Requirement of protein synthesis for the coupling of histone mRNA levels and DNA replication

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H1 and core histone mRNA levels have been examined in the presence of portein synthesis inhibitors with different mechanisms of action. Total HeLa cell RNAs were analyzed by Northern Blot hybridization using cloned human histone genes as probes. Inhibition of DNA replication resulted in a rapid decline in histone mRNA levels. However, in the presence of cycloheximide or puromycin, H1 and core mRNAs did not decrease in parallel with DNA synthesis, but were stabilized and accumulated. Inhibition of DNA synthesis with hydroxyurea after the inhibition of protein synthesis did not lead to a decline in histone mRNA levels. These results suggest that synthesis of a protein(s) – perhaps a histone protein(s) – is required for the coordination of DNA synthesis and histone mRNA levels.

Histone mRNA *DNA replication* *Histone gene* *Protein synthesis* *Cell cycle* *Polysome*

1. INTRODUCTION

Many studies [1–9] have shown the close coordination of DNA replication and histone gene expression, but the molecular basis for this relationship is not yet known. In addition to the temporal coupling of histone synthesis, DNA replication and cellular levels of histone mRNAs, it has been well documented in numerous biological systems that inhibition of DNA replication results in a rapid decline in the cellular representation of histone mRNAs.

A requirement for protein synthesis for the selective destabilization of histone mRNA was initially suggested by studies [4] which showed that treatment of cells with cycloheximide prevented the cellular loss of 7–9 S RNAs (histone-enriched RNA fraction) which followed inhibition of DNA synthesis. We have recently confirmed this observation in HeLa S3 cells by using homologous cloned histone gene probes to establish that cycloheximide blocks the hydroxyurea-mediated destabilization of core and H1 histone mRNAs in human cells [10]. A critical point for evaluating the molecular implications of these results is to determine whether protein synthesis per se is required for the cellular loss of histone mRNAs which occurs in conjunction with DNA synthesis inhibition, or if the cycloheximide-induced retention of nascent histone polypeptides on polysomes is prerequisite. We have therefore compared protein synthesis inhibitors with different mechanisms of action. Our data suggest that destabilization of histone mRNAs in response to DNA synthesis inhibition is prevented when protein synthesis is inhibited either by cycloheximide or puromycin – the latter causing a release of nascent polypeptides. The implications of these results for autogenous control of cellular levels of histone mRNAs are discussed.

2. MATERIALS AND METHODS

[*methyl*-^{3}H]Thymidine (20.0 Ci/mmol, L-[4,5-^{3}H]leucine (60 Ci/mmol) and [α-^{32}P]dCTP (approx. 3000 Ci/mmol) were purchased from New England Nuclear. Hydroxyurea and puromycin

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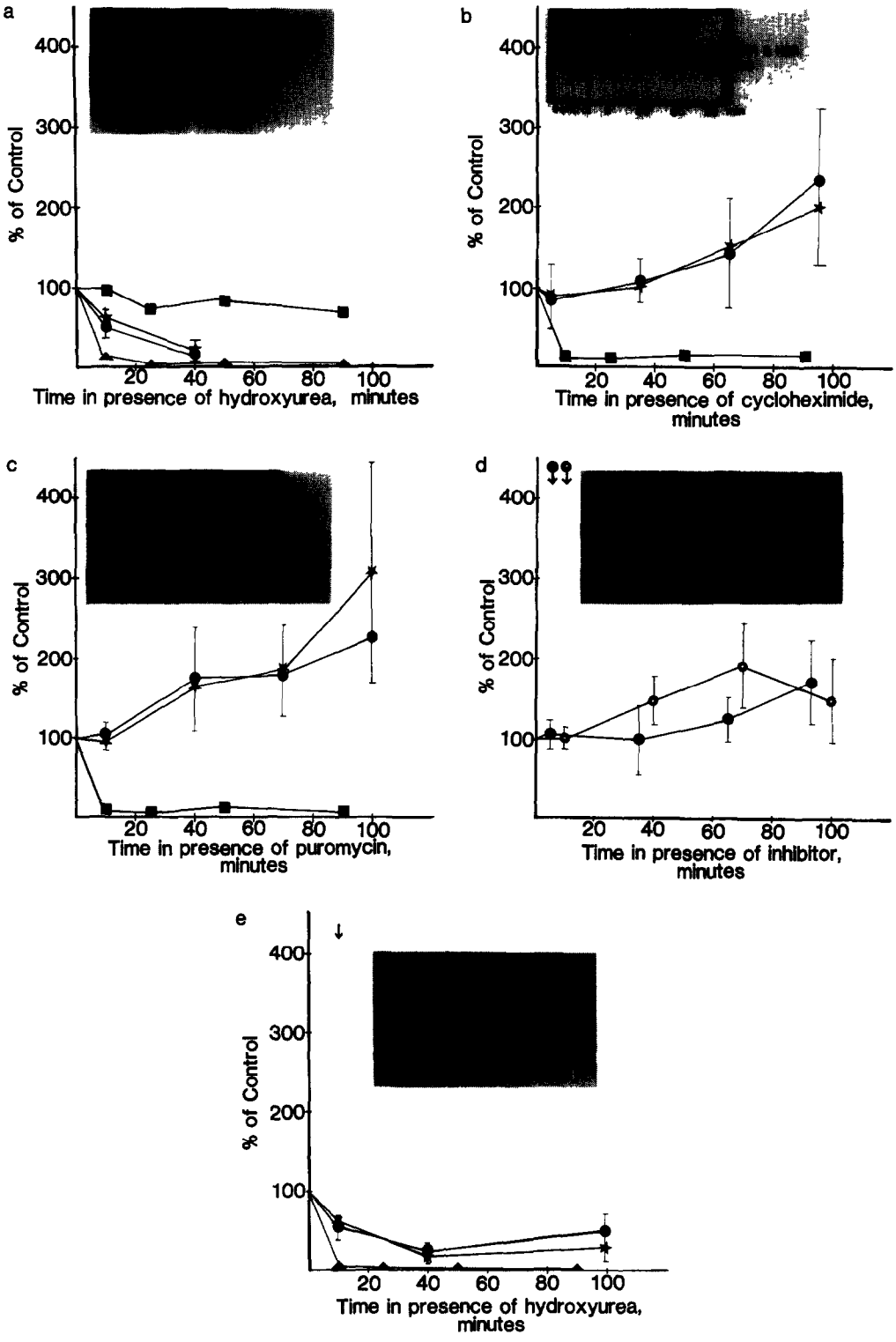
a
400
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% of Control
20 40 60 80 100
Time in presence of hydroxyurea, minutes
b
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20 40 60 80 100
Time in presence of cycloheximide, minutes
c
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100
% of Control
20 40 60 80 100
Time in presence of puromycin, minutes
d
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300
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% of Control
20 40 60 80 100
Time in presence of inhibitor, minutes
e
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200
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% of Control
20 40 60 80 100
Time in presence of hydroxyurea, minutes

were from Sigma. Cycloheximide was provided by Upjohn Co.

HeLa S3 cells were grown in suspension culture at 37°C and were maintained in exponential growth (2.5–5 × 10^5 cells/ml).

Relative rates of DNA or protein synthesis in the presence or absence of metabolic inhibitors were monitored by incorporation of [^{3}H]thymidine or [^{3}H]leucine (1 μCi/ml), respectively, into trichloroacetic acid-precipitable material [10% (w/v) trichloroacetic acid for DNA, 20% for protein]. Labelling was initiated at various times following the addition of the inhibitor to 2 ml of exponentially growing HeLa cells (5 × 10^5 cells/ml) and continued for 20 min at 37°C. Trichloroacetic acid-precipitable material was collected on 0.45 μm nitrocellulose filters (Millipore type HA). The filters were dissolved in 1 ml ethylene glycol monoethyl ether (cellosolve) and the radioactivity was measured by liquid scintillation spectrometry after the addition of 10 ml cellosolve scintillation cocktail [11].

Exponentially growing cells were divided into 7 sets and treated as follows: (1) no inhibitors were added (control); (2) 1 mM hydroxyurea; (3) 1 mM hydroxyurea followed by the addition of cycloheximide to 0.04 mM after 10 min; (4) 0.04 mM cycloheximide; (5) 0.04 mM cycloheximide followed by the addition of 1 mM hydroxyurea after 5 min; (6) 0.4 mM puromycin; (7) 0.4 mM puromycin 10 min prior to treatment with 1 mM hydroxyurea. Total cellular RNA [8] was extracted and subsequently analyzed for histone mRNA content by electrophoretic fractionation under denaturing conditions in 1.5% (w/v) agarose–6% (w/v) formaldehyde gels [12] and Northern blot hybridization [13]. Prehybridization, hybridization, and washes were carried out as in [8], except that prehybridization and hybridization were carried out at 42°C for H1 histone mRNA and at 50°C for core histone mRNAs. The filters were exposed to preflashed Kodak XAR X-ray film at −70°C. Hybridization was quantitated by densitometry of the autoradiograms and/or liquid scintillation spectrometry of the bands cut from the nitrocellulose filters.

The isolation and characterization of the λCh4A recombinant phage containing human histone genes have been described [14,15]. Genomic restriction fragments were subcloned into pBR322 [16]; the hybrid plasmids contained H1 (pFN C16), H2A + H2B (pFF 435B), H3 (pFF 435C), and H4 (pFO 108A). Plasmid DNAs were radiolabelled by nick translation as in [17] using [α-^{32}P]dCTP (2.0–5.6 × 10^8 cpm/μg).

3. RESULTS

The rate of DNA synthesis in control cells or cells in the presence of hydroxyurea, cycloheximide, or puromycin was monitored by [^{3}H]thymidine uptake. Within 25 min DNA synthesis de-

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Fig.1. (a) Relative amounts of core (●) and H1 (★) histone mRNA levels, [^{3}H]leucine incorporation (■) and [^{3}H]thymidine incorporation (▲) after treatment of HeLa cells with 1 mM hydroxyurea. The points represent the average of 6 separate hybridizations. The autoradiogram is a representative Northern blot hybridized with pFF435B (H2A + H2B) and pFO108A (H4). (b) Relative amounts of [^{3}H]leucine incorporation (■), core histone mRNA levels (●) and H1 mRNA levels (★) after treatment of HeLa cells with 0.04 mM cycloheximide. Shown is the average of 6 separate hybridizations. A representative autoradiogram shows hybridization with pFF435B (H2A + H2B) and pFO108A (H4). (c) Relative amounts of core (●) and H1 (★) histone mRNA levels and [^{3}H]leucine uptake (■) after treatment of HeLa cells with 0.4 mM puromycin. A representative autoradiogram shows total cellular RNA from control cells and cells treated with 0.4 mM puromycin hybridized to pFNC16 (H1) and pFF435B (H3). Hybridization of pFNC16 to 7 S RNA reflects annealing to a repetitive DNA sequence within the cloned fragment containing the human H1 histone gene. The 7 S RNA species does not vary in its representation during the cell cycle, nor is its level influenced by inhibition of DNA replication [22]. (d) Relative amounts of core histone mRNA levels after serial treatment with cycloheximide (●) or puromycin (○) and hydroxyurea (added at the time indicated by the arrow). A representative autoradiogram shows hybridization with pFF435B (H2A + H2B) and pFO108A (H4) after treatment with puromycin and hydroxyurea. (e) Relative amounts of [^{3}H]thymidine incorporation (▲), core histone mRNA levels (●) and H1 histone mRNA levels (★) after sequential treatment with hydroxyurea and cycloheximide (added at time indicated by arrow). The autoradiogram shows a representative Northern blot hybridized to pFNC16 (H1) and pFF435C (H3).

creased to 5% of control levels in the cells treated with hydroxyurea (fig.1a) or puromycin and to 15% of control levels with cycloheximide. The relative rates of protein synthesis as determined by [^{3}H]leucine uptake were 15% of control level after 10 min in the presence of cycloheximide (fig.1b) and 8% after 10 min in the presence of puromycin (fig.1c). Protein synthesis remained at 80% of the control levels for at least 90 min in the presence of hydroxyurea (fig.1a). The residual [^{3}H]leucine uptake detected in the presence of puromycin or cycloheximide may in part represent charged tRNAs.

Previous work by several groups [4–6,8,10, 18–20] has shown that inhibition of DNA synthesis leads to a rapid decrease in cellular histone mRNA content to a low, basal level. This apparent destabilization of histone mRNA was confirmed in the present studies, which show that cellular levels of core histone mRNAs were reduced to 15% of control after 40 min in the presence of hydroxyurea. DNA synthesis inhibition resulted in a similar decline in the cellular representation of H1 histone mRNAs, with 17% of the control levels observed after 40 min of treatment (fig.1a).

When cells were treated with protein synthesis inhibitors alone (cycloheximide or puromycin), there was an accumulation of both core and H1 histone mRNAs, despite the observed inhibition of DNA synthesis, with histone mRNA levels showing an approx. 2-fold increase over controls after 100 min (fig.1b,c). Although their mechanisms of action differ, there are no apparent differences in the effects of cycloheximide and puromycin on histone mRNA levels. Cellular levels of H1 and core mRNAs appear to be similarly affected (fig.1b). When hydroxyurea was added after addition of protein synthesis inhibitors, both core and H1 histone mRNAs remained at or above control levels (fig.1d). In contrast, when DNA synthesis was inhibited prior to inhibition of protein synthesis, there was an initial decrease in both core and H1 histone mRNA levels to approx. 20% of that observed in the untreated controls. However, by 100 min there was an increased cellular level of histone mRNAs (fig.1e) which appeared to be more pronounced for core (50% of control) than for H1 species (30% of controls).

4. DISCUSSION

We have used two different inhibitors of protein synthesis, cycloheximide and puromycin, to investigate the mechanism by which histone gene expression and DNA replication are functionally coupled. Cycloheximide inhibits the translocation of ribosomes resulting in the stabilization of the polysomal complex. Puromycin, however, is an aminoacyl-tRNA analog which results in early release of nascent polypeptides from the polysomes. Our results indicate that both inhibitors prevent the destabilization of human histone mRNAs which occurs in conjunction with inhibition of DNA synthesis. We therefore conclude that it is the inhibition of protein synthesis rather than an effect of a particular protein synthesis inhibitor which results in histone mRNA stabilization, even though blocking protein synthesis inhibits DNA replication. These results are in agreement with a recent report [21] that core histone mRNA degradation is prevented by several protein synthesis inhibitors and extend the observations to include human H1 mRNAs.

If DNA replication is blocked in cells pretreated with a protein synthesis inhibitor, cellular levels of histone mRNAs are not reduced below that of untreated controls; i.e., the stability of the histone mRNAs is not altered. Hydroxyurea may inhibit further histone gene transcription but does not affect the increased histone mRNA half-life associated with protein synthesis inhibition. Our experiments in which protein synthesis was inhibited following inhibition of DNA replication suggest that once the process of histone mRNA destabilization has been initiated it is not readily reversible.

These findings, together with our previous results which indicate that histone mRNA destabilization does not require transcription [10], suggest that a post-transcriptional process is necessary for the coordinate and selective destabilization of histone mRNAs which occurs when DNA synthesis is inhibited. It is reasonable to postulate that the translation of the histone proteins is required for autogenous regulation of cellular histone mRNA levels. Alternatively, the synthesis of some other regulatory protein may be required as part of the feedback mechanism providing a functional coupling of DNA replication and histone protein synthesis.

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