Histone H2A.F/Z mRNA Is Stored in the Egg Cytoplasm and Basally Regulated in the Sea Urchin Embryo

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The sea urchin H2A.F/Z histone is a member of a subclass of highly conserved H2A variants. Sequence analysis confirms that H2A.F/Z mRNA is polyadenylated. In situ hybridization studies demonstrate that maternal H2A.F/Z message is stored in the egg cytoplasm and present at equal levels in all cells of the mesenchyme blastula-stage embryo, suggesting that H2A.F/Z is not coordinately regulated with DNA synthesis. When blastula-stage embryos were exposed to DNA synthesis inhibitors, no effect on the steady-state level of H2A.F/Z mRNA was observed, while the level of late class H2B mRNA decreased substantially. These results provide evidence that the basal mode of regulation of this unusual histone variant is conserved evolutionarily.

INTRODUCTION

Histone genes are known to be regulated in a replication-dependent or replication-independent fashion (reviewed by Marzluff and Pandey, 1988), and recent studies have identified variant genes that encode alternate mRNA forms that are replication dependent and independent (Cheng et al., 1989; Mannironi et al., 1989). In the sea urchin embryo, the expression of the major classes of histone genes is well documented (reviewed by Davidson, 1986), but little is known of the regulation of polyadenylated, minor histone variants in this system. The H2A.F/Z variant is of particular interest since this protein is unusually conserved, and phylogenetic analysis suggests that it is one member of a separate evolutionary lineage of H2A proteins (van Daal et al., 1990). Previously, we reported that H2A.F/Z mRNA is expressed at all embryonic stages of development and in the adult (Ernst et al., 1987). In this study, we determined that maternal H2A.F/Z mRNA is located in the cytoplasm of the sea urchin egg, in contrast to the pronuclear localization of most maternal histone messages, and established that the mRNA of this minor histone variant is polyadenylated and basally regulated in the embryo.

METHODS

Embryo culturing. Strongylocentrotus purpuratus adults were obtained from Marinus Inc., Long Beach, California. Spawning, fertilization, and embryo culturing were as previously described (Nocente-McGrath et al., 1989). Blastula-stage (21-hr) embryos were exposed to the DNA synthesis inhibitors hydroxyurea (5 mM) or 5-fluoro-2-deoxyuridine (2 μM) for a period of 2 hr. In some experiments, a 5-min incubation in emetine (25 μg/ml) preceded the addition of hydroxyurea.

Isolation, sequencing, and analysis of H2A.F/Z cDNA clones. Plasmid Sp108 (Ernst et al., 1987) was used to isolate additional H2A.F/Z cDNA clones from a λgt10 gastrula-stage library and DNA sequence was determined using standard methods. Computer analysis was carried out with the aid of the Molecular Biology Computer Resource (MBCRR) at the Dana Farber Cancer Institute.

Northern blot analysis. Total embryonic RNAs were isolated by the guanidine hydrochloride/cesium chloride procedure, size fractionated by electrophoresis in formaldehyde-agarose gels, and blotted to nitrocellulose (Nocente-McGrath et al., 1989). Filters were hybridized with 32P-labeled probes specific for H2A.F/Z or late class H2B (Maxson et al., 1983) mRNA. Conditions for prehybridization/hybridization were 40% formamide, 5× SSPE, 5× Denhardt's, 0.5% SDS at 42°C, and for washing, 0.2× SSC, 0.5% SDS at 65°C.

RNA quantitation. Two micrograms of each RNA sample were denatured at 65°C for 30 min in 6× SSC, 7.4% formaldehyde and applied to a nitrocellulose filter using a BRL Hybri-Slot manifold. Hybridizations with specific probes were as described for the Northern blots. Message abundance was determined by counting excised slots in a scintillation counter.

In situ hybridization. In situ hybridizations were as previously described (Nocente-McGrath et al., 1989).
Antisense and sense probes were synthesized from pGEM-7Zf(+) (Promega Biotech) containing a 3'-specific EcoRI-HindIII fragment from pSp108. Amersham SP6 grade 35S-labeled rCTP was used in labeling reactions following instructions provided in the Promega Biotech riboprobe kit.

RESULTS AND DISCUSSION

Extensive sequence analysis of replication-dependent histones has resulted in the identification of both primary and secondary structural characteristics that are either uniquely conserved within each class of histones or shared by all members of this multigene family (Wells, 1987). In comparison to replication-dependent histones, conspicuous features of basal variant histone mRNAs are the absence of the canonical, replication-dependent 3' stem-loop, their larger more variable size, and poly(A) tails. We determined the complete 3' untranslated cDNA sequence of HBA.F/Z (Fig. 1) and compared its sequence and structural characteristics to those of known replication-dependent and -independent histone mRNAs. Three putative polyadenylation signal motifs were identified, but since we have never observed more
than a single message size and the poly(A) tail of several independently isolated cDNA clones begins 20–30 nucleotides from the most distal site, the first two signals appear to be nonfunctional (Fig. 1). Of the H2A F/Z-type messages described to date, the sea urchin mRNA is the longest (1.7 kb) and the chicken mRNA the shortest (0.82 kb), with the size difference largely attributed to the 3′ untranslated sequence. No significant similarities between sea urchin H2A.F/Z mRNA and other H2A.F/Z-like mRNAs that might mediate regulatory aspects of expression were identified by computer analysis (data not shown).

Maternal localization and cell cycle-regulated embryonic expression of the H2A.F/Z mRNA were evaluated by in situ hybridization (Fig. 2). Examination of the images for antisense probe hybridization to the mature egg demonstrates that H2A.F/Z mRNA is uniformly distributed in the cytoplasm (Figs. 2B and E). Thus, in contrast to the pronuclear localization of the early class histone mRNAs (DeLeon et al., 1983; Showman et al.,

Fig. 2. In situ distribution of H2A.F/Z mRNA in the egg and blastula-stage embryo. (A–C) Phase-contrast images; (D–F) dark-field images. Sense probe hybridization to the egg (A, D) as a nonspecific control. Antisense probe hybridization are shown for the egg (B, E) and mesenchyme blastula embryo (C, F). Bar equals 50 μm.
As a direct test of H2A.F/Z mRNA regulation, Northern blot analysis and RNA slot blot quantitation were used to examine steady-state H2A.F/Z mRNA levels in blastula-stage embryos exposed to inhibitors of DNA synthesis (Fig. 3). Exposure of blastula-stage embryos to the DNA synthesis inhibitors hydroxyurea or 5-fluoro-2-deoxyuridine had no effect on the steady-state level of H2A.F/Z mRNA, while late class histone H2B (LH2B) levels were reduced substantially by these treatments (Figs. 3A, lanes 3 and 5, and 3B). Pretreatment with the protein synthesis inhibitor emetine prevented the rapid reduction in the level of the replication-dependent LH2B mRNA (Figs. 3A, lane 4, and 3B). These data provide evidence for the replication-independent synthesis of the H2A.F/Z variant by demonstrating that the level of H2A.F/Z mRNA is not downregulated in response to DNA synthesis inhibitors.

Analysis of the regulation and utilization of histone variants provides one means of assessing the purpose of these isotypes. Basal regulation of the H2A.F/Z-type variants is evolutionarily conserved (Hatch and Bonner, 1990) and suggests a replacement function in the cell. However, reports of a limited distribution of this variant protein in chromatin (reviewed by Ernst et al., 1987; Risdale and Davie, 1987) and the strict evolutionary constraint to maintain this unusual H2A gene lineage (reviewed by van Daal et al., 1990) suggest that a more complex function may also exist.

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