Multiple, independently regulated, polyadenylated messages for histone H3 and H4 in *Tetrahymena*

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ABSTRACT

Heterologous probes for yeast H4 and H3 histone genes have been used to study the corresponding histone mRNAs in growing and starved Tetrahymena. Histone mRNAs in both physiological states are polyadenylated. Two types of H4 protein and two types of H3 protein have been previously identified in Tetrahymena. Two size classes of H4 messages and three classes of H3 messages have been detected by northern analysis. Southern blot analyses indicate that the number of different kinds of H3 and H4 genes is the same or slightly greater than the number of different messages, suggesting that each message is derived from a different gene.

Growing cells have ~30 times more histone mRNA than starved cells, even though their total mRNA content is only 4 times greater. The relative abundance of different H4 and H3 messages in growing and starved cells is different, demonstrating that the different messages for a particular type of histone are regulated non-coordinately. In starved cells the presence of a single size class of H3 messages correlates with the preferential synthesis of a previously described macronuclear-specific H3 variant.

The fraction of histone messages loaded in growing and starved cells is the same as for bulk mRNAs, and the relative concentrations of the multiple messages for H4 and H3 are the same in polysomal and total RNAs of each cell type. These observations suggest that histone synthesis in Tetrahymena is controlled largely at the level of message abundance, and that very little, if any, control occurs at the translational level.

INTRODUCTION

Histone mRNAs have been studied in a variety of organisms (see 1 for review). In most cells, histone messages are small, lack 3' poly A tails, and their expression is coordinately regulated in the cell cycle and development. Multiple primary sequence variants and multiple mRNAs for each histone have been demonstrated either within a single cell type or in different developmental stages of an organism (1-8). Where studied previously, multiple histone messages are either expressed co-ordinately as in HeLa cells (9) and yeast (10, 11) or sequentially as in developing sea urchin embryos (12). To our knowledge, differential regulation of multiple messages within a single cell type has not been described. However, recent studies have indicated

that, under certain circumstances, the synthesis of histone proteins is not co-ordinately regulated. H2B is preferentially synthesized over other histone proteins in heat shocked Drosophila tissue culture cells (13) and in mammalian cells a subset of the histone variants made in S phase are synthesized throughout the cell cycle (14) while a different subset appears to be synthesized in non-growing cells (15). In these cases it has not yet been determined whether non-coordinate synthesis is due to translational controls or to differential regulation of the abundance of different histone mRNAs.

In Tetrahymena thermophila, a ciliated protozoan, the histone proteins of the transcriptionally active macronucleus and the transcriptionally inert micronucleus have been characterized (16-18). A full complement of similar, if not identical major core histone proteins (H2A, H2B, H3 and H4) exists in both nuclei in similar amounts. Many differences between macro- and micronuclear histone proteins can be attributed to post-translational modifications such as acetylation (19) phosphorylation (20) or proteolytic processing (17). However, Allis et al. (16) have also found quantitatively minor primary sequence variants hv1 and hv2 specific to the macronucleus. In addition, the micronucleus does not contain the macronuclear H1. Instead three H1 analogs (α, β, γ) appear to be associated with linker regions in micronuclear chromatin. Since the S phases of macro- and micronuclei do not coincide (21, 22) it is likely that histone synthesis for the two nuclei does not occur at the same time.

These observations indicate that the synthesis and sites of deposition of histones in Tetrahymena must be highly regulated and led us to initiate studies on the regulation of histone mRNAs in logarithmically growing (DNA synthesis) and starved (little or no DNA synthesis) Tetrahymena. In this report we describe the findings that 1) the mRNAs for Tetrahymena histones H3 and H4 are largely if not entirely polyadenylated; 2) there are multiple mRNAs and multiple genes for histone H4 and H3; 3) the abundance of different transcripts for H4 and H3 (presumably from different genes) is regulated non-coordinately in growing and starved Tetrahymena. We also present evidence that histone synthesis in growing and starved cells is regulated at the level of message abundance and that in both physiological states, the fraction of histone message loaded onto polysomes is similar to that of other poly A+ mRNAs.

MATERIALS AND METHODS

Cells and Culture Conditions

Tetrahymena thermophila (strain B-1868-VII) were grown axenically in

enriched proteose peptone (23) at 28°C. Cells were starved by placing them in 10 mm Tris (pH 7.4) for a period of 18-24 hours.

RNA Isolation, Northern Gels, and Dot Blots

Tetrahymena RNA and polysomes were isolated according to the methods of Calzone et al. (24). RNA for northern gels was denatured in RNA resuspension buffer (RRB; 50% formamide, 20 mM phosphate buffer (pH 6.08), 0.5 mM EDTA) and then fixed by adding an equal volume of RNA fixation buffer (RRB with 4.4 M formaldehyde). RNA was heated to 60°C for five minutes before being fractionated on formaldehyde agarose gels (1% agarose, 1 mM EDTA, 20 mM phosphate buffer (pH 6.08) and 2.2 M formaldehyde). After electrophoresis, gels (with no pretreatment) were blotted to nitrocellulose in 20 x SSPE (20 x SSPE is 20 mM Na_2EDTA, 160 mM NaOH, 200mM $NaH_2PO_4 \cdot H_2O$, 3.6 M NaCl). Dot blots were prepared according to procedures described by Calzone et al. (24).

DNA Isolation

Macronuclei from T. thermophila were isolated as described by Gorovsky et al. (23). Nuclei were washed once in RSB (0.01 M Tris pH 7.5, 0.01 M NaCl, 0.003 M $MgCl_2$) and then lysed in room temperature NETS (0.5 M NaAcetate, 0.025 M EDTA, 0.1 M Tris (pH 8.0), 0.01 M SDS). Pronase was added to a concentration of 1 mg/ml and nuclei were digested for one hour at 50° C. Digested nuclei were extracted with an equal volume of 50 mM Tris (pH 8.0) saturated phenol-chloroform-isoamyl alcohol (25:24:1) and then twice with chloroform-isoamyl alcohol (24:1). DNA was concentrated by ethanol precipitation and then resuspended in TE buffer (10 mM Tris pH 8, 1 mM EDTA). RNAse A was added to 0.1 mg/ml and the DNA was incubated at 37°C for one hour. The preparation was digested with pronase and extracted with phenol-chloroform-isoamyl alcohol as described above. DNA was stored as an ethanol precipitate at -20° for future use.

Yeast Histone Gene Probes

The heterologous probes used in this study were isolated from a plasmid (pMS 191) constructed by M. Mitchell Smith. pMS191 contains one copy of yeast H4 and H3 histone genes cloned into the HindIII site of pBR322. Digestion of the plasmid with Hind III and HhaI produced an 810 bp fragment containing the H4 coding region and a 2000 bp fragment containing the H3 coding region. The H4 coding region was flanked by 250 bp of non-coding yeast DNA. The H3 coding region was flanked by non-coding yeast DNA on the 5' end and by an unknown protein coding gene, designated SMT1, on the 3' end. SMT1 does not code for a histone protein (M. Mitchell Smith, personal communication). No hybridization of an SMT1-specific probe to Tetrahymena RNA or DNA was observed at criteria

used for the histone genes. Since it was difficult to obtain pure H3 coding sequences, we used the total 2000 bp fragment to detect Tetrahymena H3 transcripts. These inserts were isolated by electrophoretic elution from agarose gels into DE81 paper (25).

Hybridizations

All northerns, Southerns, and dot blots were hybridized either with the heterologous yeast H4 probe, H3 probe or poly U. The yeast H4 and H3 probes were nick translated (25) to a specific activity of 10^8 cpm/μg DNA and poly U (sheared to ~40 NT) was kinase labelled (27) with γ labelled ^{32}P ATP to a specific activity of 2 x 10^{-} cpm/μg RNA. Histone probes were hybridized to filters at 60°C in 5 x SSPE, 1 mg/ml BSA, 1 x SPED (1 x SPED is 0.1% ficoll, 0.1% PVP 360, 0.1% BSA, 6 mM SDS, 2 mM Na_4 pyrophosphate, and 2 mM H_4EDTA). After incubation for 12 hours, the filters were washed in six changes of room temperature 2 x SSPE, 0.2% SDS. A final wash was done at 60°C in the same solution. Poly U was hybridized to nitrocellulose and the filters washed according to the methods of Calzone et al. (24). The filters were dried and put up for autoradiography against pre-flashed Kodak XAR-5 x-ray film with one intensifying screen at -80°C.

Quantitation of Filters

The amount of poly A^+ RNA on dot blots was quantitated by poly U hybridization as previously described (24). After hybridization with histone or poly U probes, filters were autoradiographed and densitometric scans were made of the x-ray film on an LKB soft laser densitometer. Areas were calculated using a Tektronix 4956 digitizer and these values used to calculate the relative concentrations of histone sequences in log and starved cells (Table 1) and their relative concentrations in polysomal and nonpolysomal fractions (Table 2).

Southern Blots and DNA Restriction Digests

Transfer of DNA from agarose gels to nitrocellulose was accomplished according to published procedures (28). DNA restriction digests were done at 37°C in 50 mM NaCl, 6 mM Tris pH 7.4, 6 mM $MgCl_2$, 6 mM β-mercaptoethanol, and 1 mg/ml BSA. Usually 1 unit of enzyme was used for every microgram of DNA.

Tetrahymena Histone Proteins

Cells were labelled for 30 minutes with ^3H-lysine about 24 hours after being placed in starvation media (10 mM Tris, pH 7.4). Histone proteins were isolated from nuclei and electrophoresed on two dimensional gels (triton acid urea followed by SDS) according to the methods of Allis et al. (17).

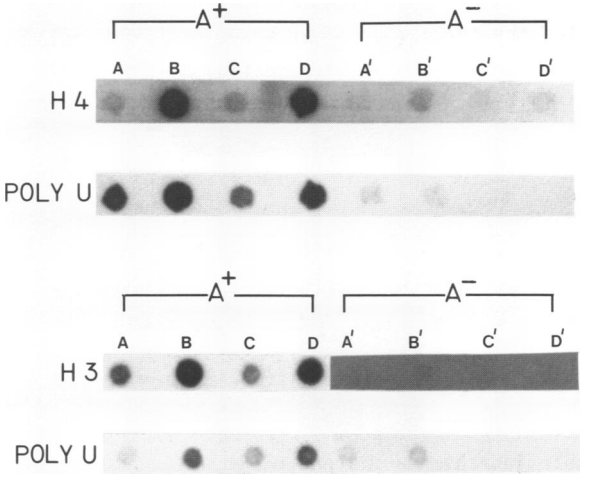

Figure 1. Tetrahymena Histone mRNA is Polyadenylated
Tetrahymena RNA from starved (A, C, A', C') or growing (B, D, B', D') cells was fractionated on poly U sepharose (A, B, A', B') or oligo dT cellulose (C, D, C', D'). RNA from these fractions was bound to nitrocellulose filters and probed with the yeast H4 or H3 sequences and poly U. Similar amounts of poly A+ RNA was loaded while about four times this amount of poly A- RNA was loaded. The weak hybridization signal over the A-fractions when using the poly U or histone probes is probably due to contamination of this fraction by poly A+ RNAs with short tails. The autoradiograms in this figure were overexposed so that all hybridization signals could be viewed on one print. Data obtained from properly exposed autoradiograms for determining the relative concentrations of histone sequences in log and starved cells are contained in Table 1.

RESULTS

Tetrahymena Histone mRNA is Polyadenylated

Most of the histone mRNA in growing or starved Tetrahymena cells contains a poly A tail of sufficient length to be retained on oligo dT cellulose or on poly U sepharose (Figure 1). As seen in Fig. 1, some poly A- fractions hybridized to a small extent with the histone probes. However, the poly A- dots contained 4 times as much RNA as those with poly A+ RNA, and positive histone hybridization in this fraction was always accompanied by a low level of hybridization with poly U. Therefore, it is likely that even the small amount of histone sequences found in the poly A- fraction is due to contamination of this fraction, presumably by poly A+ RNAs with short tails.

Multiple Histone mRNAs

When total cytoplasmic RNA from log or starved cells was electrophoresed on denaturing agarose gels, blotted, and probed with yeast H4 or H3 sequences

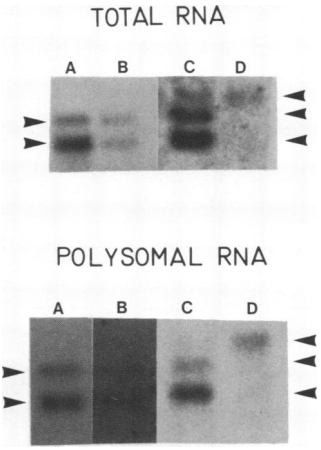

Figure 2. Multiple Tetrahymena Histone mRNAs
Northern blots of 1.0% agarose formaldehyde gel of starved (B and D) or log (A and C) cell RNA probed with the yeast H4 (A and B) or H3 (C and D) sequence. Both forms of the H4 mRNA were found on polysomes in log and starved cells. All forms of the H3 mRNA were found on polysomes in log cells. In starved cells the two smaller H3 mRNAs were undetectable.

(Figure 2), multiple bands were observed. With yeast H4 probes, two bands were evident that migrated as a 870 b and 1000 b species. Since H4 and H3 can be encoded in only 300-500 nucleotides and poly A tails in Tetrahymena are, on average, only about 30 bases long (29), these histone messages must contain unusually large untranslated regions. When northern blots were probed with yeast H3, three bands were evident that migrated at 950 b, 1050 b, and 1400 b. Approximately 85% of the H4 messages in a log cell existed as the 870 b species with only 15% in the 1000 b species. This was in contrast to starved cells where an approximately equal representation of both H4 messages was found. 53% of the H3 messages existed as the 950 b species with 32% and 15% found in the 1050 b species and 1400 b species, respectively. In starved cells the two smaller H3 mRNAs (950 b and 1050 b) were undetectable. These results suggest that the steady state concentration of multiple forms of both the H4 and H3 mRNA can be independently regulated in Tetrahymena. Furthermore, the presence of only the 1400 b H3 message on starved cell polysomes correlates with the preferential synthesis and nuclear deposition of hv2, an H3 primary sequence variant (Fig. 3). Since no other H3 protein was synthesized and deposited (Fig. 3) and no other H3 message was observed on polysomes during starvation (Fig. 2), this correlation strongly implies that the 1400 b H3 message codes for hv2.

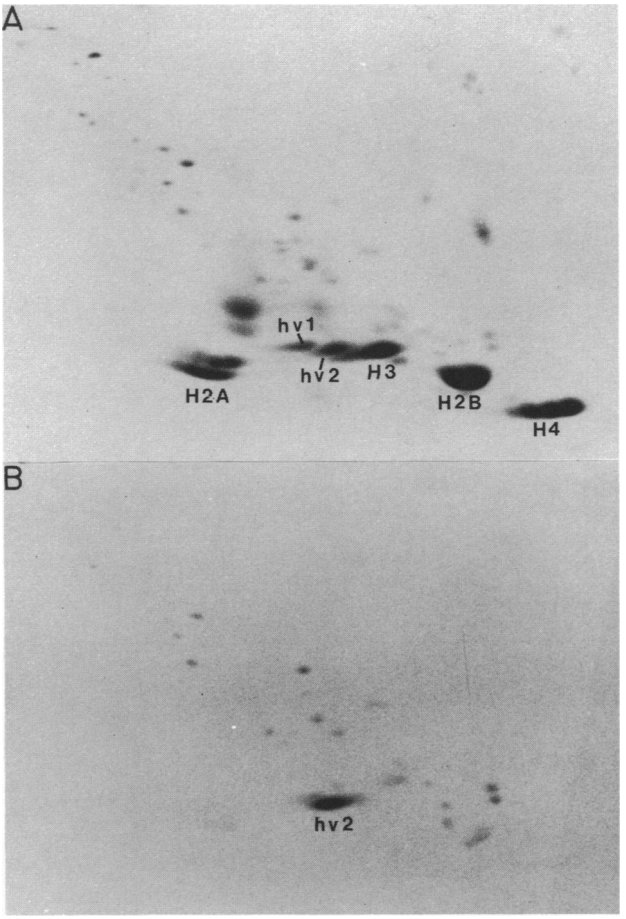

Figure 3. 2-Dimensional Gel (Triton-acid-urea by SDS) of Histones Synthesized by Starved Cells

Cells were labelled for 30 minutes about 24 hours after being placed in starvation media. The stained protein pattern (A) and its corresponding fluorograph (B) are shown. Note that hv2 is the only H3 like protein synthesized and deposited in starved cells. (Proteins were assigned to a histone group according to the rationale of Allis et al. (17).

A possible explanation for the existence of multiple messages coding for the same histone protein is that a precursor-product relationship exists between the different forms. To test this possibility we probed polysomal RNA with the yeast H3 and H4 sequences, reasoning that only the mature forms of the histone mRNAs would be found on polysomes. It is evident from these experiments (Fig. 2) that both H4 mRNAs and all three H3 mRNAs are on polysomes

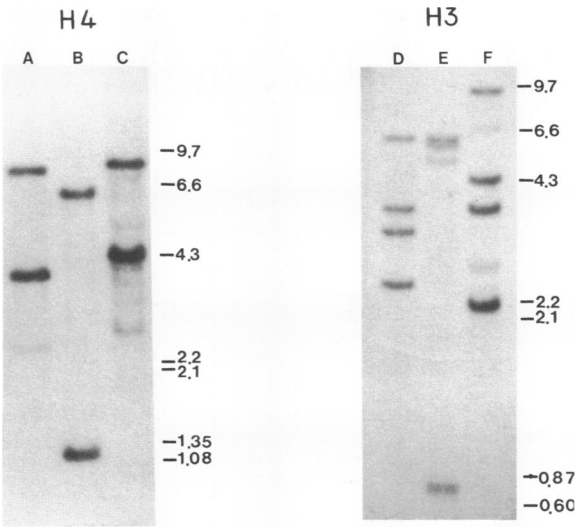

Figure 4. H3 and H4 Sequence Organization in Tetrahymena
Southern blot of macronuclear DNA digested with Hind III (lanes A and D) or Hha I (lanes B and E) or Eco R1 (lanes C and F) and probed with yeast H3 or H4 sequences. Marker DNA is Hae III cut ϕx174 and Hind III cut λ with fragment sizes expressed in kilobase.

in log cells, making it unlikely that a precursor-product relationship explains the multiple forms of Tetrahymena histone mRNAs.

Multiple Histone Genes

Since there were at least two H4 mRNAs and three H3 mRNAs by northern analysis, we suspected that each message might be the product of a different gene. To determine if our assumption was correct, Tetrahymena macronuclear DNA was digested with a variety of restriction endonucleases, electrophoresed on 0.8% agarose gel, blotted and probed with the yeast H4 or yeast H3 sequences. For most restriction enzymes, yeast H4 hybridization produced 2 major bands and yeast H3 hybridization produced at least three major bands (Figure 4). The size of the H4 bands ranged from approximately 8.6 kb to about 1 kb. Since we think it unlikely that three different enzymes which cut the highly AT rich Tetrahymena genome infrequently would restrict one H4 gene into two fragments containing approximately equal amounts of coding sequences, the simplest explanation for these results is that there are two genes coding for histone H4.

Yeast H3 hybridization to restricted macronuclear DNA in each digest resulted in at least four bands, with as many as six bands evident in an Eco R1

digest (Figure 4). The size of the H3 bands ranged from about 9 kb to 0.7 kb, with three bands of similar intensity in each digest. The other hybridization bands may be due to the restriction enzyme cutting within an H3 gene or may represent minor H3 gene variants which are present in lower amounts than the major bands. These results argue that there are two different H4 genes and at least three different H3 genes in the Tetrahymena genome. It should be noted that two types of H4 protein have been reported and two primary sequence variants of H3 have been detected in Tetrahymena (3, 16; Bowen and Gorovsky, unpublished observations). Thus, the number of genes (and messages) equals the number of known proteins in the case of H4 but exceeds the number of known proteins for histone H3.

Histone mRNAs are Differentially Regulated When Compared to General Message Populations of Log and Starved Cells

We compared the amounts of histone message in log and starved cells with the amounts of other poly A+ messages. Starved cells have about one fourth as much poly A+ mRNA as growing cells (24) and synthesize little or no DNA (Allis, Colavito-Shepanski and Gorovsky, manuscript in preparation). Equal amounts of cytoplasmic RNA from log and starved cells were fixed on nitrocellulose filters and probed with the yeast H4 or H3 sequence. Again, hybridization with poly U was used to quantitate the amount of poly A+ RNA that was loaded on the filters. On average, the concentration of H4 sequences (H4 hybrdization/Poly U hybridization) in poly A+ RNA of log cells is about 10 times greater than in starved cells; the concentration of H3 sequences is about 8 times greater (Table 1). Therefore, starved cells have 30-40 fold less (one eighth to one tenth the amount of histone mRNA/unit poly A^+ RNA x one quarter as much total poly A^+ RNA) histone message per cell than growing cells. Since the abundance of histone mRNAs and of bulk messages can be independently regulated in log and starved cells, it seemed possible that these two message populations might also be differentially loaded onto polysomes. Calzone et al. (24) have shown that ~60% of the total cellular mRNA is associated with polysomes in log cells while only 4% is loaded on polysomes in starved cells. When similar studies were performed using the yeast H3 or H4 probes, we discovered that the concentrations of histone messages in polysomal and non-polysomal RNA was similar to the value obtained for poly A+ RNA (Table 2). Thus, the loading of H4 and H3 mRNAs onto polysomes in both growing and starved cells must parallel that of the total mRNA population. When polysomal and whole cell RNAs of both growing and starved cells are compared on northern blots, it is also clear that the

Table 1. Relative Concentrations of Histone Sequences in Growing and Starved Cells

Method	Histone	Growing (histone/Poly A)	Starved (histone/Poly A)	Ratio (G/S)
Total Cytoplasmic RNA	H4	1.15	0.28	4.1
	H4	1.81	0.29	6.3
	H4	1.92	0.19	10.3
	H3	1.80	0.14	12.8
Oligo dT Cellulose	H4	1.05	0.09	11.7
	H3	1.48	0.34	4.4
poly U Sepharose	H4	1.01	0.07	15.3
	H3	0.73	0.11	6.7

Tetrahymena RNA (either total cytoplasmic RNA or poly A+ RNA) was isolated and bound to nitrocellulose filters. These filters were probed with the yeast H4 or H3 sequences followed by hybridization with poly U (to quantitate the amount of poly A+ RNA loaded on the filters). The histone to poly A+ ratio for growing (G) or starved (S) cells was calculated (See Material and Methods) and the ratio of histone mRNA contents determined (G/S). The concentration of histone mRNAs in growing cells is 8-10 times greater than in starved cells. Growing cells have approximately four times as many poly A+ messages as starved cells (Calzone et al., 1982), and most if not all of the histone messages in both cell types is poly A+. Therefore, the H4 and H3 histone mRNA content of growing cells is 30-40 times greater than in starved cells.

Table 2. Relative Concentrations of Histone mRNAs on Polysomes in Growing and Starved Cells.

Histone	Cells	Polysomal (histone/polyA)	Non-Polysomal (histone/poly A)	Ratio (P/N)
H4	Log	0.09	0.10	0.94
H4	Starved	0.28	0.22	1.29
H3	Log	0.49	0.65	0.77
H3	Starved	0.25	0.24	1.04

Tetrahymena cytoplasmic RNA was fractionated into excluded (polysomal) and included (non-polysomal) portions on a Biogel A15 column according to the methods of Calzone et al. (1982). Equal amounts of RNA from these fractions were bound to nitrocellulose filters, probed with yeast H4 or H3 sequences and poly U. Poly U was used to quantitate the amount of poly A+ RNA present in each fraction. The histone to poly A ratio was then calculated for polysomal (P) fractions (histone/poly A) and for non-polysomal (N) fractions (histone/poly A). The ratios of P/N were very close to one, indicating that histone mRNA fractionated the same as the general mRNA population in log and starved cells.

different size classes of H3 and H4 mRNAs are found on polysomes in the same relative amounts as in whole cell RNA (Fig. 2).

DISCUSSION

When using a heterologous probe, it is important to determine that hybridization has occurred with the desired gene or gene product. Unfortunately we and others (unpublished observations) have been unsuccessful in obtaining accurate translation of Tetrahymena mRNAs in vitro, precluding definitive identification by hybrid-selected translation. However, we have used highly characterized yeast histone H4 and H3 probes (M.M. Smith, personal communication) to identify Tetrahymena histone genes and mRNAs. Recently we have cloned a distinct Tetrahymena DNA fragment containing an H4 gene and also sequences homologous to other histone genes (unpublished observations). This result indicates that the yeast probes recognize (histone) sequences which are clustered in the Tetrahymena genome.
Furthermore, all of the yeast probes hybridize to RNA found in small polysomes and to RNA which is found in much greater abundance in growing cells than in non-growing cells. All of this evidence (cross-reactivity with well-characterized heterologous probes, clustered distribution in the genome, mRNAs found in small polysomes, mRNA regulated in growing versus non-growing cells) argues strongly that the yeast histone sequences are hybridizing to Tetrahymena histone genes and transcripts. Finally, where data are available, the number of known protein variants is similar to the number of genes and mRNAs we have detected coding for that histone.

The lack of poly A tails was once thought to be a distinguishing characteristic in the identification of histone mRNAs (1). However, the occurrance of poly A^+ histone message is not totally unprecedented. In both amphibian oocytes and yeast a large portion of histone mRNA is polyadenylated. The poly A^+ histone message of Xenopus oocytes is replaced by poly A^- histone mRNA at fertilization, leading to the speculation that polyadenylation may be a factor in storage of this mRNA during oogenesis (30). The yeast S. cervisiae has > 90% of its histone message in the polyadenylated form (31). Tetrahymena, another lower eucaryote, has the majority of its histone H3 and H4 mRNA in the polyadenylated form. Preliminary studies utilizing yeast probes for histones H2A and H2B indicate that all of the Tetrahymena core histone mRNAs are largely polyadenylated. No change in histone mRNA polyadenylation was observed when Tetrahymena was shifted from log phase where most histone mRNAs (>60%) are loaded to starvation conditions where most

histone messages (>95%) are not on polysomes. This suggests that polyadenylation is not simply related to storage or to loading of histone messages. It remains to be determined whether histone mRNAs in all lower eukaryotes are polyadenylated as in yeast and Tetrahymena.

Multiple histone H4 messages have been found in sea urchin embryos (5, 6) and HeLa cells (7, 8, 32). In both cases the multiple H4 mRNAs are encoded in different H4 genes, eliminating the possibility that they are identical primary transcripts which are processed differently. In Hela cells, multiple forms of the H4 mRNA are on polysomes during the S phase of the cell cycle. In the case of sea urchin embryos, developmental regulation appears to play a role in which H4 mRNA is available for translation. Grunstein (33) has shown that the early (blastula) histone H4 mRNA is replaced by a late (post-hatching) H4 mRNA which is encoded by a different H4 gene. In Tetrahymena there are at least two H4 messages and three H3 messages which are all found on polysomes in log cells. Correspondingly, there are at least two different H4 genes, shown by the appearance of two hybridization bands of similar intensity in a variety of macronuclear DNA digestions. At least three different H3 genes are detected by Southern analysis. Thus, while it is possible that the multiple mRNAs for a single histone are produced by alternative processing of transcripts from a single gene, it seems more likely that they are the products of distinct genes. However, the steady state concentrations of the three H3 mRNAs and two H4 mRNAs are not coordinately regulated when log cells are placed in starvation conditions. Log phase and starved cells have different steady state concentrations of the multiple forms of H4 and H3 mRNAs. This is most striking for H3 where three messages are loaded onto polysomes in log cells and only one message in starved cells. Our observation that histone protein hv2 (an H3 primary sequence variant; see 16 for details) is the only detectable H3 protein synthesized and deposited during this time is consistent with our identifying this mRNA as coding for hv2 and supports the argument that different histone messages are derived from different genes.

While this work was in progress there were three reports of non-coordinate regulation of histone synthesis. Wu and Bonner (14) reported that 10% of total histone synthesis was due to basal histone synthesis which took place throughout the S, G2 and G1 phases of the Chinese hamster ovary cell cycle. Basal histone synthesis was due to the preferential production of H2A variants Z and X and H3 variant 3. They also found that histone H2B and H4 participated in basal synthesis. Wu et al. (15) showed that quiescent

mammalian tissue culture cells synthesize a sub-set of histone variants different from that synthesized during S or during G_1 or G_2. Sanders (13) has found that histone H2B is a heat shock protein of cultured Drosophila cells. Synthesis of histone H2B was induced threefold while that of the other major histone proteins were reduced two- to tenfold. All of these studies demonstrate non-coordinate regulation of histone synthesis at the protein level. To our knowledge, we are the first to report non-coordinate regulation at the histone mRNA level. This differential regulation could be at the level of histone mRNA transcription, processing, or turnover. Studies are now in progress to discern between these possibilities.

In a number of cell types the concentration of histone messages is tightly regulated and does not always follow the general message population. In Hela S3 cells the synthesis of histone mRNA has been shown to occur during the S phase of the cell cycle (34). Once S phase has ended, histone message becomes undetectable in the cytoplasm of the cell. In yeast the synthesis of histone message occurs immediately preceeding S phase (10) followed by rapid transport from the nucleus to the cytoplasm where it is loaded on polysomes. In yeast, as in HeLa, histone messages become undetectable in the cytoplasm after the S phase. In early sea urchin embryos, as in starved Tetrahymena, histone synthesis is not coupled to DNA synthesis. Sea urchin eggs contain large amounts of stored histone mRNA whose utilization after fertilization is under translational control. The time at which most of the histone H3 messages are loaded on polysomes occurs about 8 hours after fertilization (35); in contrast to the general message population where loading on polysomes occurs about two minutes after fertilization (36).

In Tetrahymena we have shown that the steady state concentration of histone H3 and H4 mRNAs are also regulated differently from the general message population. However, the percentage of histone messages loaded on polysomes in log and starved cells closely parallels the loading of general messages on polysomes (24). This implies that in Tetrahymena, histone synthesis is largely regulated at the level of message abundance and that very little regulatory control is exerted at the translational level. It is rather striking that there is no preferential loading of histone messages in growing cells or unloading in starved cells, even though these different physiological states require 30-fold different levels of histone mRNA. This result, coupled with similar observations for tubulin messages in growing and starved cells (Calzone and Gorovsky, manuscript in preparation) suggest that, in

Tetrahymena, mechanisms for preferentially loading different mRNAs may not exist.

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NOTE: Sequence analysis of a Tetrahymena gene cloned by using the heterologous yeast H4 gene indicates that it codes for a Tetrahymena H4 protein. This result shows that heterologous yeast histone probes cross-hybridize with Tetrahymena histone genes and messages.

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