°C than at 33°C (Zimmermann *et al.*, 1983). Thus our findings strongly indicate that the observed low processing efficiency in G1 extracts was due to a limitation in the heat-labile component and that this effect was a true regulatory response to the proliferative state of the cells.

To analyze the distribution of snRNPs of the Sm serotype, the other previously identified component of the processing apparatus (Gick *et al.*, 1986), in the various extracts, we carried out additional mixing experiments with snRNP-depleted extracts of both exponentially dividing and G1-arrested cells. As indicated in Figure 4, such snRNP-depleted extracts were completely inactive on their own (lanes 1 and 11), as were also the heat-inactivated ones (Figure 3). The snRNP-depleted exp NE could be complemented equally well by heat-inactivated exp NE (lane 12), native (lane 6) or heat-inactivated arr NE (lane 13), indicating that all these extracts contained similar amounts of snRNPs. However, when a fully active exp NE was mixed with a snRNP-depleted exp NE, the resulting total activity was not more than additive (lane 4). This suggested that the snRNP component (or a snRNP-associated component) was limiting in the active exp NE and that the heat-labile component provided by the snRNP-depleted extract therefore could not increase the processing efficiency. As already seen earlier, when an exp NE was mixed with an arr NE (Figure 4, lane 5), the obtained activity was more than additive by approximately one equivalent of exp NE. This demonstrated that the activating component was distinct from Sm-type snRNPs and, again, that it was present in excess in exponentially growing cells. It further showed that the snRNP concentration in the arr NE was at least as high as in the exp NE. Possibly it was even a little higher in this particular extract preparation as shown by the amount of processed material obtained by a mixture of exp and arr NE compared to twice the amount of exponential extract (e.g. Figure 3, lanes 3 and 5). A snRNP-depleted arr NE could not be activated by any of the other extracts as expected (lanes 7, 11, 14 and 15).

In conclusion, our results indicate that exp and arr NE contain similar amounts of the active Sm-type snRNP component, but that the heat-labile component is present in excess in exp NE and virtually absent in arr NE.

Discussion

We previously demonstrated that, in a temperature-shift experiment with the 21-Tb cell cycle mutant, histone gene regulation is mostly due to post-transcriptional regulatory mechanisms (Lüscher *et al.*, 1985). We have now analyzed the role of histone mRNA 3′ processing in this regulation, both in the *in vivo* situation and in an *in vitro* system. Our RNA mappings indicate, by direct measurements of the relative concentrations of precursor and mature histone mRNA, that the precursor RNA accumulates in G1-arrested cells where the steady-state level of the mature RNA is minimal. This correlation strongly indicates that the conversion of the precursor to the mature RNA, i.e. RNA 3′ processing, has become inefficient in G1-arrested cells and that this, in turn, may be causing the low abundance of mature histone mRNA.

The data from our *in vitro* processing experiments with nuclear extracts from exponentially dividing and G1-arrested cells are completely consistent with this view: processing efficiency in extracts from G1-arrested cells was very poor or hardly detectable, because of the virtual absence of the heat-labile component of the processing apparatus. In contrast, exp NE efficiently processed exogenously added precursors and the heat-labile component was in excess. The activity associated with Sm-type snRNPs was shown to be present in similar amounts in both types of extracts.

Taken together with our previous demonstration that a 3′-terminal 80-bp fragment of the mouse H4 histone gene containing all the essential sequences for 3′ processing was sufficient for correct down-regulation in G1-arrested 21-Tb cell cycle mutant (Stauber *et al.*, 1986), our new results clearly establish that RNA 3′ processing is of major importance for histone gene regulation under these conditions. Although the histone genes are still being transcribed in G1-arrested cells at about one-third of the rate of exponentially dividing cells (Lüscher *et al.*, 1985), the regulatory mechanism we have described apparently determines, whether or not the primary transcripts become processed and available for translation or get discarded in the nucleus.

Is RNA 3′ processing generally involved in cell cycle- and proliferation-dependent histone gene regulation?

We are presently investigating to what extent RNA 3′ process-

ing is also involved in the regulation of histone gene expression under other conditions that block cell proliferation or during a *bona fide* cell cycle. Preliminary results indicate that the observed regulation is not just a consequence of the temperature-shift protocol or a speciality of 21-Tb cells, because the difference in histone mRNA levels between quiescent and exponentially dividing mouse C127 fibroblasts shows a similar dependence on 3′-terminal sequences (C.Stauber and D.Schümperli, unpublished results).

As far as histone gene regulation in synchronized cells is concerned, Alterman *et al.* (1984) have shown by pulse-chase experiments with mouse erythroleukemia cells separated by centrifugal elutriation into cell cycle-specific stages, that the turnover rates of H3 histone mRNA are similar in S and G2 phase. However, the periodic accumulation and decay of H3 mRNA seemed to be controlled largely by the rate of appearance of newly synthesized mRNA in the cytoplasm. Although this study did not identify any particular mechanism as being responsible for histone mRNA regulation, it was entirely compatible with the possibility that RNA 3′ processing might play an important role.

How might regulation of processing be achieved?

The heat-labile component obviously functions as an essential co-factor for histone pre-mRNA processing in addition to Sm-type snRNPs. It will be important to determine its precise function in the processing reaction. For instance, it might interact with the snRNPs and histone pre-mRNA in a stoichiometric fashion. Alternatively, it could function by transactivation of an inactive processing complex or by shuttling the snRNPs from an inactive to an active state. In the latter case, the heat-labile component would not have to be present in high amounts which would greatly favor its role as a regulatory molecule. The fact that it is thermodynamically unstable also makes the heat-labile component an attractive candidate for a short-lived regulatory factor, since it may be subject to rapid turnover *in vivo*.

One can also imagine different mechanisms by which the heat-labile component could be modulated. As a co-factor of the processing apparatus, it could be subject to activation or inactivation by post-translational modifications, e.g. by phosphorylation or dephosphorylation, proteolytic activation or degradation. Another, less attractive, possibility would be if its synthesis was being controlled. These and other questions may soon become amenable to experimentation.

Materials and methods

Plasmid constructions for SP6 probes

The 3′-terminal *Hpa*II fragment of the cloned mouse H4 gene (Seiler-Tuyns and Birnstiel, 1981) was provided with *Sal*I linkers and cloned into the *Sal*I site of the SP62 polylinker sequence, such that SP6 polymerase would transcribe the non-coding strand. SP6 run-off transcripts were performed with templates cut with *Eco*RI (probe 1), *Tth*111.1 (probe 2) or *Sac*II (probe 3) in the presence of [α-^{32}P]rGTP (410 Ci/mmol) as described by Green *et al.* (1983).

RNA purification and SP6 mapping

Total RNA was isolated by hot acid phenol extraction (Scherrer, 1969), precipitated with ethanol and extensively digested with DNase I (purified according to Picard and Schaffner, 1983). For the preparation of nuclear and cytoplasmic RNA, cells were lysed according to Carneiro and Schibler (1984). Nuclei were separated from the cytoplasm by centrifugation through a sucrose cushion and lysed by pressing them three times through a 18-g needle. Both nuclear and cytoplasmic fractions were then digested with proteinase K by the addition of an equal volume of 2 × pK solution (1 mg/ml proteinase K, 300 mM NaCl, 25 mM EDTA, 20 mM Tris−HCl pH 7.5, 2% SDS) and incubation at 45°C for 30 min. RNAs were extracted with phenol, phenol/chloroform and chloroform and precipitated with ethanol prior to DNase I digestion as above.

For SP6 mappings, 100 μg of total, cytoplasmic or nuclear RNA were co-precipitated with 2 × 10^5 c.p.m. of gel-purified SP6-RNA and hybridized overnight in 30 μl hybridization buffer (Weaver and Weissmann, 1979) at 65°C (probe 1 and 2) or at 58°C (probe 3). RNase digestions were done with 8 μg/ml RNase A and 16 U/ml RNase T1 (Böhringer Mannheim) for 1 h at 33°C in 350 μl of the buffer described by Melton *et al.* (1984). The samples were then digested with proteinase K for 20 min at 33°C by the addition of 10 μl 20% SDS and 5 μl proteinase K (20 mg/ml) and extracted with phenol /chloroform and chloroform before ethanol precipitation. They were analyzed on 0.5-mm polyacrylamide gels (5%, 8.3 M urea in 1 mM EDTA and 100 mM Tris adjusted to pH 8.3 with boric acid).

Cell cultures

COS-1 cells were cultured as described by Lüscher *et al.* (1985) and transfected by the DNA/calcium phosphate coprecipitation method (Weber *et al.*, 1984). The temperature-sensitive cell cycle variant 21-Tb of the P815-X2 mouse mastocytoma cell line (Zimmermann *et al.*, 1981, 1983) was cultured as described previously (Lüscher *et al.*, 1985). For G1 phase nuclear extracts, exponentially growing 21-Tb cells were shifted to 39°C for 44 h.

Nuclear extract preparation/heat inactivation and snRNP deletion

Nuclear extracts and S100 supernatants from both exponentially dividing and G1-arrested cells were prepared according to Dignam *et al.* (1983). For heat inactivation, the extracts were incubated for 15 min at 50°C (O.Gick and M.L.Birnstiel, in preparation). Depletion of extracts by antisera of the Sm-serotype was performed according to Krämer *et al.* (1984).

In vitro processing reactions

The capped precursor RNA from plasmid SP65-H4-119/70 (a generous gift of O.Gick) and incubation conditions in 30 μl reactions were the same as described by Gick *et al.* (1986), with the modification that ATP and creatine phosphate were omitted. In mixing experiments, 5 μl of each of the indicated extracts were added to each other and the volume was adjusted to 15 μl with buffer D (Gick *et al.*, 1986). Reactions were mixed on ice and incubated for 2 h at 30°C, extracted as described, precipitated with ethanol, and digested with DNase I for 10 min. They were extracted with phenol/chloroform, precipitated with ethanol and analyzed on polyacrylamide gels (6%, 8.3 M urea, in 1 mM EDTA and 100 mM Tris adjusted to pH 8.3 with boric acid).

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