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A signal regulating mouse histone H4 mRNA levels in a mammalian cell cycle mutant and sequences controlling RNA 3′ processing are both contained within the same 80-bp fragment

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Fragments from the 3′ end of a mouse histone H4 gene, when introduced into transcription units controlled by the SV40 early promoter, yield correctly processed RNA with histone-specific 3′ ends, both in monkey and mouse cell lines. The processed RNA is regulated in parallel with endogenous H4 mRNAs in 21-Tb cells, a temperature-sensitive mouse mastocytoma cell cycle mutant that is specifically blocked in G1 phase at the non-permissive temperature. Mutational analyses of the H4 gene fragment indicate that the minimal sequences for this regulation and for RNA 3′ processing are both contained within the same 80 bp. This fragment contains two histone-specific, highly conserved sequence elements that are located at the 3′ end of histone mRNA and in the adjacent spacer region, respectively. Our data suggest that the observed cell cycle regulation is achieved either at RNA 3′ processing or at some later step involving the conserved 3′-terminal sequence element of mature histone mRNA.

Key words: histone mRNA/3′ end generation/cell cycle/post-transcriptional regulation

Introduction

The histone genes of higher eukaryotes are a family of moderately repeated genes, most of which are coordinately regulated during the mitotic cell cycle (reviewed in Stein *et al.*, 1984; Schümperli, 1986). The steady-state levels of histone mRNAs are regulated by a combination of transcriptional and post-transcriptional mechanisms and closely parallel the DNA synthetic activity of the cell (DeLisle *et al.*, 1983; Heintz *et al.*, 1983; Plumb *et al.*, 1983; Sittman *et al.*, 1983).

In attempts to delimit the sequences responsible for the post-transcriptional control of histone mRNA metabolism, we have previously found that a fragment from the 3′ end of a mouse H4 gene, when fused to the early promoter of SV40, directed the synthesis of a fusion RNA with histone-specific 3′ ends. This RNA was regulated in parallel with endogenous H4 mRNAs in a mouse mastocytoma cell cycle mutant that is specifically blocked in G1 phase at the non-permissive temperature (Lüscher *et al.*, 1985). A distinctive structural feature of the 3′ ends of all replication-dependent histone mRNAs is the absence of a poly(A) tail and the presence of a palindromic sequence that is highly conserved throughout evolution (Busslinger *et al.*, 1979; Hentschel and Birnstiel, 1981). The mature histone mRNA is generated from longer precursor transcripts by a post-transcriptional processing reaction involving the 3′-terminal palindrome, some adjacent spacer sequences, and U7 snRNPs (Birchmeier *et al.*, 1984; reviewed in Birnstiel *et al.*, 1985). It seemed therefore possible that the regulation observed with the 3′-terminal fragment was due to this highly conserved processing signal and that the post-transcriptional regulation might be effected at the level of RNA processing.

To investigate this possibility, we constructed deletion mutants of the 3′-terminal H4 gene fragment and tested their competence for RNA 3′ processing and cell cycle regulation by transient expression assays in COS1 cells and by temperature-shift experiments with stably transformed cells of the 21-Tb mouse mastocytoma cell cycle mutant, respectively. Our results indicate that the minimal sequences required for regulated expression in the cell cycle mutant reside within the same small piece of DNA as the RNA 3′ processing signal and could be identical to it.

Results

Construction of plasmids and deletion mutants

We had previously introduced a 3′-terminal H4 gene fragment (fragment −230+232, Figure 1) into the 5′-untranslated region of the SV40/*galK* (*Escherichia coli* galactokinase) transcription unit of plasmid vector pEL1gpt (Figure 1; Lüscher *et al.*, 1985). We had analysed RNA from this transcription unit in stably transformed 21-Tb mouse cells isolated by selection for the bacterial xanthine:guanine phosphoribosyl transferase (gpt) gene present on pEL1gpt. However, further pEL1gpt-derived constructions often produced gpt-transformed cells that expressed very low or even non-detectable levels of the SV40/histone/*galK* fusion RNAs. It is possible that these plasmid sequences that had not been selected for were lost or destroyed during the selection procedure, possibly by recombination. To maximize the expression of the desired RNA in transformed cell lines, we have developed an alternative vector system, the plasmid pgptCX (Figure 1). Only the 5′ part of the bacterial DNA fragment present in gpt expression vectors (Mulligan and Berg, 1980) actually contains gpt coding sequences (Nüesch and Schümperli, 1984). Some 400 bp at the 3′ end are dispensible for gpt gene expression in mammalian cells. We engineered two synthetic oligonucleotide linkers into this region to create unique restriction sites for *Xba*I and *Cla*I, spaced 169 bp apart. Various derivatives of the H4 gene fragment were constructed by external deletion with nuclease *Bal*31 or by internal deletion with suitable restriction enzymes, and then inserted as *Taq*I fragments into the *Cla*I sites of pgptCX (Figure 1). However most fragments were also introduced into pEL1gpt (e.g. compare Figures 2A and B) and, where tested, essentially identical results were obtained wtih both vectors, with respect to both 3′ processing and cell cycle regulation.

Delimitation of the RNA 3′ processing signal by mutational analysis and transient expression in monkey COS1 cells

The competence of the various H4 gene fragments (Figure 1) for histone-specific RNA 3′ processing was tested by transient expression assays and S1 analysis in monkey COS1 cells. These cells reproducibly produced higher transient RNA levels than

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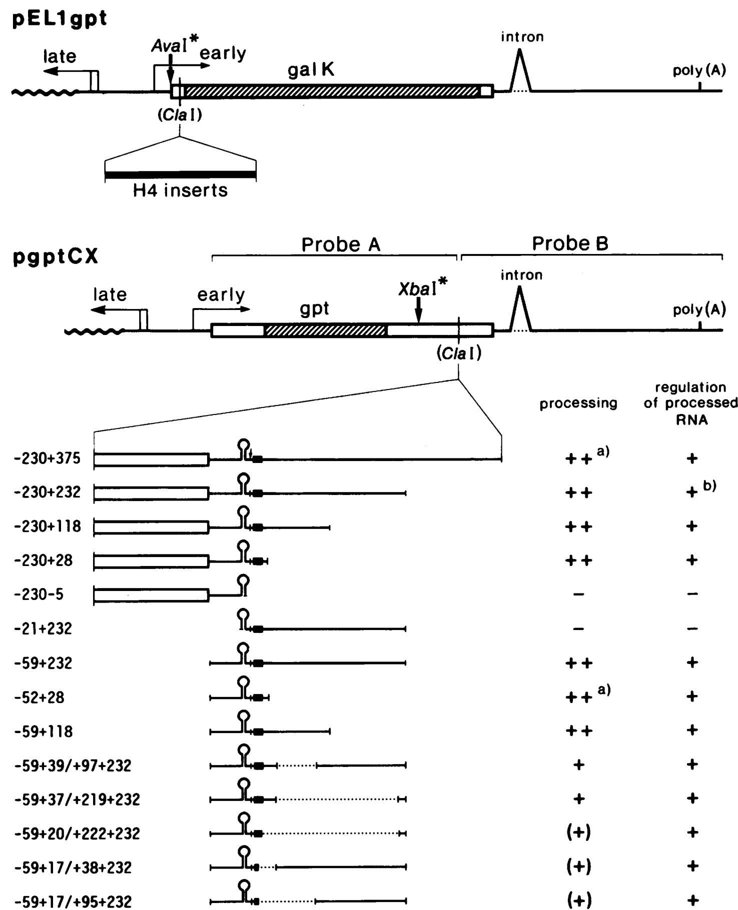

Fig. 1. Structure of recombinant plasmids and deletion mutants of the 3′-terminal mouse H4 gene fragment. Vectors: the structures of the relevant *galK* (galactokinase) and gpt (xanthine:guanine phosphoribosyl transferase) transcription units of plasmids pEL1gpt and pgptCX, respectively, are shown. pELgpt contains an additional gpt transcription unit. Thick bar: bacterial DNA. Hatched areas: *galK* and gpt protein coding sequences. Wavy lines: bacterial plasmid sequences. Straight lines: SV40 sequences. Horizontal arrows: SV40 late and early transcription start sites. Intron: intervening sequence of SV40 small t antigen transcripts. Poly(A): SV40 early polyadenylation site. Asterisk: position of *Ava*I and *Xba*I sites used for 3′ end-labeling of hybridization probes for S1 analysis. H4 gene fragments: open bar: H4 protein coding sequence. Straight line: 3′ trailer and spacer sequence. Stem–loop structure and black box: conserved sequence elements involved in RNA 3′ processing (see Figure 3). Fragment nomenclature: each fragment is designated by the numbers of its first and last nucleotide according to Figure 3. For fragments with internal deletions, the last nucleotide before and the first nucleotide after the deletion are separated by a slash. Processing (tested by transient expression in COS1 cells): ++: efficient processing. +: reduced efficiency of processing. (+): processed RNA bands barely detectable. −: no processing. Regulation (tested in stably transformed 21-Tb cells): +: levels of processed RNA significantly lower in G1-arrested versus exponentially dividing cells. −: no processed RNA detected. (a) Processing only tested in 21-Tb cells; (b) readthrough RNA reduced ~5-fold in G1-arrested cells, but processed RNA reduced even more (see text).

mouse cells, probably because of the high copy number amplification of any plasmids carrying an SV40 origin of replication (Gluzman, 1981). As will be seen, COS1 cells correctly interpreted the RNA 3′ processing signal from the mouse H4 gene and quantitative comparisons between various deletion mutants yielded similar if not identical results as in exponentially dividing, stably transformed 21-Tb cells.

The types of S1-protected bands produced by RNA from the hybrid transcription unit can be illustrated in the case of fragment −230+232 (Figure 2A, for numbering and nomenclature of fragments see legends to Figures 1 and 3). They are from top to bottom of the gel: (rt), a band corresponding to transcripts extending through the entire H4 insert; (spl), a band mapping to position +41 and presumably corresponding to RNA that is being spliced from this position to an undefined 3′ splice site further downstream [the sequence of positions +39 to +45 is CAGGTAA (Figure 3) and thus closely resembles the consensus sequence for 5′ splice sites (Breathnach and Chambon, 1981)]; and (proc), the band corresponding to histone-like processed RNA. Whenever the specifically processed RNA from several mutant fragments could be mapped by S1 analysis with the same probe, RNAs corresponding to bacterial plasmid sequences transcribed from the SV40 late promoter (Figure 1; bands labelled as 'ref' in Figure 2) were also quantitated on the same gel as an internal control to detect variations in transfection efficiencies.

We first defined the 3′ border of the histone-specific RNA processing signal by external deletions (Figure 2A). All fragments containing 28 or more nucleotides of uninterrupted H4 3′ spacer sequences produced high and similar amounts of 3′ processed RNA (fragments −230+232, −230+118 and −230+28). In contrast, no processed RNA was produced with fragment −230−5, i.e. when the last five nucleotides of mature histone mRNA had been deleted. To define the 3′ border more precisely, we tested a series of fragments with internal spacer deletions (Figure 2B). Again, efficient RNA 3′ processing was observed when 118 bp of uninterrupted H4 3′ spacer DNA were present (fragment −59+118). However, the fragments −59+37/+219+232 and −59+39/+97+232 had significantly lower efficiencies of RNA 3′ processing. For the fragments −59+17/

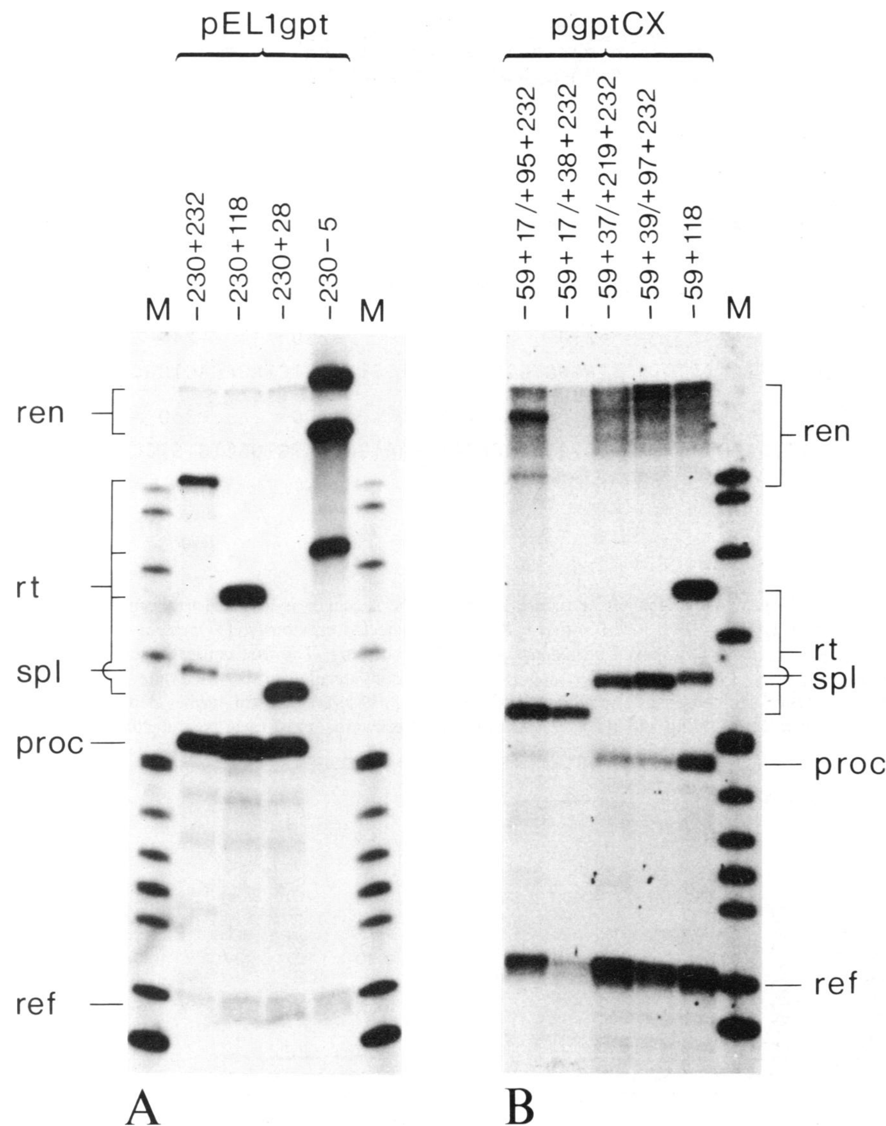

Fig. 2. Competence of mutant H4 gene fragments for RNA 3′ processing. Total RNA from COS1 cells transfected with the indicated plasmids was used for S1 mapping with a mixture of 5′- and 3′-end-labelled probes. **ref**: reference RNA initiated at SV40 late promoter mapped with a 5′-end-labelled probe from pBR322. All other bands were mapped with 3′-end-labelled probes that were either only partly homologous to the input DNA (pEL1gpt plasmids) or extended beyond the SV40 small t intron (pgptCX plasmids), so that readthrough RNAs could be accurately measured. **proc**: RNA with correctly processed H4-specific 3′ ends; **spl**: RNA spliced from a 5′ splice site at position +41 to an undefined 3′ splice site; **rt**: readthrough RNA extending to the end of the transcription unit (see Figure 1); **ren**: renatured hybridization probes; **M**: end-labelled DNA size markers (*Hpa*II digest of pBR322).

+95+232, −59+17/+38+232 (Figure 2B), or −59+20/+222+232 (not shown), the amounts of processed RNA were reduced even further to barely detectable levels (even after correction of the data in Figure 2B for the varying levels of internal reference RNA). Thus, RNA 3′ processing is virtually abolished in deletions that contain no adjacent spacer sequences downstream of the second histone-specific conserved sequence element ending at position +20 or in deletions that even removed the last nucleotides of this element (see sequence in Figure 3). Better, but variable, processing is observed with fragments containing spacer sequences up to positions +28, +37 or +39, respectively. This may indicate that the efficiency of the processing signal, which must lie upstream of +28, can be modulated by the sequences to which it has been fused (see Discussion).

The 5′ border of the processing signal was characterized by external deletions. In fragments −230+232 (Figure 2A) and −59+118 (Figure 2B), RNA 3′ processing was quite efficient. However fragment −21+232 (Figure 1, data not shown) had completely lost the ability to process RNA at the histone-specific position. The smallest fragment, −52+28, was not tested in COS1 cells, but it must be inferred from the experiments carried out with transformed 21-Tb cells, that RNA 3′ processing is still quite efficient in this mutant (Figure 4C). Therefore the 5′ border of the processing signal must lie between positions −52 and −21.

Delimitation of sequences conferring cell cycle regulation in stably transformed 21-Tb cells

We tested the competence of the various H4 gene fragments for correct regulation of the short, specifically processed SV40/H4 RNA in stably transformed 21-Tb cells. These cells can be specifically blocked in the G1 phase of the cell cycle by incubation at 39.5°C (Zimmermann *et al.*, 1983). Total cellular RNA was isolated from transformed cells that had been incubated either at 33°C (the permissive temperature) or for 48 h at 39.5°C

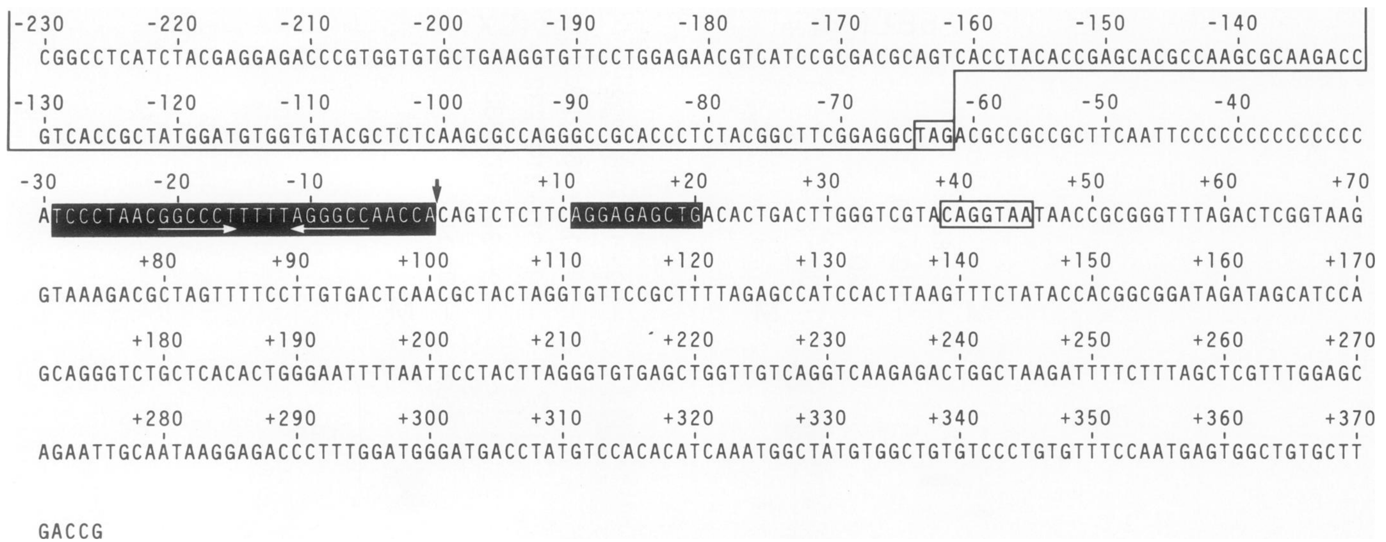

Fig. 3. Nucleotide sequence of relevant 3′-terminal region of the mouse H4 gene. The sequence is identical to the published one (Seiler-Tuyns and Birnstiel, 1981), except for a 34-bp *Hin*fI fragment (position +60 to +93) that had not been detected previously. H4 protein coding sequences are framed. Two black boxes represent highly conserved sequence elements involved in histone mRNA 3′ processing. The first conserved element extends further upstream than previously published (Hentschel and Birnstiel, 1981; Birchmeier *et al.*, 1983), for reasons detailed in the discussion. An open box (position +39 to +45) indicates a sequence similar to the 5′ splice site consensus (Breathnach and Chambon, 1981; see text for further details). Arrow: position of H4 mRNA 3′ ends (Gick *et al.*, 1986). Numbering of mRNA coding and spacer sequences starts backwards and forwards from this position, respectively.

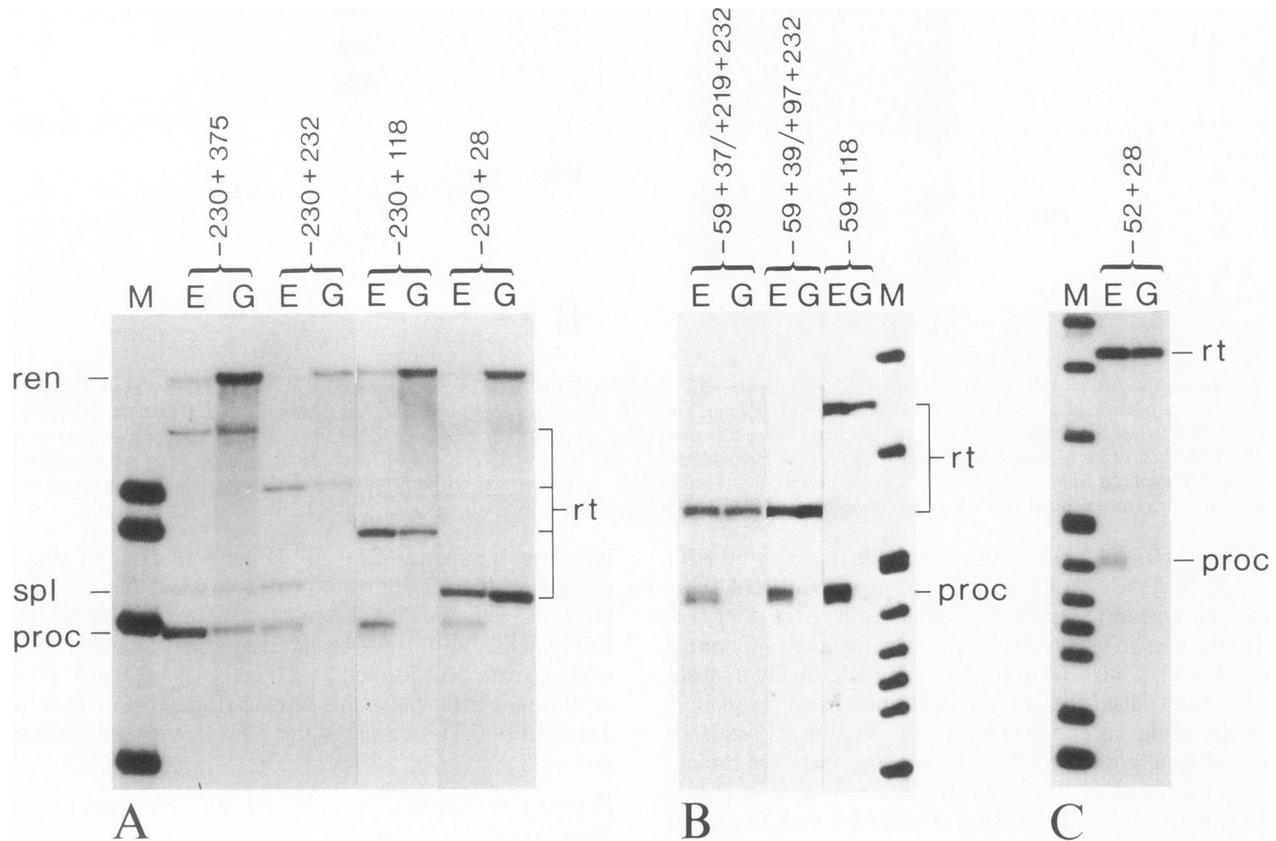

Fig. 4. Regulation of gpt/H4 fusion transcripts in 21-Tb cells. Total RNA from cells stably transformed with the indicated pgptCX recombinants was used for S1 mapping with probes that had been 3′-end-labelled at the *Xba*I site and extended beyond the SV40 small t intron and early polyadenylation sequences (Figure 1). **E**: RNA from exponentially dividing cells incubated at 33°C; **G**: RNA from cells arrested in G1 phase by incubation at 39.5°C for 48 h; **proc**: RNA with correctly processed H4-specific 3′ ends; **spl**: RNA spliced from a 5′ splice site at position +41 to an undefined 3′ splice site; **rt**: readthrough RNA extending to the end of the transcription unit (see Figure 1); **ren**: renatured hybridization probes; **M**: end-labelled DNA size markers (*Hpa*II digest of pBR322); RNA amounts used for S1 mapping were measured and adjusted according to OD at 260 nm and to visual examination of ribosomal RNA bands after agarose gel electrophoresis. Parts **A** and **B** are composite pictures taken from different gels.

(G1-arrested) and this RNA was analysed by S1 mapping.

In all of the transformants that produced some specifically processed RNA, the level of this RNA was drastically reduced in G1-arrested as compared to exponentially dividing cells (Figure 4A–C). Most significantly, even in the case of fragment −52+28, the short SV40/H4 fusion RNA was faithfully regulated (Figure 4C). Quite in contrast, most transformed cell lines contained similar amounts of the readthrough RNA, irrespective of whether the cells had been incubated at 33°C or at 39.5°C. This confirms our previous finding that regulation in 21-Tb cells is only observed for the short, processed RNA, although the readthrough RNA contains the same regulatory sequences (Lüscher *et al.*, 1985).

For the pgptCX recombinant containing fragment −230+232, the amount of readthrough RNA was reduced about 5-fold in G1-arrested cells. This phenomenon may be related to the findings of Imbert *et al.* (1984), who found that one group of SV40-transformed FR 3T3 rat cells accumulated T-antigen or SV40 early transcripts throughout the cell cycle, whereas in another group their levels were significantly lower in G1 phase. In other words, transcription from the SV40 early promoter may be cell cycle-regulated in some, but not all SV40-transformed rodent cell lines. Whatever may be the reason for this phenomenon, we have scored the short, processed RNA of −230+232 as being regulated in 21-Tb cells (Figure 1), because the difference in S1 signals was much more dramatic for 3′ processed than for readthrough RNA. Moreover, in our previous analysis of transformants obtained with a pEL1gpt recombinant of fragment −230+232, no difference in readthrough RNA levels had been observed, whereas the processed RNA had been strongly down-regulated in G1-arrested cells (Lüscher *et al.*, 1985).

In the case of pgptCX/−230+375, the amount of processed RNA was reduced in G1-arrested cells, albeit not as dramatically as for the other recombinants. However, in this case, the level of readthrough RNA was somewhat higher in G1-arrested than in exponentially dividing cells. It is therefore possible that the amounts of RNA used in the S1 mapping procedure or the amounts of material loaded onto the gel were not exactly the same for the two samples from this recombinant.

It can be concluded from this mutational analysis that the signal for histone-specific regulation of the short RNA must be identical with or entirely contained within the RNA 3′ processing signal as defined in the previous section.

Discussion

Minimal sequences required for 3′ processing of histone H4 mRNAs cause histone-specific regulation of a heterologous gene

We have previously demonstrated that sequences in the 3′-terminal part of a mouse H4 gene can faithfully regulate gene expression in the 21-Tb cell cycle mutant (Lüscher *et al.*, 1985). We have now dissected this region of the gene by deletion mutagenesis to determine if the two functions controlled by it, RNA 3′ processing and regulation in 21-Tb cells, can be attributed to separate regulatory sequences. The results presented above suggest the contrary: whenever a particular mutant produced some specifically processed RNA, this RNA was also properly regulated in the 21-Tb cell line.

The smallest fragment obtained that still controls both functions is only 80-bp long. It contains 52 nucleotides normally present in H4 mRNA plus 28 nucleotides of 3′ spacer sequences. Some of its salient features are (Figure 3) a stretch of 14 consecutive C residues contained in the 3′-untranslated region of H4 mRNA and two sequence elements (positions −29 to −1 and +11 to +20, respectively) which are highly conserved among histone genes (Busslinger *et al.*, 1979; Hentschel and Birnstiel, 1981; Turner and Woodland, 1982; Birnstiel *et al.*, 1985; see below). We do not know if the C-stretch is required for either RNA 3′ processing or cell cycle regulation. It might, in fact, be dispensible, because similar sequences are not commonly found in other histone genes and our deletion analysis only indicates that more than the last 21 nucleotides are required, whereas the last 52 nucleotides are sufficient for either function.

It is evident from our deletion analysis that the other two, conserved sequence elements are required, at least for RNA 3′ processing (the target for down-regulation in G1-arrested cells might be smaller and completely contained within the one for RNA 3′ processing). Similar mutational analyses have previously been carried out for the RNA processing signals of sea urchin H2A and H3 histone genes that were tested by injection into frog oocyte nuclei (Birchmeier *et al.*, 1982, 1983; Georgiev and Birnstiel, 1985). The results from these analyses are very similar to ours but not completely identical. For instance, a mutation in the H2A gene analogous to our completely deficient fragment −21+232 was still competent for correct 3′ end generation (Birchmeier *et al.*, 1983). It is possible that some important sequences have been functionally replaced by linker sequences in the case of the sea urchin but not the mouse gene. However, this result could also indicate slightly different sequence requirements for RNA 3′ processing between sea urchin and mouse histone genes at the first conserved sequence motif. In fact, a recent compilation of 29 published vertebrate histone gene sequences (Wells, 1986) yields the following distribution for the eight nucleotides immediately preceding the 3′-terminal palindrome:

A	18	16	6	5	5	22	28	23
C	6	9	20	18	20	6	0	6
G	1	0	1	1	1	0	1	0
T	4	4	2	5	3	1	0	0
Consensus:	A	A	C	C	C	A	A	A

A similar comparison of 17 published sea urchin histone gene sequences (Birchmeier *et al.*, 1983; Wells, 1986) yields the following nucleotide distribution for the same eight nucleotides:

A	8	3	9	7	10	17	15	1
C	3	7	5	5	6	0	1	16
G	2	2	1	0	0	0	0	0
T	4	5	2	5	1	0	1	0
Consensus:	N	N	A	non-G	A	A	A	C

Thus, the eight nucleotides immediately upstream of the 3′-terminal palindrome are conserved among vertebrate histone genes, whereas this is only true for the last four nucleotides in the case of the sea urchin histone genes (Hentschel and Birnstiel, 1981; Birchmeier *et al.*, 1983). Moreover, the consensus sequence for these last four nucleotides differs between the vertebrate and sea urchin genes.

The mouse H4 and sea urchin H2A and H3 genes yield virtually identical results as far as the requirements for spacer sequences for specific RNA processing are concerned. Deletions to +24, +23 and +28 still yield correctly processed RNA for the sea urchin H2A, H3 and mouse H4 genes, respectively. In the present analysis, some, albeit drastically reduced, amounts of correctly processed RNA were even obtained when the last three nucleotides of the conserved spacer motif had been deleted. The mutational analysis of the sea urchin H3 gene had demon-

strated the importance of the spacer motif for both the specificity and the efficiency of 3′ processing (Georgiev and Birnstiel, 1985). Our data suggest that not all nucleotides in the conserved motif may be absolutely required. A similar conclusion can also be drawn from a point mutation analysis of the spacer motif of the sea urchin H3 gene (Schaufele *et al.*, 1986). It is also interesting to notice that the efficiencies of 3′ end generation are highly dependent on what sequences are positioned downstream of the conserved spacer motif. For instance, there is an apparent discrepancy between our mutants containing 28 or 37 nucleotides of contiguous H4 spacer sequences, respectively: 3′ end generation for the former is as efficient as when 118 nucleotides are present (Figure 2A), whereas for the latter it is somewhat reduced (Figure 2B). Similarly, for the sea urchin H3 gene, the efficiency of 3′ end generation in a mutant containing 23 nucleotides of contiguous spacer sequences varied between 15 and 90% of the wild-type efficiency, depending on what sequences were fused to the truncated spacer (Georgiev and Birnstiel, 1985). This probably does not reflect a requirement for specific sequences in addition to the conserved spacer motif, but rather indicates that 3′ processing may be more sensitive to some sequences than to others, perhaps because the corresponding precursor transcripts assume different secondary structures.

An important question is whether the 3′-terminal regulatory mechanism we have characterized in 21-Tb cells is also operating in other systems used to study cell cycle-related phenomena. Preliminary data indicate that the short specifically processed RNA from pgptCX recombinants is also faithfully regulated in stably transformed C127 mouse fibroblasts, when such cells are arrested by serum starvation (C.S. and D.S., unpublished results).

The fact that small fragments from the 3′ end of the H4 gene may confer cell cycle regulation to an otherwise constitutively expressed gene, may also be of interest for the construction of specific expression systems. One could try to replace the polyadenylation signal from a gene of interest by the histone 3′-terminal regulatory region, to obtain S phase-specific expression of that gene on a non-polyadenylated mRNA.

Possible mechanisms for post-transcriptional regulation of histone mRNA levels in the cell cycle

Based on the results of our mutational analysis, it is tempting to speculate that histone RNA metabolism might be controlled in the cell cycle at the level of RNA 3′ processing. However, the conserved terminal palindrome might also be important for other functions, e.g. in mRNP formation, nucleocytoplasmic transport, or in controlling the stability of the mature histone mRNA. Cell cycle regulation might then operate at any one of these levels.

If post-transcriptional regulation were to occur at some step in the nucleus, one testable prediction would be that the differences in the rate of appearance of newly synthesized histone mRNA in the cytoplasm should be larger than the reported 2- to 5-fold difference in histone gene transcription. This has indeed been observed. In mouse erythroleukemia cells, the incorporation of [^{3}H]uridine into cytoplasmic H3 mRNA in 15 or 60 min pulses was found to rise sharply during the transition from G1 to S and then to fall as cells approached G2 (Alterman *et al.*, 1984). Further predictions based on the assumption that the post-transcriptional regulation of histone gene expression operates specifically at the RNA 3′ processing level can also be tested. For instance, the availability of an *in vitro* system to study RNA 3′ processing (Gick *et al.*, 1986) should be of great help to investigate this possibility.

Whatever the actual mechanism may be, making use of the conserved 3′-terminal sequence motifs is a very economical solution that allows the cell to regulate the levels of all the replication-dependent histone mRNAs by a single mechanism. No other genes have so far been found to contain the same sequence elements or to be processed by the same mechanism. Interestingly, those histone genes that are not subject to cell cycle regulation, e.g. those for the avian erythroid cell-specific histone H5 (Krieg *et al.*, 1982) or for the replacement variant histone H3.3 (Brush *et al.*, 1985; Wells and Kedes, 1985), also do not contain the conserved 3′-terminal sequence motifs, and their mRNAs are polyadenylated.

Materials and methods

Plasmid constructions

Nuclease *Bal*31 and the synthetic linkers *Cla*I [d(CATCGATG)] and *Xba*I [d(CTCTAGAG)] were purchased from Boehringer Mannheim.

The 3′-terminal *Hpa*II-fragment (−230/375) of the cloned mouse H4 gene (Seiler-Tuyns and Birnstiel, 1981) was resected either from the 5′ or the 3′ end by nuclease *Bal*31 digestion and the exact endpoints of individual deletion mutants were determined by DNA sequencing according to Maxam and Gilbert (1981).

A fragment lacking all H4 protein coding sequences (−59/+232) was used to construct further mutants containing internal spacer deletions. It was digested with several restriction enzymes and appropriate fragments were isolated and religated. All mutant H4 gene fragments were inserted into vector plasmids pEL1gpt and/or pgptCX (see Figure 1). In the pEL1gpt vector, a 'filler' fragment had to be inserted in front of those H4 gene fragments lacking H4 protein coding sequences, in order to obtain long enough hybridization probes in S1 mapping experiments. The 309-bp *Hpa*II fragment from pBR322 was used for this purpose.

Cell culture

The heat-sensitive cell-cycle variant 21-Tb of the P815-X2 mouse mastocytoma cell line (Zimmermann *et al.*, 1981, 1983) was cultured as described previously (Schaer and Schindler, 1967; Lüscher *et al.*, 1985). Transfections were performed by protoplast fusions (Schaffner, 1980; Rassoulzadegan *et al.*, 1982) and stable transformants were selected for the expression of the *E. coli* xanthine:guanine phosphoribosyl transferase (gpt) gene essentially as described (Mulligan and Berg, 1981), using a selection medium with 10% dialysed horse serum, 1 μg/ml mycophenolic acid, 0.25 μg/ml xanthine, 0.45 μg/ml aminopterin, 80 μg/ml inosine, 7.5 μg/ml glycine and 24 μg/ml thymidine.

Monkey COS1 cells (Gluzman, 1981) were cultured in Dulbecco's modified minimal essential medium supplemented with 5% fetal calf serum. Transfections were carried out by a modified DNA/calcium phosphate co-precipitation method (Weber *et al.*, 1984) and two days later transfected cells were harvested to isolate RNA.

RNA-purification and S1 nuclease mapping

Total cellular RNA was isolated by hot acid phenol extraction (Scherrer, 1969). Hybridization was carried out overnight at 50°C with 40 μg RNA and 0.03 pmol end-labeled DNA probe. S1 nuclease analysis was performed as described (Berk and Sharp, 1977; Weaver and Weissmann, 1979).

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