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Cell Cycle Regulation of a Mouse Histone H4 Gene Requires the H4 Promoter

ANNE SEILER-TUYNS† AND BRUCE M. PATERSON*

Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892

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The mouse histone H4 gene, when stably transformed into L cells on the PSV2gpt shuttle vector, is cell cycle regulated in parallel with the endogenous H4 genes. This was determined in exponentially growing pools of transformants fractionated into cell cycle-specific stages by centrifugal elutriation, a method for purifying cells at each stage of the cell cycle without the use of treatments that arrest growth. Linker additions in the 5′ noncoding region of the H4 RNA or in the coding region of the gene did not affect the cell cycle-regulated expression of the modified H4 gene even though the overall level of expression was altered. However, replacing the H4 promoter with the human α-2 globin promoter, so that the histone transcript produced by the chimeric gene remains essentially unchanged, resulted in the constitutive expression of H4 mRNA during all phases of the cell cycle with no net increase in H4 mRNA levels during the G1-to-S transition. From these results we conclude that (i) all the information necessary for the cell cycle-regulated expression of the H4 gene is contained in the 5.2-kilobase subclone used in these studies with 228 nucleotides of 5′-flanking DNA and that (ii) the increase in H4 mRNA during the G1-to-S transition in the cell cycle is mediated by the H4 promoter and not by the increased stability of the H4 RNA.

Histone expression involves de novo synthesis of histone mRNA at the onset of DNA replication (1, 8, 11, 13, 23; unpublished data). Various levels of control are possible and may involve changes in the half-life of histone mRNA during the cell cycle, processing and transport of the newly synthesized histone RNA into the cytoplasm, or variations in the rate of transcription. Both mRNA half-life and the rate of transcription have recently been implicated in histone gene regulation in yeasts (12, 13, 18) and in mammalian cells (1, 11, 21, 28).

In a preliminary study, using DNA-mediated gene transfer to study the expression of a cloned mouse histone H4 gene (25) reintroduced into CV1 cells, we have shown that a restriction fragment carrying the histone H4 gene contains all the necessary information to generate a correct mouse histone H4 mRNA (27). Furthermore, the 3′ regulatory signal (4–6) is also functional in this H4 isolate. Here we investigate the role of transcription on the cell cycle expression and regulation of H4 mRNA with linker-modified or chimeric H4 genes transcribed under the control of the human α-2 globin promoter. These gene constructs, carried by the eucaryotic vector PSV2gpt (17), were reintroduced into mouse L cells by the calcium phosphate transformation procedure (17). Pools of cells corresponding to approximately 100 to 500 independent integration events were analyzed by primer extension to measure the amounts of the specific H4 RNA present in exponentially growing cells fractionated into cell cycle-specific stages by centrifugal elutriation. This procedure obviates those problems associated with treatments that arrest cell growth to study cell cycle regulation of histone mRNA metabolism. Variations in the expression of the introduced H4 gene constructs were compared with the endogenous H4, *gpt*, and β-actin transcripts to control for cell cycle-regulated expression and provide markers for cell cycle-independent gene expression. Neither the expression of β-actin nor that of *gpt* is cell cycle regulated.

MATERIALS AND METHODS

Cell culture, DNA transformation, and selection of transformed cells. L cells (kindly provided by R. Greenberg) were grown in Dulbecco modified Eagle medium plus 10% fetal calf serum. Cells were transformed and selected by the

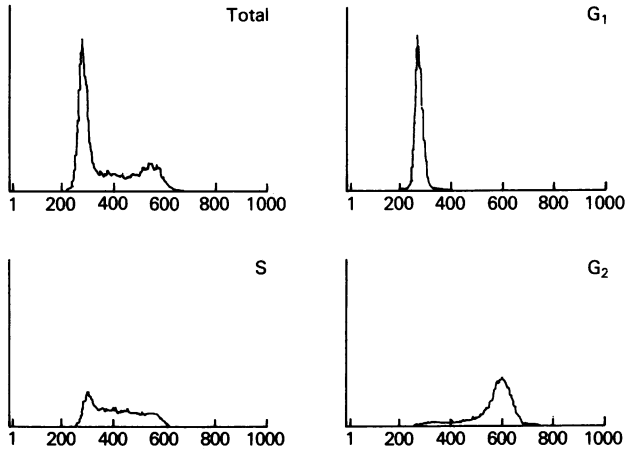

FIG. 1. DNA content of the cells fractionated by centrifugal elutriation into the various cell cycle stages. Approximately 10^6 cells were analyzed from each fraction and represent total cells, cells in G1, cells in S, and cells in G2 plus M. The profiles shown represent transformants with construct 1 (see Fig. 2). The numbers on the *x* axis are units of DNA fluorescence, and the *y* axis shows units of cell number.

* Corresponding author.

† Present address: Institut de Biologie Animale, Batiment de Biologie, Universitae de Lausanne, CH-1015 Lausanne, Switzerland.

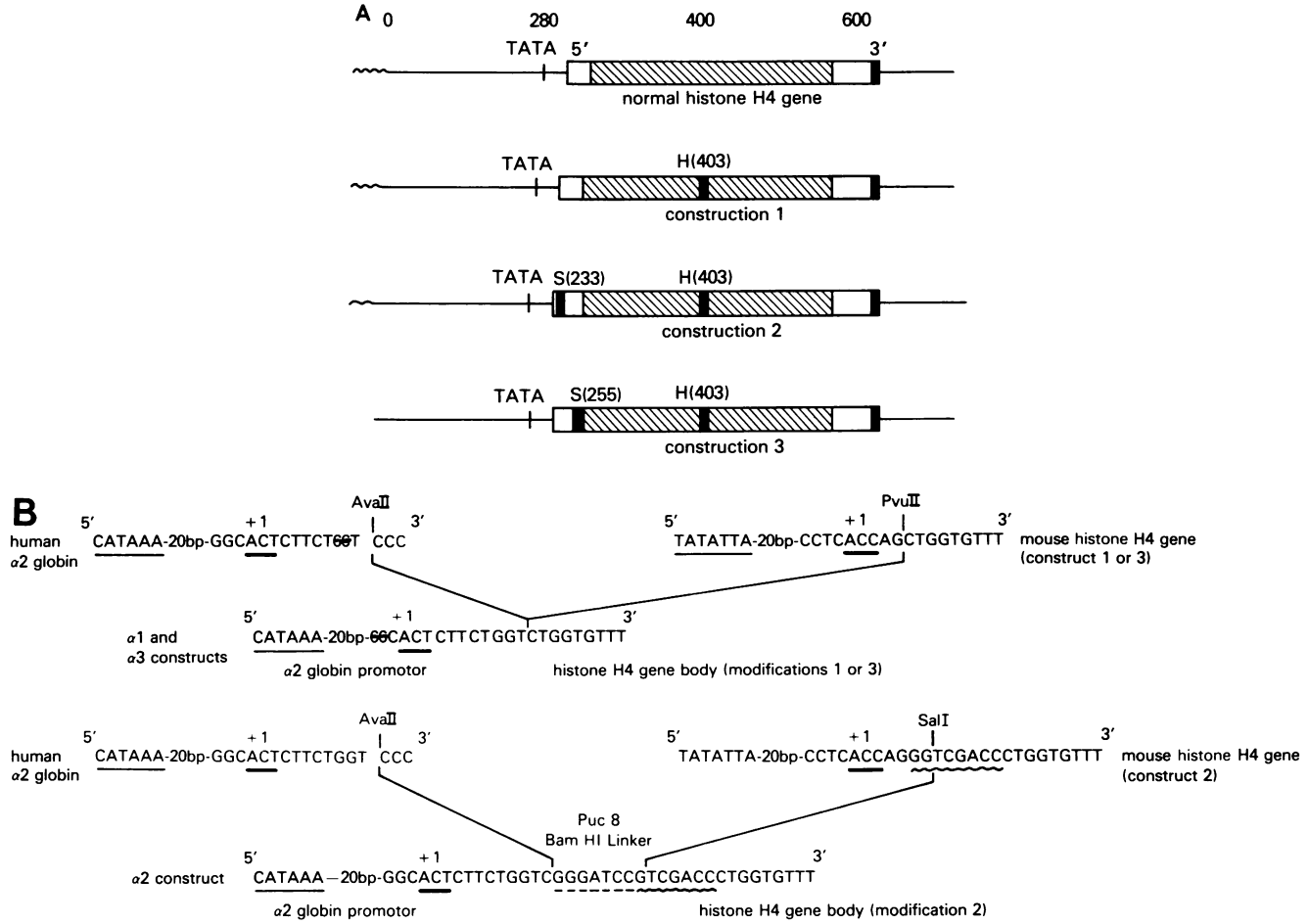

FIG. 2. Description of the different H4 gene constructs with either linker or the human α-2 globin promotor. (A) Linker additions. All the constructs contain a 12-base-pair *Hin*dIII linker at position 403 (25) indicated with the letter H; constructs 2 and 3 have an 8-base-pair *Sal*I linker at position 233 (+5) and at position 255 (+27), respectively, marked with the letter S. The numbers at the top of the figure indicate base pairs from the 5' *Eco*RI site of the subclone (25). The boxed area defines the mRNA transcript, and the shaded portion marks the coding region. (B) Promoter exchange constructs with the human α-2 globin gene. A 582-base-pair (bp) *Pst*I-*Ava*II fragment containing the globin promoter was converted to an *Eco*RI-*Ava*II fragment in pUC8. The *Ava*II end was filled with Klenow polymerase and flush-end ligated to the *Pvu*II site at position 233 (+5) in the H4 gene for constructs α1 and α3. This extends the H4 RNA transcript an additional six nucleotides on the 5' end. For the α2 construct, the flushed *Ava*II end of the globin promotor was modified with *Bam*HI linkers (octamer), reclaimed from pUC8 as an *Eco*RI-*Sal*I fragment, and joined to the *Sal*I linker at position 233 (+5) in construct 2. This extends the H4RNA transcript an additional 14 nucleotides on the 5' end.

method of Mulligan and Berg (17). Briefly, 36 to 48 h after transformation by the standard calcium phosphate procedure, cells were transferred into *gpt* selection medium and subcultured in that medium for 3 weeks. The cells were then grown for two passages in Dulbecco modified Eagle medium containing hypoxanthine (20 μM) and thymidine (10 μg/ml). Finally, cells were transferred back into regular medium. Spinner cultures were grown in 1-liter bottles in modified Eagle medium plus 10% fetal calf serum and harvested at a density of 4×10^5 cells per ml for centrifugal elutriation.

Centrifugal elutriation of L cells. Approximately 60×10^6 to 80×10^6 cells were loaded onto a Beckman elutriator rotor (model JE 6B) at 1,760 rpm with a pump rate of about 10 ml/min at 20°C. Cells were washed in the rotor with 300 ml of phosphate-buffered saline, and 12 fractions of 100 ml were eluted by increasing the pump rate from 14 to 30 ml/min. G1 cells were in fractions 4 to 6, S cells were in fractions 7 to 10, and G2 plus M cells were in fractions 11 to 12. Cells were pelleted and resuspended in a small volume for Coulter counting, and 10^6 cells were fixed for FACS analysis. The remaining cells were extracted for RNA as described below. Approximately 50% of the input cells were recovered for RNA extraction and analysis.

FACS analysis of elutriated cell fractions. Cells were fixed with ethanol immediately after the phosphate-buffered saline was decanted from the final wash. Saline G (0.3 ml) (phosphate-buffered saline plus 1 g of glucose per liter) was added to the cell pellet followed by 0.9 ml of cold 100% ethanol. The ethanol was added dropwise while the cell suspension was being vortexed. After the ethanol was decanted, a solution containing 15 μg of propidium iodide per ml and 1,000 U of RNase A per ml in 1 ml of saline G was added to each cell pellet. After a 20-min incubation at room temperature, the cells were analyzed. Approximately 15,000 cells were counted for each distribution. Fluorescence of PI-stained cells was monitored on the Cytofluorograf System 60H interfaced to a 2150 computer system. The data were expressed as a plot of fluorescence intensity (represented by

channel number) versus the number of cells. The percentage of cells containing G1, S, or G2 plus M DNA content was estimated by a program developed for the 2150 computer by Dean and Jett (personal communication).

RNA extraction. RNA was prepared as previously described (25). Total RNA was prepared by lysing L cells in isotonic buffer (150 mM NaCl, 10 mM Tris hydrochloride [pH 7.6], 2 mM $MgCl_2$ containing 1 mg of proteinase K per ml and 1% sodium dodecyl sulfate). After two phenol extractions and one chloroform extraction, the RNA was pelleted through a CsCl step gradient as follows. After the addition of sarcosyl (0.4%) to the extracted RNA solution, 1 g of solid CsCl was added per ml of extract. This solution was then layered on a 1.2-ml CsCl cushion (1 g of solid CsCl per final ml in 0.1 M EDTA, pH 7.5) and centrifuged in an SW50. 1 rotor at 35 krpm for 12 h at 20°C. The resulting RNA pellet was dissolved in 0.2% sodium dodecyl sulfate, precipitated with ethanol, redissolved in water, and used for primer extension.

Construction of linker-modified and chimeric histone genes. Linker additions were as described by Maniatis et al. (15). The human α-2 globin gene as a 1.5-kilobase *Pst*I fragment was kindly provided by Barbara Felber and Dean Hamer (9). The constructions are described in detail in Fig. 2. All H4 gene constructs were cloned into the *Eco*RI site of the PSV2gpt vector in the same orientation as the *gpt* gene.

Preparation of end-labeled primers. Restriction enzymes and T4 DNA ligase were purchased from New England BioLabs, Inc. (Beverly, Mass.), T4 polynucleotide kinase was from P-L Biochemicals, Inc. (Milwaukee, Wis.), reverse transcriptase was from life Sciences, Inc. (St. Petersburg, Fla.), and linkers were from Collaborative Research, Inc. (Waltham, Mass.). Standard procedures were as described by Maniatis et al. (15).

Primer extension reaction. Total RNA (10 μg) was mixed with 5,000 Cerenkov counts of each of the DNA primers and hybridized at the appropriate temperature (45 to 50°C overnight) in 10 μl of 80% formamide–0.4 M NaCl–40 mM PIPES [piperzine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.5)–4 mM EDTA (3). After hybridization, 100 μl of 0.3 M NaCl–10 mM $MgCl_2$ was added, and the hybrids were precipitated with 2 volumes of ethanol. The pellet was suspended in reverse transcriptase buffer, and extension was performed at 41°C with all four deoxynucleotides (19, 26). The reaction was stopped with 25 mM EDTA and 0.3 M NaOH. The RNA was hydrolyzed at 41°C for 30 min, neutralized with the appropriate amount of 1 M Tris (pH 7.5), and precipitated. The pellet was washed, dried, suspended in sample buffer, and loaded onto a DNA-sequencing gel.

RESULTS

Cell cycle analysis by centrifugal elutriation. Centrifugal elutriation provides the optimal physiological conditions for the preparative fractionation of L cells into the various phases of the cell cycle and allows one to analyze the regulation of histone gene expression in an exponentially growing cell population. This procedure avoids any potential problems that may arise with the various synchronization methods that involve growth arrest with drugs or other chemical treatments (22). The DNA content of L cells in different stages of the cell cycle fractionated by centifugal elutriation is shown in Fig. 1. Virtually all the cells in the G1 phase of the cell cycle contain the 2C amount of DNA (Fig. 1, G1) The DNA content of the various fractions increases as larger cells are elutriated and the degree of cross contamination of the various phases of the cell cycle becomes evident. Since we are looking at events during the G1-to-S transition the purity of the G1 fraction is most important to our results. This point will be emphasized throughout the text. It should be noted that even under the best conditions G1 cells prepared by centrifugal elutriation contain 10 to 20% early-S-phase cells (1), as determined by thymidine pulse-labeling. Similarly, the latest G2 cell fractions contain 20 to 30% of cells synthesizing DNA. The levels of H4 expression we report should be considered with these observations in mind.

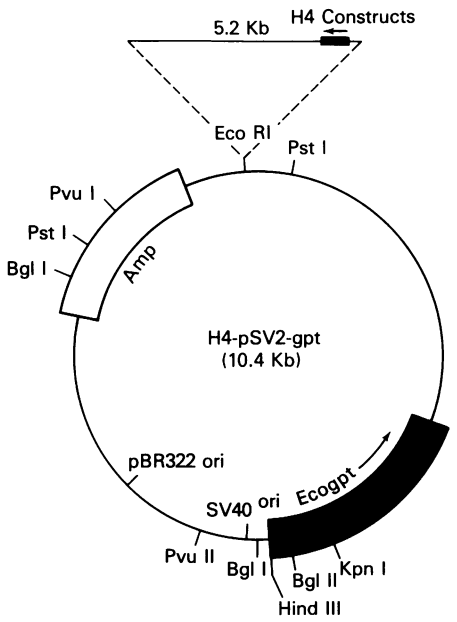

FIG. 3. Schematic representation of the H4-PSV2gpt constructs. Both *gpt* and the various H4 inserts shown in Fig. 2 are on the same coding strand. The direction of transcription is indicated by the arrows. The 1.5-kilobase (kb) *Pst*I fragment containing the human α-2 globin gene was modified with *Eco*RI linkers (octamer) and also cloned in the same orientation as the *gpt* gene. ori, Origin.

In pools of H4-PSV2gpt transformants the introduced H4 gene is cell cycle regulated. To distinguish H4-PSV2gpt transcripts in the mouse L-cell background, we inserted a *Hin*dIII linker, a 12-mer, at position 403 in the middle of the H4 coding region (construct 1, Fig. 2A) to provide a marker for primer extension analysis without disrupting the reading frame. It should be noted that a small percentage of the endogenous H4 transcripts hybridize with the primer and provide a convenient marker of endogenous H4 RNA. These likely represent variant H4 genes somewhat more homologous to the primer, although this has not been rigorously established. The pattern is highly reproducible (see Fig. 4) and was therefore used to monitor endogenous H4 regulation. This is not taken as a quantitative measure of total endogenous H4 RNA. A 5.2-kilobase *Eco*RI fragment containing the linker-modified gene was used to make the H4-PSV2gpt vector (Fig. 3). The recombinant vector was transfected into mouse L cells, stable transformants were selected for *gpt* expression, and pools of 100 to 500 independent transformants were expanded and grown in suspension. After centrifugal elutriation, RNA was extracted from the appropriate cell cycle fractions and analyzed by primer extension. There was significant expression of the intro-

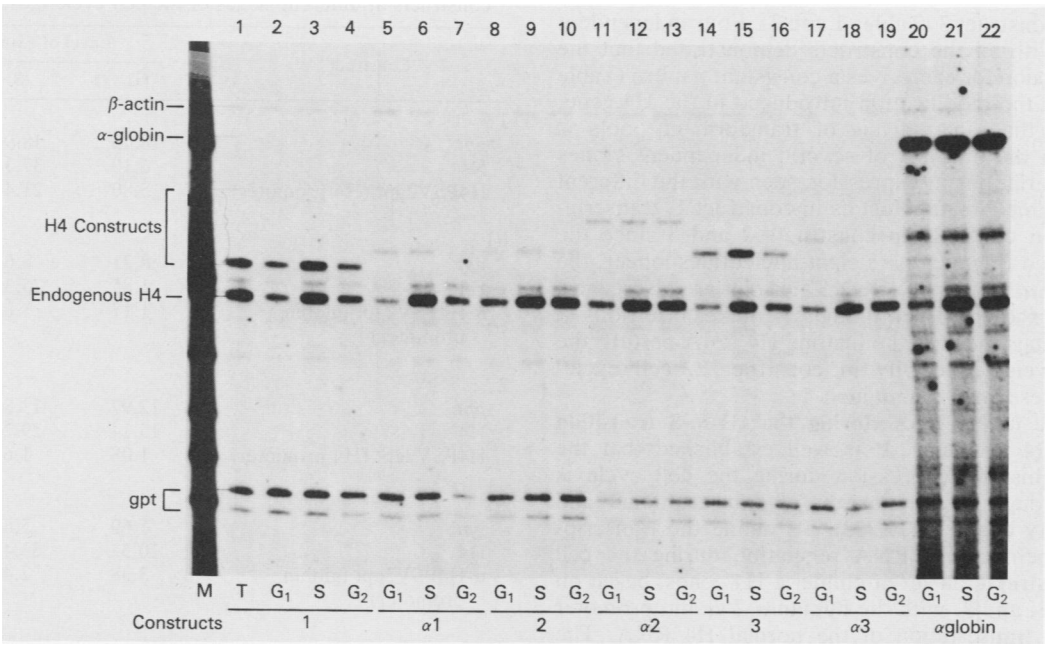

FIG. 4. Typical primer extension analysis of the various stable H4 transformants described in Tables 1 and 2. The following primers and their extended lengths and labeled ends (*) are given as follows. The *gpt* primer, from PSV2gpt, is the 120-base-pair **Bgl*II-*Hin*dIII fragment which extends to a length of 170 nucleotides; the histone H4 primer, from construct 1, is the 70-base-pair **Tth*I-*Hin*dIII (linker) fragment which extends to a length of 240 nucleotides; the β-actin primer, from the chicken β-actin gene, is the 109-base-pair **Bgl*II-*Hae*III fragment which extends to a length of 350 nucleotides; the human α-2 globin primer, from the α-2 globin gene, is the 131-base-pair **Hin*dIII -*Taq*I fragment which extends to a length of 308 nucleotides. Although endogenous histone RNA should not have led to an extension of the histone primer because nucleotides in the *Hin*dIII linker region are not homologous to the RNA at the extended end, there is a useful background which represents a small percentage (1 to 3%) of expression from the endogenous genes. Two or three H4 RNA variant species seem to hybridize to the primer and give extension bands around the expected size of 240 nucleotides. The H4 RNA from the introduced gene in a given construct is indicated by the construct number and the band within the bracket. For construct 1, the extension will be 6 nucleotides longer (half the *Hin*dIII linker) than endogenous H4 RNA, and it will be 14 nucleotides longer (half the *Hin*dIII linker plus the *Sal*I linker) for constructs 2 and 3. For the promoter exchange constructs, α1 and α3, the RNA will be 6 nucleotides longer than the corresponding modified H4 RNA (Fig. 2) and 14 nucleotides longer for construct α2 (Fig. 2). Cell cycle analysis of the cell fractions used for the RNA preparations analyzed in construct 1 is shown in Fig. 1. Numbers over the lanes are for clarity. M, PBR *Hpa*II markers; T, total RNA; G1, S, G2, RNA from the corresponding cell cycle fractions. The signal corresponding to the β-actin RNA is weak since the primer used is from another species (chicken versus mouse).

duced gene from the correct transcription start point (27) (Fig. 4, lanes 1 to 4). Furthermore, the level of expression of the introduced gene was consistently lower in G1 cells compared with S cells when normalized to either *gpt* or β-actin expression (Table 1, construct 1; Fig. 4). Thus, the level of expression of the introduced H4 gene when analyzed in pools of transformants reflects the particular phase of the cell cycle regulates in parallel to the endogenous gene: during the G1-to-S transition there is a sixfold increase in the endogenous H4 expression compared with a three- to four-fold increase in H4-PSV2gpt transcripts (Table 1, construct 1). This moderate increase in H4 transcription likely reflects the fact that histone synthesis begins very early in the S phase, and the G1 cells are contaminated with early S cells. However, our values are in good agreement with those of Alterman et al. (1) for elutriated cells. The level of regulated expression from H4-PSV2gpt is probably further reduced since we are analyzing pools of more than 100 transformants, some of which may not regulate appropriately.

The histone H4 RNA synthesized from construction 1 represents a few percentages (about 3%) of the total H4 RNA (determined by primer extension with an *Xmn*I-*Sau* 96 universal primer from nucleotides 434 to 539; data not shown), yet the transcripts are correctly initiated (see Fig. 4 for details) and processed (27) at the histone-specific element of dyad symmetry at the 3′ end of the gene (S1 data not shown).

We conclude that (i) the level of histone RNA from H4-PVS2gpt is cell cycle regulated for the average gene in the pool of transformants, reflecting a pattern of regulation similar to the endogenous gene; and that (ii) the correct histone transcript is produced from the introduced gene.

Linker modification of 5′ noncoding region of H4 RNA reduces the level of expression. In addition to the *Hin*dIII linker already present in the coding region, *Sal*I linkers were introduced at two different positions in the 5′ noncoding region of the H4 RNA. One linker was inserted (Fig. 2A, construct 2) five nucleotides downstream from the transcriptional start site (+) at position 233 (9) and the second was inserted (Fig. 2B, construct 3) two nucleotides to the 5′ side of the translational start at position 255. An *Eco*RI fragment containing either construct was inserted in the PSV2gpt vector as before. Following the procedures described for construct 1, expression and regulation were assayed by primer extension. Both constructs were cell cycle regulated (Table 1; Fig. 4, lanes 8 to 10 and 14 to 16); however, the levels of expression were distinctly different: both constructs had low levels compared with those of construct 1, and the amount of RNA produced from construct 2 was consistently three to five times less then the levels tran-

scribed from construct 3 (Tables 1 and 2). Four independent experiments with all the constructs demonstrated that the level of expression for each gives a consistent pattern (Table 2) that reflects the modification introduced in the H4 gene. We conclude that an analysis of transformant pools is comparable to the analysis of several independent clones and that the variations in expression seen with the different constructs are meaningful. Just as in construct 1, transcription is initiated correctly in constructs 2 and 3 since the primer-extended fragments are eight nucleotides longer, the length of the *SalI* linker. Correct 3′ termini are also generated (data not shown). Even though the linker insertions in the 5′ noncoding leader of the histone H4 RNA perturb the expression levels, especially in construct 2, cell cycle-regulated expression is maintained.

The increase in H4 RNA during the G1-to-S transition requires the H4 promoter. It is well established that the regulation of histone expression during the cell cycle is controlled through a combination of transcriptional and mRNA stability changes (11, 28). To define the transcriptional component of H4 RNA regulation during the cell cycle, we constructed a set of chimeric H4 genes, based on constructs 1, 2, and 3, with the human α-2 globin promoter regulating the transcription of the normal H4 RNA. The constructs and nucleotide changes are presented in Fig. 2B. Utilizing these different promoter constructs, one can delineate transcriptional differences from changes in H4 RNA stability since the basic H4 transcript is similar in all cases and only the promotors have been exchanged. The human α-2 globin promoter did not cause the increased expression of the chimeric H4 RNA during the G1-to-S transition for any of the α-constructs (Fig. 4; Table 1); however, all the constructs with the H4 promoter showed increased expression of H4 RNA with the G1-to-S transition in parallel with the endogenous H4 genes. We interpret this to mean that the normal increase in H4 RNA as the cells go from G1 to S is dependent on an increased rate of transcription from the H4 promoter. By comparison, L cells transformed with the α-globin PSV2gpt construct showed a progressive increase in expression of globin RNA during the G1, S, and G2 phases of the cell cycle (Fig. 4, lanes 20 to 22; Table 1). This implies that the decrease in chimeric H4 RNA produced under the control of the globin promoter reflects posttranscriptional changes in the stability of the globin-H4 RNA during the cell cycle.

Taking these two observations together, we conclude that the increase in H4 RNA levels during the G1-to-S transition reflects increased transcription from the H4 promoter, not an increase in the stability of the H4 mRNA.

TABLE 1. Levels of expression for the various histone H4 gene constructs at different stages of the cell cycle (absolute values)

Construct	Level of expression at:		
	G1	S	G2
1			
gpt	18.43	18.96	18.25
H4	6.10	37.38	15.04
H4PSV2gpt (H4 promoter)	5.80	21.44	8.88
α1			
gpt	8.21	8.64	8.65
H4	1.85	25.35	17.38
α-H4PSV2gpt (globin promoter)	1.17	1.09	0.74
2			
gpt	12.97	13.86	12.61
H4	15.14	29.24	17.42
H4PSV2gpt (H4 promoter)	1.08	1.62	1.01
α2			
gpt	5.60	5.42	5.47
H4	10.32	33.45	14.89
α-H4PSV2gpt (globin promoter)	3.38	2.42	1.70
3			
gpt	11.84	11.10	10.76
H4	5.64	33.52	11.80
H4PSV2gpt (H4 promoter)	6.20	17.58	4.72
α3			
gpt	10.22	10.74	13.00
H4	4.50	37.82	17.97
α-H4PSV2gpt (globin promoter)	1.65	1.19	0.97
α-globin			
gpt	5.87	5.27	6.18
H4	3.29	28.51	17.46
α-PSV2gpt	39.28	80.58	98.14

DISCUSSION

We clearly demonstrated that the 5.2-kilobase histone H4 subclone (25) introduced into L cells on the PSV2gpt vector is correctly expressed and regulated in parallel with the endogenous gene throughout the cell cycle. The H4 gene isolate used in our studies contains just 228 base pairs to the 5′ side of the transcription start point, yet H4 RNA is transcribed and processed correctly and is expressed in parallel to the endogenous H4 genes; the level of endogenous H4 RNA increased sixfold on average compared with a consistent three- to fourfold increase for the introduced gene (construct 1) during the G1-to-S transition (Table 1). We performed experiments with elutriated pools of transformants to minimize variations owing to gene copy number per cell, random integration of the H4 gene within the genome, and changes in regulated expression introduced by mutation during the transformation procedure (7). It may well be that a fraction of the transformants obtained were not regulated properly owing to these factors, introducing a slight bias to the results reported here. Support for this interpretation is based on several reports in which single transformant clones were analyzed and only a subset of the various clones was found to be properly regulated (16, 24). Even with this qualification, we feel that the analysis of transformant pools is equivalent to the analysis of several clonal isolates since the introduced H4 gene is regulated in parallel to the endogenous gene. Furthermore, the reproducible nature in the expression of the different constructs supports this interpretation (Table 2).

Luscher et al. (14) have also recently reported the regulated expression of the same H4 gene in stable transformants of 21-Tb cells, a line temperature sensitive for the cell cycle. However, the 980-fold-increased level of H4 RNA expression reported by these authors during the Go-to-S transition is extremely high compared with our results and those of others in the 10- to 50-fold range. Our values are in good agreement with those of Heintz et al. (11) and Sittman et al. (28) for core histones.

The results obtained with construct 2 (Fig. 1), which inserts a *SalI* linker five nucleotides downstream from the +1 position, suggest that the DNA sequences very near the

TABLE 2. Levels of expression for histone H4 gene modified in the 5' noncoding end of the mRNA in exponentially growing cells

Construction	Expt 1		Expt 2		Expt 3		Expt 4		Avg ratio
	Scan	Ratio	Scan	Ratio	Scan	Ratio	Scan	Ratio	
1									
gpt	37.9		60.1		50.1		30.0		
H4	9.1	0.89	11.1	2.05	10.4	0.99	7.2	1.64	1.39
H4PSV2gpt	10.2		5.4		10.5		4.4		
2									
gpt	33.7		56		36.9				
H4	10.3	10.3	5.5	13.75	8.5	12.14			12.06
H4PSV2gpt	1.0		0.4		0.7				
3									
gpt	27.4		56.4		38.1		26.3		
H4	8.8	1.27	3.6	1.71	7.2	3.79	7.1	2.84	2.40
H4PSV2gpt	6.9		2.1		1.9		2.5		

transcription initiation site play a role in facilitating higher levels of H4 RNA expression but do not alter the pattern of regulation (Tables 1 and 2). By comparison, a *Sal*I linker inserted two nucleotides to the 5' side of the initiator ATG (construct 3) produced only slight changes in the level of expression (Tables 1 and 2), again suggesting that the linker in construct 2 is in a critical region. Similarly, the low level of expression of H4 RNA in the promoter exchange constructs likely reflects the disruption of the critical region delineated by the linker in construct 2.

To distinguish the relative importance of transcriptional and mRNA stability changes in the increased expression of H4 RNA during the G1-to-S transition, we constructed three chimeric H4 genes, based on constructs 1, 2, and 3 (Fig. 2), with the human α-2 globin promoter regulating the transcription of H4 mRNA. With these constructs the histone RNA produced with either the H4 or globin promoter was similar: constructs α1 and α3 were six nucleotides longer at the 5' end, and construct α2 was 14 nucleotides longer at the 5' end. Our results with the chimeric genes demonstrate that the three- to fourfold increase in histone mRNA during the G1-to-S transition is transcriptionally controlled and is not due to an increase in the stability of the mRNA since the level of chimeric histone mRNA produced under the control of the globin promoter does not increase during this transition (Fig. 4; and Table 1). This increase in the histone RNA transcriptional rate measured by promoter exchange agrees with the in vitro nuclear incubation or runon experiments previously published for histone genes (1). Furthermore, the level of chimeric H4 RNA produced under the globin promoter decreases during the cell cycle (Table 1), in support of earlier studies which suggest that histone RNA stability decreases during the cell cycle (1, 13). Our results imply that this decrease is not due to the globin promoter per se since the level of α-globin mRNA actually increases during each phase of the cell cycle (Table 1; Fig. 4), but reflects the intrinsic stability of H4 mRNA during cell replication.

Luscher et al. (14) report that the faithful cell cycle regulation of the same mouse histone H4 gene is controlled by sequences in the 3'-terminal part of the gene. This conclusion was based on a plasmid construct, pEL1gpt, with the simian virus 40 early promoter transcribing the 3'-terminal half of the H4 gene in stable transformants of 21-Tb cells. However, these authors were not able to demonstrate a net increase in the total H4 RNA transcripts as the cells entered the S phase with the temperature shift. Instead they observed a decrease in the level of readthrough transcription with the simultaneous appearance of the correctly processed 3' end of the H4 RNA transcript. On the other hand, our results with the chimeric H4 genes clearly indicate that the H4 promoter is essential for the proper net increase in the level of H4 mRNA during the G1-to-S phase of the cell cycle. This is in agreement with a recent report on a chimeric construct with the hamster H3 promoter and the *neo* gene (2) which was used to demonstrate that *neo* RNA levels increase as the cells go from G0 into S. The Luscher report (14) does suggest that the 3' processing of H4 RNA is cell cycle regulated since the correct 3' end of the pEL1gpt chimeric transcript is only seen once the cells have entered S. It is unlikely that our results reflect 3' processing since the mRNA from the different constructs is essentially the same; only the α-globin promoter has been exchanged for the H4 promoter.

The recent report by Heintz and Roeder (10) using an in vitro transcription system with cell extracts prepared from S- and non-S-phase HeLa cells supports our in vivo results with the chimeric globin-H4 genes with the following observations. Transcription of the human H4 gene is 3- to 10-fold greater in S-phase extracts than in extracts from non-S-phase cells; however, the late simian virus 40 transcription unit is equally transcribed with both extracts. Factors that affect processing of the correct 3' end of the H4 RNA or RNA stability appear to come into play during the S phase of the cell cycle.

Utilizing the globin-H4 chimeric gene constructs, we were able to observe the transcriptional component and mRNA stability changes in H4 RNA expression during the cell cycle in actively dividing cells. The utility of this approach should make it possible to study those regions in the H4 gene responsible for transcriptional control separately from regions involved in mRNA stability.

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