3' processing of pre-mRNA plays a major role in proliferation-dependent regulation of histone gene expression

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SUMMARY

A short histone-like fusion RNA, generated when the RNA 3' processing signal from a mouse histone H4 gene is inserted into a heterologous transcription unit, becomes correctly down-regulated in G1-arrested cells of a temperature-sensitive mouse mastocytoma cell cycle mutant (21-Tb; Stauber et al., EMBO J. 5, 3297-3303 [1986]), due to a specific deficiency in histone RNA processing (Lüscher and Schümperli, EMBO J. 6, 1721-1726 [1987]). In contrast, inhibitors of DNA synthesis, known to stimulate histone mRNA degradation, have little or no effect on the fusion RNA. This RNA can therefore be used to discriminate between regulation by RNA 3' processing and RNA stability, respectively. The fusion RNA is also faithfully regulated in 21-Tb cells arrested in G1 phase by the drug indomethacin or in C127 mouse fibroblasts during a serum starvation experiment. Moreover, nuclear extracts from serum-starved C127 cells show a specific deficiency in a heat-labile component of the histone RNA processing apparatus, similar to that previously observed for temperature-arrested 21-Tb cells. These results suggest that RNA 3' processing is a major determinant for the response of histone mRNA levels to changes in cell proliferation.

INTRODUCTION

Histone genes are a model system for studying the regulation of mRNA abundance during the cell cycle or in response to changes in cell proliferation. The five types of histone proteins are synthesised in stoichiometric proportions and in parallel with cellular DNA synthesis, under a wide variety of experimental conditions (synchronised cells, conditional cell cycle mutants, proliferative quiescence and stimulation, chemical inhibition of DNA synthesis). For each of these conditions, regulation has been reported to involve both transcriptional and post-transcriptional regulatory mechanisms (reviewed in 1,2).

Transcriptional regulation seems to be driven by certain transcription factors that bind to specific sequence elements of the histone gene promoters (3-6). Concerning the post-transcriptional regulation, there are reports suggesting that it acts either at the level of mature histone mRNA turnover or at the level of nuclear histone RNA 3'

processing. The first of these two mechanisms, a drastic increase in the turnover of mature histone mRNAs, can be observed when cells are treated with inhibitors of DNA synthesis (reviewed in 1,2). Recent results indicate that the target for this regulation is a conserved hairpin-loop stucture residing in the 3'-untranslated region of histone mRNAs (7,8). The second of these post-transcriptional mechanisms was first revealed by our studies of histone gene regulation in 21-Tb cells, a temperature-sensitive mouse mastocytoma cell cycle mutant that can be blocked specifically in G1 phase. The very strong histone gene regulation occurring during such temperature shift experiments is mostly due to changes at the post-transcriptional level (9). We could further show that 3'-terminal mouse H4 gene fragments, when fused to the SV40 early promoter, produced fusion RNAs with histone-specific 3' ends that were regulated like the endogenous H4 mRNAs (9). Most significantly, the minimal sequences required for this regulation essentially coincided with the histone mRNA 3' processing signal (10). Further *in vivo* and *in vitro* studies then directly demonstrated changes in histone RNA 3' processing that could account for the post-transcriptional regulation in 21-Tb cells (11). These changes were apparently due to the lack of a heat-labile component (12) of the histone RNA processing apparatus in the G1-arrested cells (11).
It was not clear, from these investigations, which of the above post-transcriptional mechanisms, i.e. selective histone mRNA degradation or regulation of histone RNA 3' processing, is most important for histone gene regulation, either during the cell cycle or when cell proliferation is arrested or stimulated. A particular problem was that the regulatory signals for the two regulatory mechanisms overlap, because the 3'-terminal hairpin is also part of the RNA processing signal (1,13). In this paper, we have analysed the expression of a gene fusion containing the 3'-terminal H4 RNA processing signal under different experimental conditions that lead to an arrest of cell proliferation in G1 (or G0). For reasons which are discussed below, the histone-like RNA generated from this construct is not subject to the stability regulation. Using this RNA as an indicator for the processing mode of regulation, we demonstrate that RNA 3' processing is an important control mechanism coupling histone gene expression to cell proliferation. This notion is also supported by *in vitro* experiments that reveal changes in the efficiency of histone RNA processing between quiescent and proliferating fibroblast cells.

MATERIALS AND METHODS

Cell culture, inhibition of DNA synthesis, indomethacin block, and serum starvation

The temperature-sensitive cell cycle variant 21-Tb of the P815-X2 mouse mastocytoma cell line (14) was grown in suspension culture as described previously (9,15). In this work, 21-Tb cells stably transformed with plasmid pgptCX/-230+118 (a gift of

B. Lüscher; Fig.1B; 10) were used. C127 mouse fibroblasts, grown as monolayer cultures (16), were transfected by the DNA/calcium phosphate coprecipitation technique (17), followed by a shock with 15 % glycerol for 3 min, and stable transformants were selected for the expression of the *E.coli* gpt gene as described (18).
DNA synthesis was inhibited in exponentially growing 21-Tb or C127 cells by incubation with 5 mM hydroxyurea. Partial synchronisation of 21-Tb cells in G1 was performed by incubation with 0.4 mM indomethacin (19). Quiescent C127 cells for RNA extraction experiments were obtained by incubating half confluent cultures for 3 days in growth medium containing only 0.5 % fetal calf serum. These cells were stimulated using fresh medium containing 10 % fetal calf serum. When arrested C127 cells were used for nuclear extract preparation, a confluent culture was incubated in growth medium with 0.5% fetal calf serum for 24 hours.
The rates of DNA synthesis were measured by the incorporation of ^{3}H-methyl-thymidine (2-10 uCi/ml; 46 Ci/mmol; Amersham) in 30 min pulses (20).

RNA-purification and S1 nuclease or RNAase mapping

Total cellular RNA was isolated by hot acid phenol extraction (21). Hybridisation and S1 nuclease analysis were carried out as described (10,22). For RNAase mappings, 20ug of total cellular RNA were coprecipitated with an excess of a uniformely labelled RNA probe obtained by run-off transcription with SP6 RNA polymerase and HindIII-cut plasmid pSP65gptCX (Fig.1B), hybridised overnight in 25ul hybridisation buffer (22) at 65^{o}C and further analysed as described previously (11).

Nuclear extract preparation and in vitro processing reaction

Nuclear extracts from exponentially dividing and quiescent C127 cells were prepared according to Dignam et al. (23). Extracts from 21-Tb cells were a gift of B. Lüscher. In vitro processing reactions were performed as described (11,24).

RESULTS

Experimental strategy

When 3'-terminal mouse histone H4 gene fragments were inserted into the gpt transcription unit of plasmid pgptCX (Fig.1B), these new constructs, after transfection into mammalian cells, gave rise to two main RNA species: a short one with histone-specific 3' ends and a longer one traversing the entire histone DNA insert and proceeding to the end of the original transcription unit (10). In temperature shift experiments with the temperature-sensitive cell cycle mutant 21-Tb, the short RNA (proc) was regulated similarly as the endogenous histone mRNAs, whereas the long RNA (rt) was maintained at roughly constant level (10). The regulation of the short RNA was shown to be controlled by the sequences constituting the histone-specific RNA processing signal (10). In line with this, we found that histone RNA processing is deficient in G1-arrested 21-Tb cells (11). To analyse the physiological relevance of this regulation by RNA 3' processing, we have now used cell lines transformed with such a pgptCX/H4 recombinant and tested under which other experimental conditions the

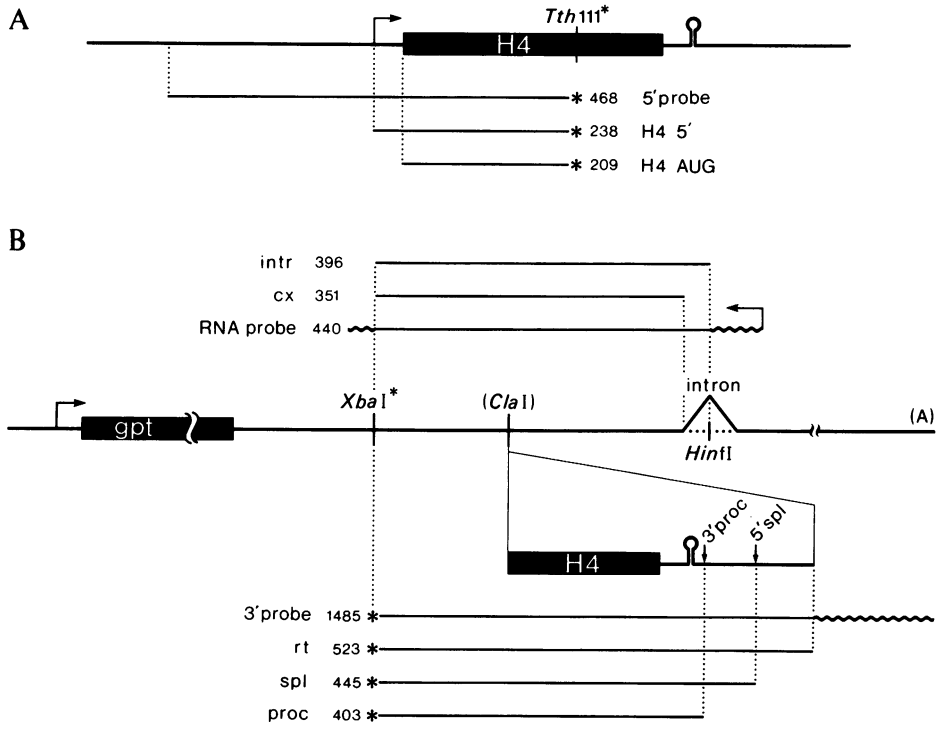

Figure 1. Structures of genes, SP6 RNA and S1 hybridization probes. (A) Structure of the mouse histone H4 gene (25). The transcription start site (arrow) and the 3'-terminal hairpin loop representing part of the RNA 3' processing signal are indicated. The S1 hybridization probe was 5'-endlabelled at a Tth111.I site within the gene. H4 5': part of probe protected by correctly initiated, fully homologous H4 mRNA. H4 AUG: part of probe protected by transcripts from nonallelic H4 genes (protein coding portion only). Numbers indicate the lengths of fragments in nucleotides. **(B)** Structure of the gpt transcription units of plasmids pgptCX and pgptCX/-230+118 (10). The original plasmid pgptCX contains a transcription unit consisting of the early promoter of SV40 (arrow), the bacterial gene for xanthine: guanine phosphoribosyl transferase (gpt), the SV40 small t intron (intron) and polyadenylation site ([A]). Uniformely labelled RNA probe for RNAase mapping of pgptCX transcripts (shown above) was obtained by run-off transcription with SP6 RNA polymerase of HindIII-cut plasmid pSP65gptCX (the latter contains a fragment extending from the XbaI site to a HinfI site within the SV40 small t intron, subcloned between the SmaI and XbaI sites of pSP65). intr: part of probe protected by unspliced transcripts still containing intron sequences. cx: part of probe protected by transcripts correctly spliced at the small t intron 5' splice site. Plasmid pgptCX/-230+118 additionally contains a mouse H4 gene fragment with the last 230 bp of H4 mRNA coding sequence and the first 118 bp of 3' spacer, inserted at the ClaI site. The S1 hybridization probe (shown below) was 3'-endlabelled at the XbaI site. rt: part of

probe protected by readthrough transcripts traversing the entire histone DNA insert. spl: part of probe protected by differently spliced readthrough RNA generated by utilisation of a cryptic 5' splice site present in the post-H4 spacer (10). proc: part of probe protected by correctly processed histone-like RNA. Wavy lines: parts of probes not present on gene constructs.

short RNA encoded by this plasmid is also regulated in parallel with endogenous histone mRNAs.

The short RNA is not destabilised by inhibitors of DNA synthesis

When 21-Tb cells transformed with pgptCX/-230+118 (Fig.1B) were treated with 5 mM hydroxyurea (HU), the levels of endogenous H4 mRNAs became drastically reduced within 30 min of the application of the drug (Fig.2). This down-regulation is known to be the consequence of a selective destabilisation of histone mRNAs (reviewed in 1,2). Unlike the endogenous H4 mRNAs, the short (proc) RNA encoded by pgptCX/-230+118 was not affected by the HU treatment (Fig.2). The level of long (rt) RNA decreased slightly in this particular experiment, but remained constant in

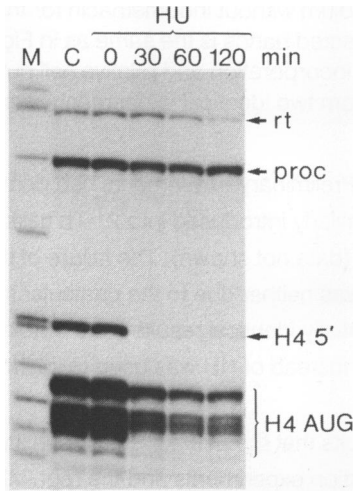

Figure 2. Mapping of RNAs in 21-Tb cells after inhibition of DNA synthesis. Total RNA from 21-Tb cells stably transformed with pgptCX/-230+118 was used for S1 mapping with a mixture of 5'- and 3'-endlabelled probes (see Fig.1). M: endlabelled DNA size markers (HpaII digest of pBR322). C: untreated control cells. HU: cells treated with 5 mM hydroxyurea for the indicated times (in min). rt: readthrough transcripts from pgptCX/-230+118 traversing the entire histone DNA insert. proc: correctly processed histone-like RNA from pgptCX/-230+118. H4 5': correctly initiated endogenous H4 mRNA from the particular gene used. H4 AUG: transcripts from nonallelic endogenous H4 genes (only protein coding region protected).

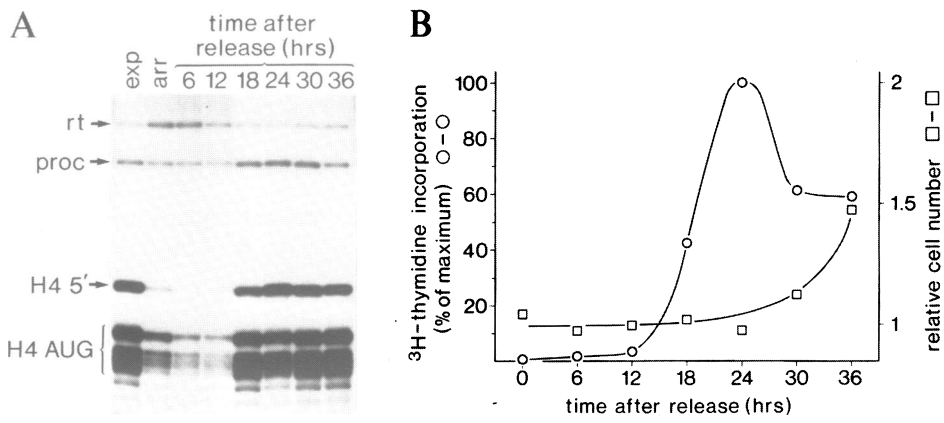

Figure 3. Mapping of RNAs in 21-Tb cells partly synchronized by indomethacin block. (A) Total RNA from 21-Tb cells stably transformed with pgptCX/-230+118 was used for S1 mapping with a mixture of 5'- and 3'-endlabelled probes (see Figs.1 and 2). exp: exponentially dividing control cells. arr: cells arrested in G1 phase by incubation in 0.4 mM indomethacin for 20 hours. 6, 12, 18, 24, 30, 36: arrested cells reincubated in growth medium without indomethacin for the indicated times (in hours). The nomenclature of protected bands is the same as in Figs.1 and 2. **(B)** Plot showing the rates of ^{3}H-thymidine incorporation and relative cell numbers during such an experiment. The results from two identical experiments were averaged.

others (data not shown). Preliminary experiments had demonstrated that a complete mouse H4 gene, when similarly introduced into 21-Tb cells, was faithfully regulated in response to hydroxyurea (data not shown). The failure of the short histone-like RNA to be properly regulated was neither due to the particular inhibitor nor to the particular cell line used, since essentially identical results were obtained in C127 mouse fibroblast cells or if aphidicolin instead of HU was used to inhibit cellular DNA synthesis (data not shown).

It appears from these results that the destabilisation of histone mRNAs occurring in such DNA synthesis inhibition experiments and the regulation of histone RNA 3' processing occurring in a temperature shift experiment are controlled by different features of the RNA (see Discussion). This implies that the regulatory behaviour of the short proc RNA encoded by pgptCX/-230+118 can be used to discriminate between these two post-transcriptional regulatory mechanisms. For any situation in which this RNA is regulated in parallel with endogenous histone mRNAs, it is therefore very likely that this regulation is mediated by changes in histone-specific RNA 3' processing.

The short RNA is correctly regulated in cells partly synchronised by a G1-specific block

To see if the down-regulation of histone RNA 3' processing in 21-Tb cells was specific for temperature shift experiments or whether it was a more general feature of a G1-specific proliferation arrest, we treated 21-Tb cells stably transformed with pgptCX/-230+118 with the G1-specific inhibitor indomethacin (19). After an overnight incubation with 0.4 mM indomethacin, cellular DNA synthesis had practically ceased (Fig.3B) and the cells represented an essentially pure G1 population, as judged by flow cytometry of ethidium bromide-stained cells (data not shown). After removal of the drug, the cells traversed S phase in a partly synchronous manner, i.e., after a lag period of about 12 hours, DNA synthesis rose to a peak at about 24 hours and then decreased again as the cells began to divide (Fig.3B). However, the activity of DNA synthesis did not return to very low levels, indicating that the cells had already lost most of their synchrony at this point. The levels of endogenous H4 mRNAs closely followed the rates of DNA synthesis (Fig.3A). More importantly, however, the short fusion RNA encoded by pgptCX/-230+118 also showed changes that followed cellular DNA synthesis activity (Fig.3A). Thus, the histone RNA processing signal appeared to modulate the levels of the histone-like fusion RNA during this experiment. However, there were two noteworthy differences to the behaviour of the same transcription unit in temperature shift experiments (10): (i) the fluctuations in the levels of short (proc) RNA were less pronounced than those of the endogenous histone mRNAs (see Discussion) and (ii) concomitant with the reduction in the short (proc) RNA during and at early times after the indomethacin block, there was an increase in the long (rt) RNA (see next section).

The short RNA is faithfully regulated in response to serum in fibroblast cells

Another experimental condition under which histone genes are strongly regulated is when fibroblast cells are arrested by serum starvation and restimulated to proliferate by addition of fresh serum. This is a relatively physiological kind of growth regulation, since it represents the withdrawal and addition of growth factors, i.e. a phenomenon also controlling cell proliferation in multicellular organisms. The C127 line of mouse fibroblasts (16) is well suited for this kind of experiment: it shows a strong contact inhibition and is completely dependent on serum growth factors for its proliferation. Moreover, after 3 days of serum starvation, endogenous H4 mRNA levels in C127 cells are reduced about 15- to 50-fold (Fig.4A, 4C and other data not shown).

To test if RNA 3' processing plays a major role in this regulation, we isolated C127 cells that were stably transformed with pgptCX/-230+118 and analysed total cellular

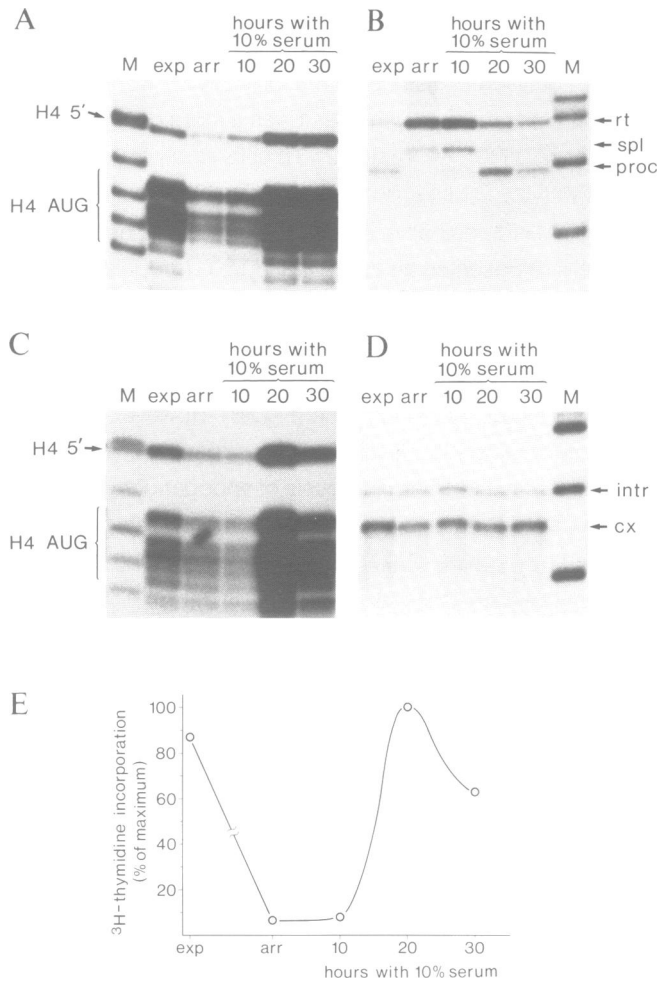

Figure 4. Mapping of RNAs in serum-starved C127 fibroblasts. Total RNA from C127 fibroblast cells stably transformed with either pgptCX/-230+118 (panels A and B) or pgptCX (panels C and D) was used (for the structure of transcription units and hybridization probes, see Fig.1). Panels A and C: S1 mapping with the H4-specific 5'-endlabelled probe; panel B: S1 mapping with the pgptCX/-230+118-specific 3'-endlabelled probe; panel D: RNAase mapping with the pgptCX-specific SP6 RNA probe; **panel E:** Plot showing the rates of ^{3}H-thymidine incorporation during an identical experiment. M: size markers. exp: exponentially dividing control cells. arr: quiescent cells obtained by incubation in growth medium containing only 0.5% fetal calf serum for 3 days. 10, 20, 30: quiescent cells refed with growth medium containing 10% fetal calf serum for the indicated times (in hours). The nomenclature of protected bands is the same as in Figs.1 and 2.

RNA by S1 mapping during a serum starvation experiment. As can be seen in Fig.4B, the short RNA (proc) encoded by pgptCX/-230+118 was indeed regulated in a very similar way as the endogenous H4 mRNA. Similarly to the previous experiment with indomethacin, however, the long RNA (rt) accumulated to relatively high levels in serum-starved cells. The same was true for an additional RNA (spl) in which a cryptic 5' splice site 42 nucleotides downstream of the histone mRNA 3' processing site was used (10). This particular behaviour of these long RNAs in this and the previous indomethacin experiments was somewhat surprising, because, in 21-Tb-cells arrested in G1 by incubation at the non-permissive temperature, the rt RNA had remained roughly constant (10). Thus it seemed either that transcription from the SV40 early promoter was specifically activated in cells arrested by indomethacin block or serum starvation or that the two processing events, splicing/polyadenylation on the one hand and histone-specific 3' processing on the other, were competing with each other, making it difficult to assess which was the primary regulated phenomenon.

To clarify this point, we isolated another pool of C127 cells transformed with the original pgptCX plasmid. In this case, the gpt mRNAs were mapped with a uniformly labelled RNA probe (Fig.1B) that detected both RNA spliced at the SV40 small t antigen 5' splice site (cx) and unspliced (intr) RNA. Neither of these RNAs showed any significant fluctuation during a serum starvation experiment (Fig.4D). Thus, the fluctuations in the levels of the long rt and spl RNAs noted above must have been caused by the introduced histone sequences. Additional experiments suggested that the rt and spl RNAs in pgptCX/-230+118-transformed cells did not simply represent unprocessed precursors to the histone-like proc RNA, because they were retained on oligo(dT) columns and because their distribution between cytoplasmic and nuclear RNA preparations did not differ significantly from that of the proc RNA (data not shown). Thus we conclude that the primary regulated event must have been histone-specific RNA 3' processing and that, for reasons that are still unclear, the generation of the rt and spl RNAs showed inverse compensatory fluctuations under conditions of indomethacin treatment and serum starvation, but not in the previous temperature-shift experiments.

Serum-dependent regulation of histone RNA 3' processing in nuclear extracts from fibroblast cells

We have previously demonstrated that, in 21-Tb cells arrested in G1 by incubation at the non-permissive temperature, histone RNA 3' processing is specifically down-regulated (11). Notably, nuclear extracts from G1-arrested cells processed an exogenous histone precursor RNA less efficiently than extracts from exponentially dividing cells (see Fig.5, lanes 4 and 3, respectively). Moreover, mixing of extracts

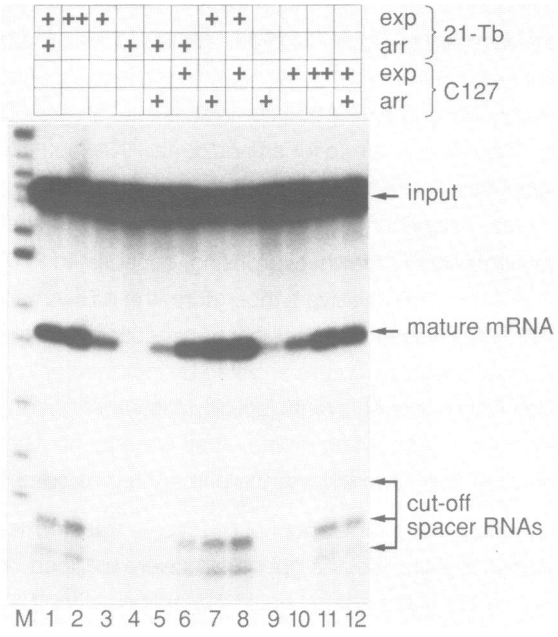

Figure 5. In vitro processing of preformed precursor RNAs. In vitro synthesized transcripts (input) corresponding to the 3'part of the histone H4 precursor mRNA (13,14) were incubated with nuclear extracts from exponentially dividing cells (exp) or G1-arrested cells (arr) of the 21-Tb cell line and of the C127 cell line. 21-Tb cells were arrested by incubation at the non-permissive temperature for 48 hours, C127 cells were growth arrested in G1/G0 by serum starvation for 24 hours. The products of processing reactions were analysed on denaturing polyacrylamide gels. M: size markers (HpaII digest of pBR322).

from arrested and proliferating cells resulted in a more-than-additive processing activity (compare Fig.5, lane 1 with lanes 3 plus 4). This had indicated that the two extracts were limiting in two different components of the processing apparatus. Further experiments had demonstrated that both extracts contained similar amounts of active small ribonucleoprotein particles (snRNPs;11). However, we had found that a heat-labile component, characterised by Gick et al. (12), was severely limiting in extracts from G1-arrested cells but present in excess in extracts from exponentially dividing cells (11).

To investigate further the apparent processing defect in serum-starved C127 cells, we prepared and analysed nuclear processing extracts. As in 21-Tb cells, there was a strong difference in processing activity between proliferating and arrested cells which was already evident after 24 hours of serum starvation (Fig.5, lanes 10 and 9, respec-

tively). Moreover, mixing of arrested and exponential extracts resulted in a similar more-than-additive processing activity as in 21-Tb cells (lane 12). Mixing of either arrested (lane 5) or exponential (lane 8) extracts from the two cell lines yielded only roughly additive activities, but both crosswise mixes (lanes 6 and 7) again gave the more-than-additive phenomenon. From this, we conclude (i) that histone-specific RNA 3' processing is down-regulated in serum-starved C127 cells and (ii) that this phenomenon is due to a defect in a specific component of the processing apparatus that is present in excess in nuclear extracts from exponentially dividing cells. Moreover, the regulated component seems to be the same as in temperature-arrested 21-Tb cells (i.e. the previously characterised heat-labile factor; 11,12), as is indicated by the mutual complementation of 21-Tb and C127 nuclear extracts. This latter conclusion was fully supported by additional experiments in which the heat-labile regulatory component was inactivated by incubation at 50^{o}C. Heat-inactivated exponential extracts, native, or heat-inactivated arrested extracts were virtually indistinguishable in terms of their ability to be reactivated/complemented by native exponential extract (data not shown).

It must also be noted that the extract isolated from C127 cells that had been starved for 24 hours still showed weak processing activity. Interestingly, several additional extracts prepared after 3 days of serum starvation were completely inactive on their own and failed to be complemented by exponential C127 extracts. However, complementation of these extracts was still possible with extracts from exponentially dividing 21-Tb cells (data not shown). This implies that the RNA 3' processing apparatus is not simply a two component system (snRNPs plus heat-labile activity) and that there are subtle differences between the two cell systems. In particular, it seems that an additional component becomes deficient in C127 cells at longer arrest times and that this component is present in excess in 21-Tb cells but not in C127 cells. However, further experiments will be necessary to clarify this point.

In summary, these *in vitro* experiments fully support the conclusion, gained from the previous analysis of cells transformed with pgptCX/-230+118, that histone RNA 3' processing is down-regulated in serum-starved C127 fibroblasts in a manner very similar to that previously observed for 21-Tb cells arrested by incubation at the non-permissive temperature.

DISCUSSION

Structural requirements for the regulation of histone mRNA levels by degradation and by RNA 3' processing

In this paper, we have monitored the expression of the short SV40/histone H4 fusion RNA under various conditions under which mammalian histone genes and mRNAs

are known to be regulated. We had previously shown this fusion RNA to be faithfully regulated in temperature-shift experiments with the temperature-sensitive cell cycle mutant 21-Tb (9,10). We could further demonstrate that this regulation was largely, if not entirely, due to fluctuations in the efficiency of histone-specific RNA 3' processing (11). We therefore argued that the expression of this fusion RNA might be taken as an indicator for the processing mode of regulation. Our finding that this RNA is not subject to regulation at the level of RNA stability, establishes the validity of this approach and independently proves that histone mRNA levels are (post-transcriptionally) regulated by at least two different mechanisms. With the possible caveat in mind that there may be other, as yet unidentified modes of post-transcriptional regulation for the histone genes, we can thus assume that, in any situation where the short RNA is correctly regulated, RNA 3' processing plays an important role in histone gene regulation.

An important implication of this is that the processing and degradation modes of regulation are controlled by different features of the RNA. Nevertheless, recent reports indicate that the selective histone mRNA degradation that occurs when cellular DNA synthesis is inhibited is controlled by the conserved hairpin loop at the 3' end of histone mRNAs (7,8). As this structure also forms part of the RNA 3' processing signal (1,10,13), this means that the two regulatory signals must at least overlap. However, it was found that the susceptibility of histone mRNA for selective degradation was also dependent upon its translation to within 300 nucleotides of the 3' end (26) and that out-of-frame translation to the very 3' end prevented selective degradation (27). These findings strongly suggested a direct coupling of histone mRNA degradation to translation and also provided an explanation for the fact that histone mRNAs are stabilised, when cells are treated with inhibitors of protein synthesis (28-30). Although the fusion RNA we have monitored did contain the 3' end of H4 mRNA, translation of the gpt coding region would have terminated about 540 nucleotides further upstream. Thus it is possible that the failure of the fusion RNA to be regulated in response to hydroxyurea is not due to the lack of a regulatory sequence in the strict sense, but rather to an inability to couple mRNA turnover to translation. Consistent with this notion is our recent finding that, when inserted into another transcription unit, such that translation in the H4 reading frame was possible, the same histone fragment produced an RNA that was correctly down-regulated by hydroxyurea (C.S., K.Bähler and D.S., unpublished result).

Thus it is remarkable that the conserved 3'-terminal hairpin loop of histone mRNAs seems to play an important role in two distinct processes which both participate in and are used to control histone gene expression. On the one hand, together with an additional conserved sequence located within the first 20 nucleotides of 3' spacer, the

hairpin loop constitutes the RNA 3' processing signal (1,10,13) and is therefore involved in the regulation of histone RNA processing (1,11). On the other hand, the hairpin loop seems to be the target for the first steps in cytoplasmic histone mRNA degradation (7,8,31), provided that the yet poorly understood translation/degradation coupling is allowed to function (26,27). This dual role is highly reminiscent of bacterial systems, where similar hairpin loop structures sometimes participate in multiple regulatory processes including transcription termination, RNA processing and RNA degradation (reviewed in 32).

Histone RNA 3' processing is coupled to cell proliferation

The main purpose of this work was to begin to investigate the physiological significance of the processing mode of histone gene regulation. Together with our previous analyses (9-11), we have now demonstrated that this regulatory mechanism plays an important role in histone gene regulation in three different experimental systems: a temperature-induced, G1-specific arrest of 21-Tb cells, a block of 21-Tb cells in G1 by indomethacin, and a G1/G0 arrest of C127 fibroblasts by serum starvation. We therefore feel confident to propose that this regulatory mechanism is generally involved in histone gene regulation under G1/G0 arrest conditions. This is of considerable physiological importance because the control of cell proliferation in multicellular organisms is most often effected by G1/G0 arrest.

Nevertheless, our conclusion that RNA processing is a major determinant of histone gene expression in such conditions does not exclude the participation of other post-transcriptional mechanisms such as mRNA degradation. For instance, the extent of regulation of the SV40/H4 fusion RNA was clearly smaller than that of the endogenous H4 mRNAs in the indomethacin experiment (Fig.3A), but roughly similar in temperature-shift experiments (9,10) or in the serum starvation experiment (Fig.4A and 4B). This could mean that, in the indomethacin experiment, either changes in the rate of histone gene transcription or an additional regulation of mRNA stability also significantly contributed to histone gene regulation.

Which mode of regulation is most prevalent in cycling cells ?

An important question is what mechanism(s) controls histone gene expression in cycling cells. Clearly, transcription plays an important role. In fact, transcriptional activation seems to be the main mechanism responsible for the increase in histone mRNA levels at the onset of S phase. Histone gene transcription is activated slightly before cellular DNA replication starts (33,34). Moreover, a histone promoter region can cause an appropriate S phase-dependent activation of a linked reporter gene (4,6,35,36). However, such a hybrid RNA is only inefficiently down-regulated at the end of S phase (35), indicating that a post-transcriptional mechanism(s) must become important at this point. This may but must not necessarily be histone mRNA degrada-

tion, because the only direct evidence, so far, for this kind of regulation comes from experiments with inhibitors of DNA synthesis. However, it is equally difficult to say if histone RNA 3' processing is the major cell cycle regulatory mechanism, because, although both indomethacin block and serum starvation resulted in partly synchronous cell populations, our present experiments are probably reflecting the arrest and stimulation of proliferation rather than a true cell cycle. One reason for taking this point of view is that the cells come out of a prolongued proliferation block and it is questionable whether all events of the first cycle would also occur in continually cycling cells. Moreover, when we synchronised 21-Tb cells with S phase-specific blockers (i.e. inhibitors of DNA synthesis) such as thymidine or aphidicolin, the SV40/H4 fusion RNA monitored in this paper was not regulated like the endogenous H4 mRNAs (C.S. and D.S., unpublished). This is in agreement with similar data of Capasso et al. (27) who found that a degradation-deficient human H4 derivative failed to be correctly regulated in cells synchronised by an aphidicolin block. From these results it would seem that histone RNA 3' processing does not play a major role in controlling histone gene expression in cycling cells. However, because the first phase of such an experiment (S phase-specific block and release thereof) is mainly reflecting the drug's known effect on histone mRNA stability, this question will require further investigation, e.g. by analysing cells that have been synchronised by methods that do not block proliferation.

A plausible current view on this problem is that histone mRNA degradation plays a major role in cycling cells, but an additional contribution by RNA processing cannot presently be excluded. In contrast, the regulation of histone RNA 3' processing now appears to be an important (perhaps even the most important) regulatory mechanism responding to changes in the proliferative state of a cell. An obvious question resulting from this is why different post-transcriptional mechanisms should be used to control histone gene expression in the cell cycle as compared to situations of proliferation arrest/stimulation. Possibly, the regulation of mRNA stability is optimal for rapid and transient changes, whereas the regulation of RNA 3' processing is better suited for a more permanent shut-down of histone gene expression.

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