MOLECULAR AND CELLULAR BIOLOGY, Mar. 1988, p. 1236–1246
0270-7306/88/031236-11$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 8, No. 3

Differential Stimulation of Sea Urchin Early and Late H2B Histone Gene Expression by a Gastrula Nuclear Extract after Injection into *Xenopus laevis* Oocytes

ROB MAXSON,[1]* MASAMICHI ITO,[1] SUSANA BALCELLS,[2] MATHEW THAYER,[1] MARTHA FRENCH,[2] FREDERICK LEE,[1] AND LAURENCE ETKIN[2]

Department of Biochemistry and Norris Cancer Hospital and Research Institute, University of Southern California Medical School, Los Angeles, California 90033,[1] and Department of Genetics, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas 77030[2]

Received 16 September 1987/Accepted 30 November 1987

Sea urchin early histone genes are active in preblastula embryos; late histone genes are maximally expressed during subsequent stages of embryogenesis. We used the *Xenopus laevis* oocyte to assay for *trans*-acting factors involved in this differential regulation. Sea urchin nuclear proteins were prepared by extracting gastrula-stage chromatin successively with 0.45, 1, and 2 M NaCl. We injected three fractions into oocytes along with plasmids bearing sea urchin early and late H2b histone genes. While neither the 0 to 0.45 M nor the 1 to 2 M salt fraction affected H2b gene expression, the 0.45 to 1 M salt fraction stimulated early and late H2b mRNA levels significantly. Late H2b gene expression was stimulated preferentially when the early and late genes were coinjected into the same oocytes. This extract did not stimulate the accumulation of transcripts of injected herpesvirus thymidine kinase genes or of the sea urchin Spec 1 gene, suggesting that the stimulatory activity is not a general transcription factor. We localized the DNA sequence required for the stimulatory effect to a region of the late H2b gene located between −43 and +62 relative to the transcription start site. A component of the 0.45 to 1 M salt wash fraction specifically bound to the 105-base-pair late gene DNA sequence and to the corresponding early gene fragment. The abundance of this binding activity decreased on a per genome basis during early development of the sea urchin.

The sea urchin synthesizes several distinct sets of histone proteins during early embryogenesis (7, 32). These include early isotypes, synthesized from fertilization to the blastula stage, and late isotypes, synthesized from the blastula stage onward. Each set of proteins is encoded by a distinct class of genes. Early histone genes are repeated several hundredfold in the genome and are organized in tandem quintets, each comprising genes for the five major histone types. In contrast, late histone genes are present in only 6 to 12 copies per genome and are arranged in irregular clusters (6, 21, 22, 27). The developmental switches in the synthesis of early and late histone isotypes are caused largely by changes in rates of transcription of the two gene sets (23, 28, 35).

The *Xenopus laevis* oocyte has proven highly useful in the analysis of transcriptional processes (9, 10). A variety of genes, including histone genes, are accurately transcribed after microinjection into oocyte nuclei, and the injection of genes with mutated promoter regions has enabled the identification of elements required for transcription in the oocyte (16). The *Xenopus* oocyte system has also been used to identify a *trans*-acting factor involved in the processing of sea urchin histone mRNAs (34) and, more recently, to identify a factor capable of stimulating sea urchin early H2b gene transcription (30).

We used the *Xenopus* oocyte to examine the mechanisms underlying the differential expression of sea urchin early and late histone genes. We found that an extract of sea urchin gastrula-stage chromatin stimulated the expression of sea urchin early and late H2b histone genes in *Xenopus* oocytes. This effect was probably not caused by a general transcription factor since the extract did not stimulate the expression of the herpesvirus thymidine kinase gene or of the sea urchin Spec 1 gene. Late H2b gene expression was stimulated to a greater extent than early gene expression, whether the early and late genes were on the same plasmid or separate plasmids. Deletion and fragment-competition experiments showed that a region of the late H2b gene containing the TATA element, the mRNA leader sequence, and a small portion of the protein-coding sequence is involved in the stimulatory effect. A component of the gastrula nuclear extract bound specifically to this same region, and its concentration decreased through early development of the sea urchin on a per genome basis.

MATERIALS AND METHODS

Preparation of chromatin fractions from sea urchin embryos. We obtained sea urchins (*Strongylocentrotus purpuratus*) from Alacrity Marine Biologicals, Redondo Beach, Calif. Gametes were collected and embryos were reared as described by Hinegardner (20).

Gastrula-stage embryos (2 liters of a 1% [vol/vol] suspension) were concentrated by centrifugation and then washed once in calcium-magnesium-free sea water and once in 1.5 M glucose. The resultant disassociated, partially lysed cells were homogenized gently in 10 volumes of low-salt buffer (4 mM magnesium acetate, 50 mM Tris [pH 7.4], 0.5% Nonidet P-40, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride [PMSF]). Usually, two to five strokes in a loose-fitting Dounce homogenizer were sufficient to complete lysis. The homogenate was overlaid (15 ml per tube) on step sucrose gradients consisting of 5 ml of 1.7 M sucrose on 5 ml of 2.2 M sucrose in 30-ml Corex tubes. The gradients were centrifuged at 5,000 rpm for 15 min, and the gray nuclei were collected from the interface between the two sucrose

* Corresponding author.

solutions. The nuclear material was diluted twofold with a buffer containing 75 mM NaCl, 25 mM EDTA, 5 mM Tris (pH 7.5), 20 mM 2-mercaptoethanol, and 0.1 mM PMSF. The insoluble material (partially lysed nuclei and chromatin) was collected by centrifugation at 10,000 × *g* for 30 min.

We used a method similar to that of Stunnenberg and Birnstiel (34) for extracting proteins from isolated nuclei. The chromatin-nuclei pellet was suspended in extraction buffer (0.45 M NaCl, 20 mM Tris [pH 7.0], 1 mM EDTA, 1 mM EGTA, 0.5 mM dithiothreitol, 0.1 mM PMSF) and vortexed for several minutes, and the insoluble material was collected by centrifugation at 10,000 × *g* for 30 min. The supernatant was set aside, and the extraction and centrifugation were repeated on the residual insoluble nuclear material with extraction buffer containing 1 M NaCl and then 2 M NaCl. The extracts were concentrated by precipitation with ammonium sulfate (0.35 g/ml), dialyzed for 48 h against several changes of injection buffer (100 mM NaCl, 20 mM Tris [pH 7.0], 5 mM $MgCl_2$, 1 mM EDTA, 0.1 mM EGTA, 5% glycerol, 0.5 mM dithiothreitol, 0.1 mM PMSF), and concentrated severalfold by dialysis against dry Sephadex or Aquacide (Calbiochem-Behring, La Jolla, Calif.). For whole-cell extracts, embryos washed as described previously were lysed with low-salt buffer. Ammonium sulfate was added to a final concentration of 0.36 M. Lysed embryonic cells were homogenized and centrifuged at 10,000 × *g* for 5 min, and the protein was precipitated by the further addition of $(NH_4)_2SO_4$ (55% saturated) to the supernatant. The precipitated protein was collected by centrifugation, and the pellet was suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.0)–0.1 mM EDTA–0.1 M KCl–20% glycerol–0.5 mM PMSF–1 mM dithiothreitol. The protein was dialyzed for 12 h against the same medium. Protein concentrations were determined by the method of Bradford (4). Extracts were stored frozen at −80°C. Repeated freezing and thawing resulted in a significant loss of activity.

Isolation of plasmid DNA. Plasmids bearing early or late histone genes were prepared by the standard cleared-lysate procedure (24) and suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The plasmids used in these studies were pCO2 (31), consisting of a single copy of the early histone repeat unit cloned in pBR313, pSpL-1, consisting of a pair of late histone genes, H4 and H2b, on a 9.5-kilobase (kb) genomic segment cloned in pBR322 (27), and several other subclones of pCO2 and pSpL-1 (see Fig. 1 and 7).

Microinjection of proteins and DNA into oocytes. *Xenopus* females were anesthetized by hypothermia, and oocytes were surgically removed. Injections and subsequent culture of oocytes were performed as previously described (11). A 10-nl sample of 0.45, 1, and 2 M salt washes (containing approximately 400 μg of protein per ml was injected into the oocyte cytoplasm. Five hours later, histone gene-containing supercoiled plasmids (0.25 to 4 ng of each) were injected into oocyte nuclei. In most experiments, Southern blots were done (12, 33) to check for uniformity between groups of oocytes in the amount of injected DNA.

Extraction of RNA from oocytes and analysis of histone gene transcripts by S1 nuclease protection or primer extension. Oocytes (5 to 20) were homogenized in 0.5 ml of homogenization buffer (0.3 M NaCl, 2% sodium dodecyl sulfate, 50 mM Tris [pH 7.5], 1 mM EDTA). The homogenate was extracted several times with a mixture of 50% phenol, 48% chloroform, and 2% isoamyl alcohol. Nucleic acid was precipitated with ethanol and collected by centrifugation, and the pellet was washed several times with 70% ethanol. DNA was removed by digestion with RNase-free DNase according to the specifications of the manufacturer (Promega Biotec, Madison, Wis.).

S1 nuclease and primer extension analyses of sea urchin H2b mRNAs were performed as described previously (1, 17, 29). Oligonucleotides specific for the herpesvirus thymidine kinase and sea urchin Spec 1 mRNAs were generous gifts of S. L. McKnight and W. Klein, respectively (15, 18). To quantitate the amounts of early and late H2b mRNA in injected *Xenopus* oocytes, we scanned autoradiograms of S1 protection or primer extension gels with a Hoefer GS 300A densitometer coupled to a Hewlett-Packard integrator. A comparison of densities of sea urchin standard mRNA preparations with experimental samples enabled us to calculate the quantities of early and late H2b transcripts in oocytes. We assumed 3×10^6 early H2b transcripts per morula-stage embryo (25) and 6.7×10^5 late H2b transcripts per sea urchin egg or embryo (M. Ito, J. Bell, G. Lyons, and R. E. Maxson, submitted for publication). Using a value of 3 ng of RNA per sea urchin egg or embryo (L. Washburn, Ph.D. thesis, University of California, Berkeley, 1971) and knowing the amount of whole-embryo RNA loaded on a given gel, we calculated the number of H2b mRNA molecules represented by the signals on the gel. By comparing these numbers with the scanning data from the injected samples, we were able to calculate the number of H2b transcripts in the injected oocytes.

Gel retardation assay for DNA-binding factors. Purified DNA fragments were labeled at their 3′ termini with T4 DNA polymerase and [^{32}P]dATP. The labeled fragments (2 ng) were incubated with chromatin extracts (usually 1 μg) in 50 mM NaCl–10 mM Tris (pH 7.4)–5% glycerol for 30 min at room temperature. Poly(dI-dC) (1 μg) was added to all reaction mixtures as a nonspecific competitor. In addition, specific competitor DNA fragments were included in some binding reactions. These fragments were: for the late H2b gene, *Bam*HI (−43 to +62) (R. E. Maxson, T. J. Mohun, G. Gormezano, and L. H. Kedes, Nucleic Acids Res., in press); for the early H2b gene, *Bam*HI-*Dde*I (−39 to +104); for the herpesvirus thymidine kinase gene, *Pvu*II (−200 to +2200). DNA-factor complexes were resolved on low-salt gels as described previously (13).

RESULTS

Sea urchin chromatin fraction stimulates early and late H2b histone gene expression in a dose-dependent manner after injection into *Xenopus* oocytes. To identify *trans*-acting factors involved in the temporal regulation of the sea urchin histone gene family, we tested the effect of sea urchin gastrula nuclear extracts on the expression of early and late H2b genes after injection of recombinant plasmids containing these genes into *Xenopus* oocytes. We isolated chromatin from gastrula-stage embryos and extracted proteins with successive washes of 0.45, 1.0, and 2.0 M NaCl. In preliminary experiments, we found that when the plasmid DNA was mixed with the protein before injection into the germinal vesicle it was often degraded and consequently was poorly transcribed. To remedy this problem, we injected the nuclear protein into the oocyte cytoplasm, waited 5 to 24 h for the nuclear proteins to migrate into the nucleus (3), and then injected the DNA into the nucleus. Gel blot analysis showed that the injected DNA remained intact over the course of the experiment (data not shown). A similar approach was used by Stunnenberg and Birnstiel (34) in the analysis of a factor involved in sea urchin H3 histone mRNA processing. After

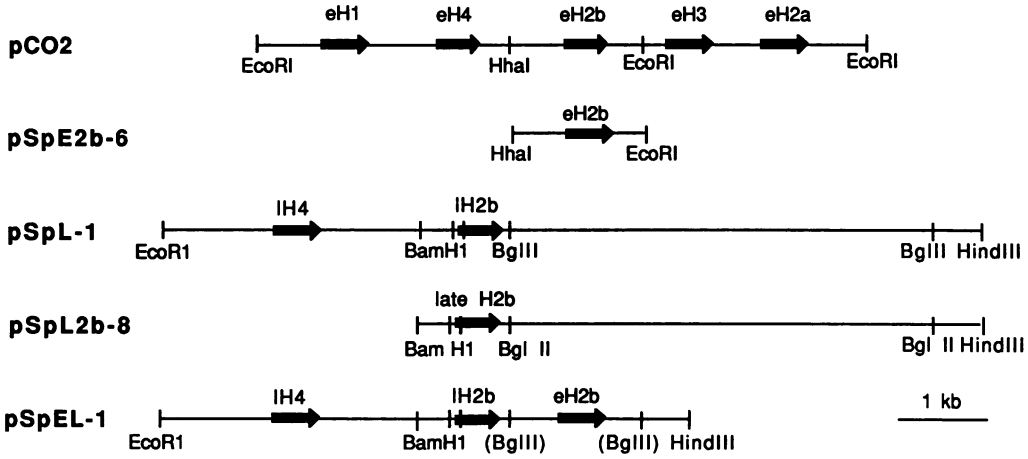


FIG. 1. Diagrammatic representations of early and late histone gene clones. pCO2 is a genomic segment containing all five *S. purpuratus* early histone genes (31). pSpE2b-6 is a subclone of pCO2 bearing the *Hha*I-*Eco*RI fragment containing the early H2b gene with about 500 bp of 5′-flanking sequences and 600 bp of 3′-flanking sequences. pSpL-1 is a genomic segment containing the late H4 and late H2b histone genes inserted in pBR322 (27). pSpL2b-8 is a subclone of pSpL-1 containing 418 bp of 5′-flanking sequence and 5 kb of 3′-flanking sequence. pSpEL-1 is a chimeric plasmid bearing both early and late H2b genes, constructed by replacing the *Bgl*II fragment 3′ to the L1 late H2b gene with the 1.8-kb early H2b *Hha*I-*Eco*RI fragment (i.e., pSpE2b-6).

DNA injection, the oocytes were cultured for 3 to 5 h, RNA was extracted, and sea urchin H2b histone transcripts were quantitated by S1 nuclease protection or primer extension.

We first tested the effect of a range of chromatin protein concentrations on the transcription of early and late H2b genes. The plasmids used in this experiment were subclones of genomic DNA segments containing only early or late H2b genes and several hundred base pairs of flanking DNA (Fig. 1). The late H2b gene, designated L1, is one of several variant late H2b genes so far characterized in *S. purpuratus*. Approximately 250 pg (0.05 fmol) of each plasmid was injected into the germinal vesicles of oocytes that had received prior injections of from 20 to 100 pg of the 0.45 to 1 M NaCl extract.

Figure 2 shows the results of a primer extension analysis of the early and late H2b transcription products. Oocytes that had received the early H2b genes contained an RNA species whose extension product comigrated with a product previously shown to be that of the early H2b mRNA (17). Oocytes that had received the L1 late H2b gene contained an RNA whose extension product comigrated with that of the L1 late H2b mRNA. This latter group of oocytes contained in addition to minor RNA species that gave rise to a more slowly migrating extension product, representing a spurious transcription initiation event approximately 50 base pairs (bp) upstream from the in vivo mRNA start site. However, most transcripts of the injected early and late H2b genes initiated at the correct site. The level of stimulation of the H2b transcripts varied between experiments (3- to 20-fold). This variation was probably due to differences in the factor preparations used in different experiments as well as to differences in the ability of oocytes of various frogs to respond to the extract (M. French and L. Etkin, unpublished observations).

The results presented in Fig. 2 show that the expression of both early and late H2b genes was stimulated in a dose-dependent manner by the 0.45 to 1 M salt extract. Also, the levels of stimulation of the two genes were similar. Neither the 0 to 0.45 M nor the 1 to 2 M salt extract contained detectable transcription stimulatory activity (data not shown; see also Fig. 5).

The 0.45 M salt wash fraction does not affect the expression of herpesvirus *tk* or sea urchin Spec 1 genes in *Xenopus* oocytes. To determine whether the 0.45 to 1 M salt extract affects histone gene expression specifically, we tested the effect of the extract on the expression of the herpesvirus thymidine kinase gene (*tk*) (14) and the *S. purpuratus* Spec 1 gene (18). The timing of Spec 1 gene expression is similar to that of the late histone genes in *S. purpuratus* development. The *tk* and Spec 1 genes as well as a control L1 H2b gene was injected separately into groups of oocytes that either had received the 0.45 to 1 M salt extract or had not. Levels of transcripts of the injected genes were monitored by primer extension with oligonucleotides specific for each mRNA. We expected primer extension products of 55 and 67 nucleotides from the Spec 1 and *tk* messengers, respectively. Figure 3A

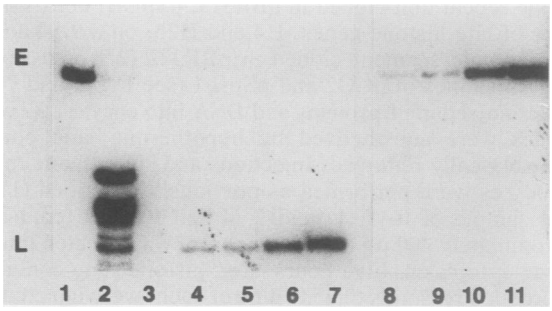


FIG. 2. Effect of factor concentration on stimulation of early (E) and late (L) H2b transcript levels in *Xenopus* oocytes. Groups of oocytes (19) were injected with 20, 50, or 100 pg of 0.45 to 1 M salt extract and then with plasmids bearing early or late H2b histone genes. There hours after DNA injection, RNA was extracted from oocytes and analyzed for early and late H2b transcripts by primer extension. Lanes: 1, RNA from sea urchin eggs; 2, RNA from sea urchin gastrula; 3, RNA from noninjected oocytes; 4, RNA from oocytes injected with plasmid pSpL2b-8 (no extract); 5, pSpL2b-8 with 20 pg of extract; 6, pSpL2b-8 with 50 pg of extract; 7, pSpL2b-8 with 100 pg of extract; 8, pSpE2b-6 with no extract; 9, pSpE2b-6 with 20 pg of extract; 10, pSpE2b-6 with 50 pg of extract; 11, pSpE2b-6 with 100 pg of extract.

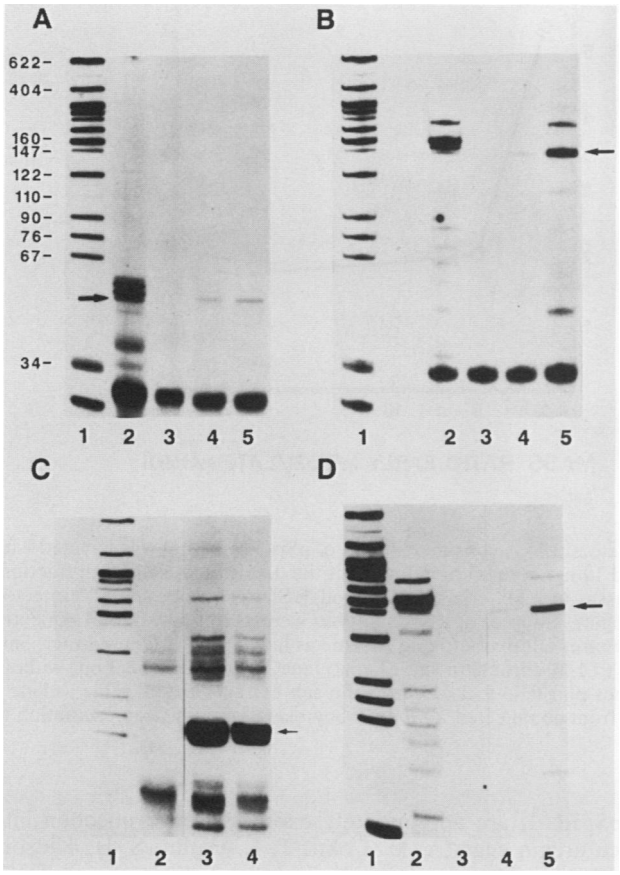


FIG. 3. Specificity of the transcription-stimulatory effect. We tested the effect of the 0.45 to 1 M salt extract on the expression of Spec 1 (A), *tk* (C), and pSpL-1 late H2b genes (B and D) in *Xenopus* oocytes. Oocytes were incubated for 4 h after injection of the DNA. The RNA was extracted and analyzed by primer extension with an end-labeled oligonucleotide specific for the Spec 1 gene transcript (A), the *tk* transcript (C), or histone H2b transcripts (B and D). The arrows point to the locations of the expected extension products of each transcript. Equimolar amounts of each gene were injected with and without the extract. (A) Lane 1, Marker DNA (pBR322 digested with *Msp*I); molecular weights (10^3) are shown to the left. Sources of RNA samples used in primer extension analyses were as follows: lane 2, sea urchin gastrula; lane 3, noninjected oocytes; lane 4, oocytes injected with the Spec 1 gene alone; lane 5, oocytes injected with the extract followed by injection of the Spec 1 gene. (B) Control analysis of the same group of oocytes as in panel A, but injected with the protein extract and the pSpL-1 plasmid. Lane 1, Markers. Sources of RNA used for primer extension analyses were as follows: lane 2, sea urchin gastrula; lane 3, noninjected oocytes; lane 4, oocytes injected with the late H2b gene alone; lane 5, oocytes injected with the extract and the late H2b gene. (C) The experimental analysis was performed exactly as in panel A, except the herpesvirus *tk* gene was injected. Lane 1, Markers; lane 2, RNA from noninjected oocytes; lane 3, oocytes injected with the *tk* gene alone; lane 4, oocytes injected with protein extract followed by injection of the *tk* gene. (D) Control analysis of the same group of oocytes as in panel C, but injected with the protein extract and the sea urchin late H2b gene. Lane 1, Markers; lane 2, RNA from sea urchin gastrula; lane 3, RNA from noninjected oocytes; lane 4, oocytes injected with the pSpL-1 clone alone; lane 5, oocytes injected with the protein extract followed by injection of the pSpL-1 clone.

and C shows that products of these approximate sizes were evident, demonstrating that transcription of the Spec 1 and *tk* genes initiated accurately in the oocytes.

The levels of L1 H2b mRNAs were stimulated 20- to 30-fold (Fig. 3B, lanes 4 and 5; Fig. 3D, lanes 4 and 5) by the 0.45 to 1 M salt extract, but neither the *tk* nor the Spec 1 transcript levels were stimulated significantly (Fig. 3A and C). The expression of these genes was probably not limited by endogenous factors since titration experiments performed with the *tk* gene showed that injection of amounts of DNA greater than that used in this experiment resulted in increased transcription (data not shown). Thus, if the stimulatory factor was capable of stimulating the expression of these genes, the genes should have responded. These results suggest that the transcription-stimulatory activity in the 0.45 to 1 M salt extract is not general.

To analyze further the specificity of the transcription factor, we asked whether it interacts with both early and late histone genes. We performed a competition experiment in which we mixed a constant amount of late histone gene plasmid (pSpL-1) with increasing amounts of early histone gene plasmid (pCO2) in ratios of 1:1, 1:5, and 1:20. These DNA mixtures were microinjected into groups of oocytes with or without the 0.45 to 1 M salt extract. RNA was extracted from these oocytes and analyzed for early and late H2b transcripts by S1 nuclease protection. The DNA probe was a 105-bp fragment spanning the transcription start site of the L1 late H2b gene. On the basis of previous experiments (17, 29), we expected a group of protected fragments of about 59 nucleotides, which are indeed evident in the autoradiogram shown in Fig. 4.

Using these radiolabeled fragments as a measure of late H2b transcript levels, we found that the relative stimulation of late H2b gene expression decreased with increasing amounts of early histone genes from 5.4-fold at the 1:1 ratio to 2.1-fold at the 1:5 ratio and 1.7-fold at the 1:20 ratio (Fig. 4A and B). This quantitation was obtained by densitometry scans of a lighter exposure of the data in Fig. 4. Neither pBR322 nor poly(dI-dC) competed for the transcription-stimulatory activity (data not shown). The relatively slight decrease in stimulation observed in the presence of 20:1 competitor as compared with 5:1 competitor suggests that a minor fraction of the transcription stimulation activity is not inhibited effectively by the early histone genes and may thus represent an activity unique to late histone genes. Nevertheless, these results demonstrate that early histone genes compete with the L1 late H2b gene for a majority of the stimulatory activity.

Expression of late H2b genes is preferentially stimulated when they are coinjected with early H2b genes. We showed previously that the factor in the 0.45 to 1 M salt wash affected early and late genes approximately equally when the two genes were injected separately into different groups of oocytes (Fig. 2). We also showed that the early histone repeat unit competes with the late H2b gene for the transcription-stimulatory factor, suggesting that a common factor is responsible for the majority of the stimulation of early and late H2b gene expression in oocytes. If the stimulatory factor is involved in the developmental regulation of early and late histone genes, we would expect the factor to affect early and late H2b genes differently in oocytes. We tested this by injecting both early and late H2b genes into the same group of oocytes, together with a limiting amount of factor. The plasmids used in this experiment were PCO2, containing an entire early histone gene repeat unit, and pSpL-1, containing a late H4 and H2b gene and several kilobases of

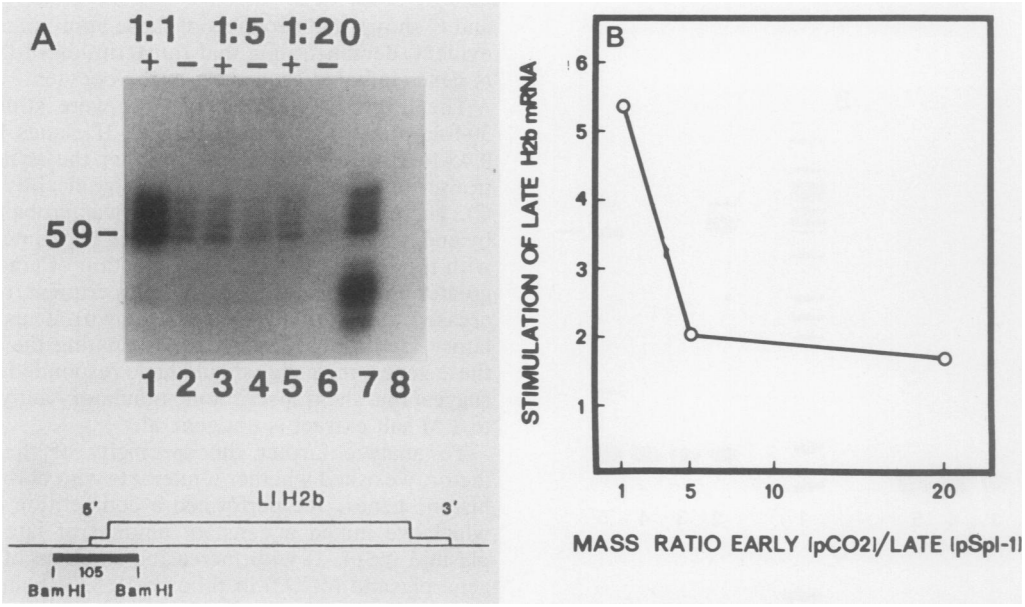


FIG. 4. Competition of early and late H2b plasmids for transcription factors. (A) A constant amount of pSpL-1 (0.5 ng) was injected with increasing amounts of competitor early histone DNA (pCO2, 0.5, 2.5, and 10 ng) both with and without the 0.45 to 1 M salt wash fraction. Analysis of late H2b transcripts was performed by S1 nuclease protection assay (see Materials and Methods). Sizes of probe and S1-protected fragment corresponding the mRNA cap are indicated at the bottom of the figure. Sources of RNA analyzed were as follows. Lane 1, Oocytes injected with 0.5 ng of late and 0.5 ng of early genes plus 0.45 to 1 M chromatin salt extract; lane 2, same as lane 1 but without extract; lane 3, oocytes injected with 0.5 ng of late and 2.5 ng of early genes plus 0.45 to 1 M chromatin salt extract; lane 4, same as lane 3 but without extract; lane 5, oocytes injected with 0.5 ng of late and 10 ng of early genes plus 0.45 to 1 M chromatin salt extract; lane 6, same as lane 5 but without extract; lane 7, sea urchin gastrula RNA (4 μg); lane 8, RNA from noninjected *Xenopus* oocytes. (B) Graphic representation of panel A.

flanking DNA. We chose these constructs because they most faithfully represent the in vivo DNA context of the early and late H2b genes. The amount of extract injected was in the linear range of the dose-response curve, i.e., the injected DNA was in molar excess over the factor. Under these conditions, the relative amounts of transcription of the early and late H2b genes should provide a measure of the relative activity of the stimulatory factor on the two genes.

We found that the early and late histone genes were transcribed with near equal efficiencies when injected into oocytes without sea urchin chromatin proteins (1.4×10^8 early transcripts versus 1.2×10^8 late transcripts [Fig. 5A, lane 4; Fig. 5B, lane 4; Fig. 5C]). Coinjection of the 0.45 to 1 M salt fraction with the early and late genes resulted in a twofold increase in early H2b transcript levels, from 1.4×10^8 to 2.5×10^8 transcripts (Fig. 5A, lane 6; Fig. 5C) and an eightfold increase in late H2b transcript levels, from 1.2×10^8 to 9.3×10^8 transcripts (Fig. 5B, lane 6; Fig. 5C). Neither the 0.45 M nor the 2 M salt extract affected H2b mRNA levels (Fig. 5A, lanes 5 and 7; Fig. 5B, lanes 5 and 7; Fig. 5C).

It seemed possible that this differential stimulation of early and late H2b gene expression in the injected oocytes was caused by differences in the DNA context of early and late H2b genes in their respective cloned DNA segments. To control for this possibility, we tested the activity of the factor on early and late H2b genes linked closely together on the same plasmid (clone pSpEL-1 in Fig. 1).

A plasmid containing both early and late H2b genes was constructed by inserting a 1.8-kb *Hha*I fragment bearing the early H2b gene into the a *Bgl*II site located 90 bp downstream from the mRNA termination site of the L1 late H2b gene (Fig. 1). The early and late H2b genes on this construct (pSpEL-1) are appropriately expressed after injection into sea urchin eggs (A. M. Colin, T. L. Catlin, S. H. Kidson, and R. E. Maxson, Proc. Natl. Acad. Sci., in press). Thus, this plasmid contains DNA sequence elements that are sufficient for the correct temporal expression of these genes during development.

After injection of pSpEL-1 DNA and a limiting amount of the stimulatory factor (100 pg) into oocytes, we monitored the expression of the injected genes by primer extension. It is evident from the data presented in Fig. 6 that although the basal level of early H2b transcription was less than that of late H2b in this construct (see below), the transcription of the late H2b gene was stimulated eightfold, while that of the early H2b gene was stimulated only threefold. Thus, the transcription-stimulatory factor selectively stimulated late H2b transcript levels even when the early and late H2b genes were closely linked. These results further suggest that when the early and late H2b genes are forced to compete for a limiting factor, the late gene is the more effective competitor.

DNA sequences required for the transcription-stimulatory effect are located proximal to position −43 in the L1 late H2b gene. As a first step toward identifying the DNA sequences required for the transcription-stimulatory effect, we tested the effects of gross deletions on the ability of the L1 H2b gene to respond to the nuclear extract. We prepared two L1 H2b gene deletion constructs, Δ1 and Δ2, whose 5′ borders were *Bam*HI sites at positions −418 and −43 (relative to the mRNA start site), respectively, and whose 3′ border was a *Bgl*II site 90 bp downstream from the mRNA termination site (Fig. 7A). We injected these constructs into oocytes that either had or had not received previously the 0.45 to 1 M salt extract, and we monitored their expression by primer extension. Deletion of flanking DNA to position −43 did not affect

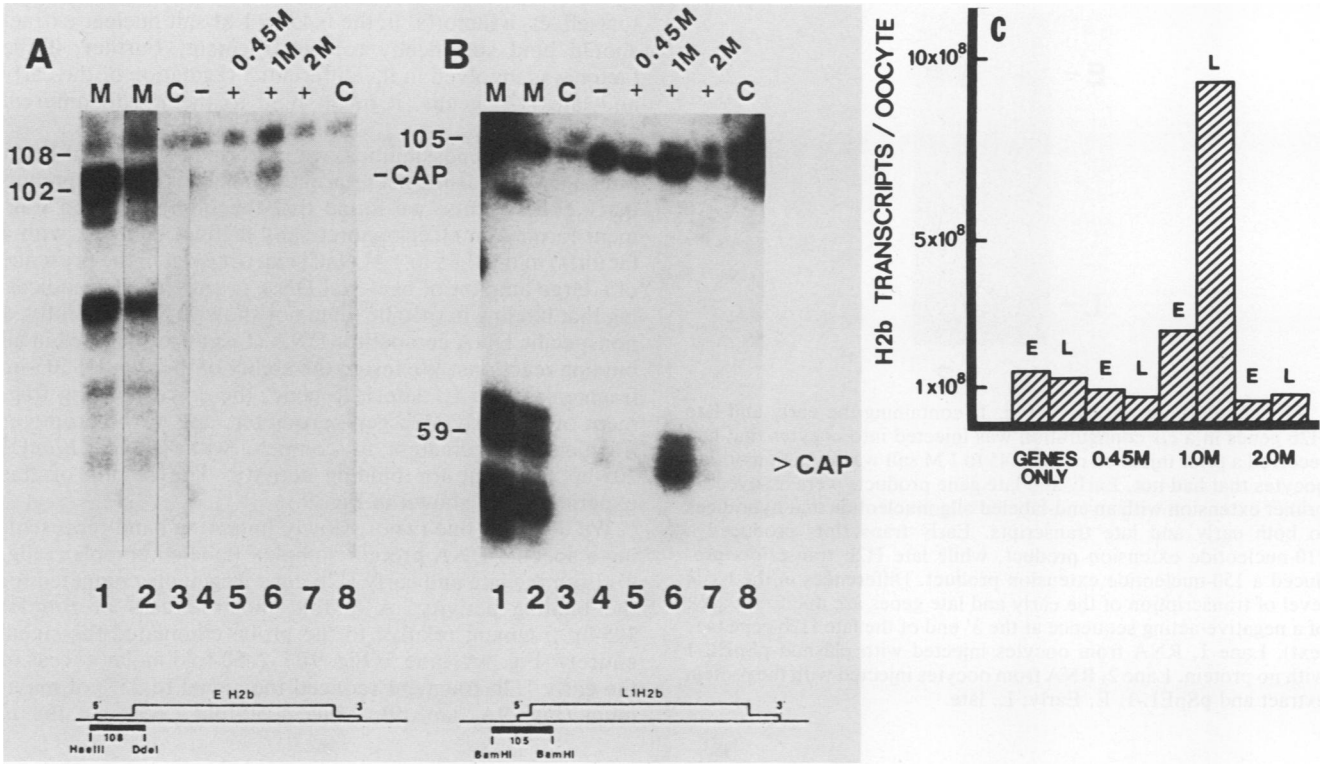


FIG. 5. Differential stimulation of early and late H2b genes coinjected on different plasmids. Sea urchin nuclear extracts were microinjected into the cytoplasm of *Xenopus* oocytes. Subsequently, equimolar amounts of early and late H2b histone genes on plasmids pCO2 and pSpL-1 were microinjected into the oocyte nuclei. Transcripts of the injected genes were analyzed by S1 nuclease protection with probes shown in schematic below panels A and B. (A) Early H2b probe. (B) Late H2b probe. Sizes (base pairs) of probe (top) and S1-protected fragment corresponding the mRNA cap (bottom) are indicated to the left of panels A and B. The same group of oocytes was used for experiments shown in panels A and B. Each lane represents 2 oocyte-equivalents of RNA (8 μg). Sources of RNA analyzed in panels A and B were as follows. Panel A, lane 1, Sea urchin morula (2 μg); lane 2, sea urchin morula (1 μg); panel B, lane 1, sea urchin gastrula (8 μg); lane 2, sea urchin gastrula (4 μg); lane 3, panels A and B, tRNA; lane 4, panels A and B, *Xenopus* oocytes injected with early and late histone genes; lane 5, panels A and B, oocytes injected with early and late histone genes plus 0.45 M salt extract of sea urchin gastrula chromatin; lane 6, panels A and B, oocytes injected with early and late histone genes plus 0.45 to 1 M chromatin salt extract; lane 7, panels A and B, oocytes injected with sea urchin early and late histone genes plus 1 to 2 M salt extract; lane 8, panels A and B, noninjected oocytes. (C) Quantitation of early (E) and late (L) H2b transcripts in *Xenopus* oocytes. The autoradiograms shown in panels A and B were scanned, and the quantities of early and late H2b mRNAs in oocytes were calculated as described in Materials and Methods.

the ability of the injected H2b gene to respond to the stimulatory factor. The expression of the pSpL-1 wild-type construction was stimulated 5-fold, while the expression both of the deletions was stimulated 4- to 4.5-fold (Fig. 7C). The Δ1 and Δ2 deletion constructs were transcribed more efficiently in *Xenopus* oocytes than the wild-type pSpL-1 construct (Fig. 7B, lanes 4 and 5). This is due to the absence of a DNA element in the Δ1 and Δ2 constructs that had a negative effect on the basal level of H2b gene transcription in the oocyte. This DNA segment comprises a 5-kb region flanked by *Bgl*II sites 3′ to the late H2b gene. Its presence or absence did not affect the ability of the gene to respond to the stimulatory extract (A. Chang and L. Etkin, unpublished observations).

Specific competition of a DNA segment located between −43 and +62 of the L1 late H2b gene for the transcription-stimulatory factor. Since the distal border of the Δ2 construct was only 10 bp upstream from the TATA sequence and since deletion into the TATA sequence would be expected to reduce greatly the basal level of transcription as well as cause initiation at inappropriate sites (16) we decided to use an alternative to the deletion approach to further localize the DNA sequences required for the transcription-stimulatory effect. We performed a competition experiment in vivo in which we tested the effect of injecting successively increasing amounts of a 105-bp *Bam*HI fragment (−43 to +62) of the late H2b gene on the stimulation of late H2b gene transcription. The distal border of this fragment was at position −43, the same as the Δ2 mutant construct, and the proximal border was located in the nontranslated mRNA leader sequence at +62 (Fig. 7A). When the late H2b gene was injected without a competitor fragment, the nuclear extract stimulated the level of H2b transcripts fourfold (Fig. 8A, lane 3, Fig. 8C). Coinjection of an equimolar amount of fragment diminished the stimulation to 3-fold, and coinjection of a fivefold molar excess of fragment reduced the degree of stimulation further to 1.4-fold (Fig. 8A, lanes 4 to 7; Fig. 8C). In contrast, injection of a ninefold molar excess of linearized pSV2CAT plasmid with pSpL-1 DNA did not affect the enhancement activity (Fig. 8B and 8C). These results show that the DNA sequences in the 105-bp *Bam*HI fragment are able to compete for the stimulatory activity and thus are probably involved in the transcription-stimulatory effect.

Preferential binding of factors in 0.45 to 1 M NaCl nuclear extract to H2b gene fragments compared with *tk* gene fragments. If the DNA sequences contained in the 105-bp *Bam*HI fragment were involved in the transcription-stimula-

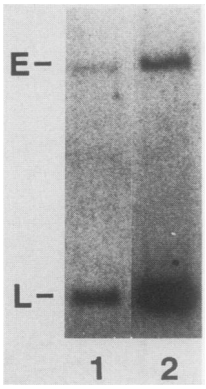
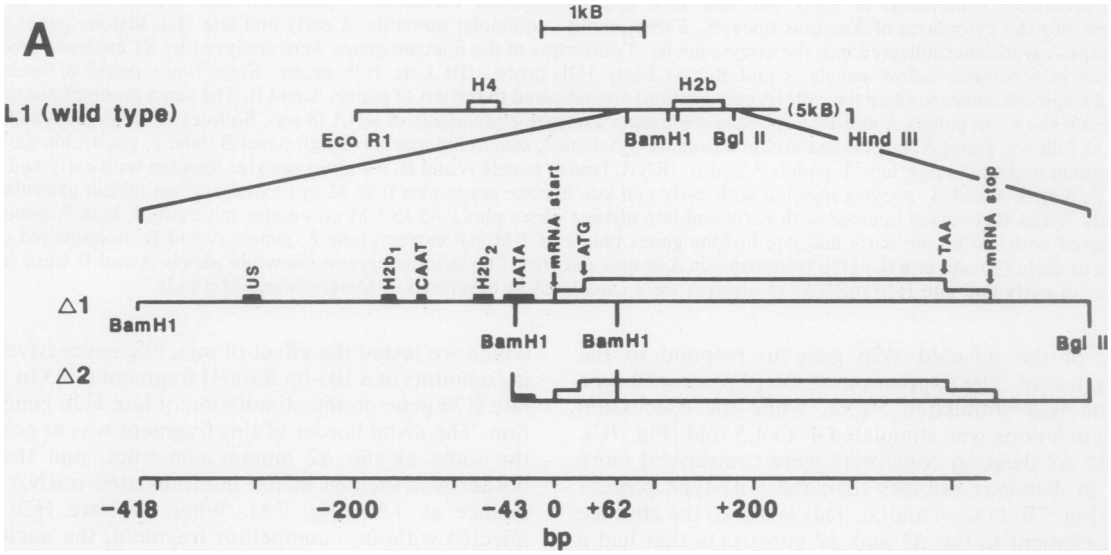


FIG. 6. Plasmid pSpEL-1 (Fig. 1) containing the early and late H2b genes in a *cis* configuration was injected into oocytes that had received a prior injection of the 0.45 to 1 M salt wash fraction or into oocytes that had not. Early and late gene products were assayed by primer extension with an end-labeled oligonucleotide that hybridizes to both early and late transcripts. Early transcripts produced a 210-nucleotide extension product, while late H2b transcripts produced a 150-nucleotide extension product. Differences in the basal level of transcription of the early and late genes are due to the lack of a negative-acting sequence at the 3′ end of the late H2b gene (see text). Lane 1, RNA from oocytes injected with plasmid pSpEL-1 with no protein. Lane 2, RNA from oocytes injected with the protein extract and pSpEL-1. E, Early; L, late.

tory effect, a factor(s) in the 0.45 to 1 M salt nuclear extract should bind specifically to this fragment. Further, if the factor was involved in the differential regulation of the early and late H2b genes, it might bind to them with different affinities.

To test these possibilities, we performed a series of DNA binding studies using gel retardation analysis (13). In preliminary experiments, we found that the *Bam*HI 105-bp fragment formed an electrophoretically distinct complex with a factor(s) in the 0.45 to 1 M NaCl extract even in the presence of a large amount of bacterial DNA or poly(dI-dC), indicating that binding is specific (data not shown). Subsequently, a nonspecific DNA competitor DNA (1 μg) was included in all binding reactions. We tested the ability of the *Bam*HI 105-bp fragment of the L1 late H2b gene, the corresponding fragment of the early H2b gene promoter, and the *tk* promoter fragment (coordinates) to compete with labeled *Bam*HI 105-bp fragment for binding activity. The results of this experiment are shown in Fig. 9.

We detected one major, slowly migrating band representing a specific DNA protein complex (labeled complex, Fig. 9). Both the late and early H2b gene fragments competed for the binding activity. A 30-fold molar excess of *Bam*HI 105-bp fragment relative to the probe eliminated the signal entirely (Fig. 9A, lane 5; Fig. 9B). A 50-fold molar excess of the early H2b fragment reduced the signal to 25% of maximum (Fig. 9A, lane 9), while a 50-fold excess of the *tk*



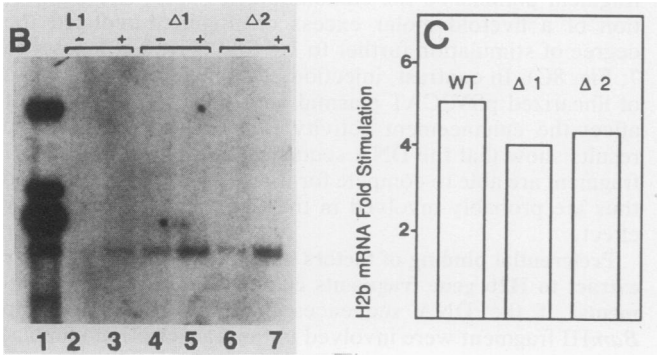


FIG. 7. DNA sequences required for the transcription-stimulatory effect. (A) Schematic maps of wild type and Δ1 and Δ2 deletion constructions. Promoter consensus sequences are indicated by solid boxes. L1 H2b DNA was cleaved at *Bam*HI sites at positions −418 and −43 and a *Bgl*II site to the right of the H2b gene (90 bp 3′). The *Bam*HI-*Bgl*II fragments were ligated into the *Bam*HI site of pUC8. (B) The effect of the stimulatory factor on these deletions was tested in the *Xenopus* oocyte assay. Levels of late H2b transcripts were monitored by primer extension. Lane 1, RNA from sea urchin gastrula; lanes 2, 4, and 6, RNA from oocytes injected with L1 and Δ1 and Δ2 constructs, respectively; lanes 3, 5, and 7; RNA from oocytes injected with the gastrula nuclear extract and subsequently with the L1 and Δ1 and Δ2 constructs, respectively. (C) Quantitation of late H2b transcript stimulation. The autoradiogram shown in panel B was scanned with a densitometer and the fold stimulation of late H2b transcript levels was calculated for the wild type and Δ1 and Δ2 constructs.

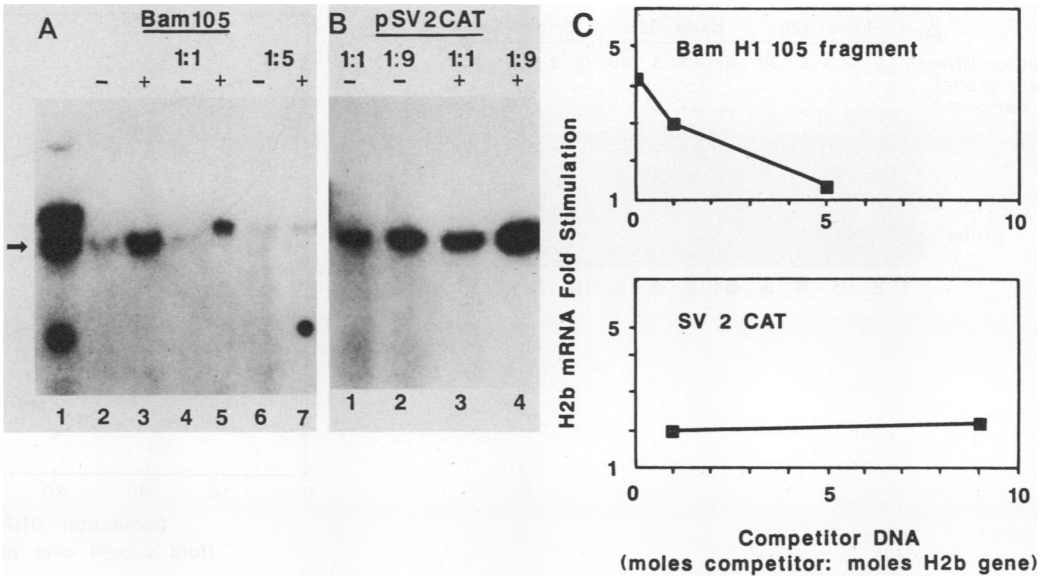


FIG. 8. The *Bam*HI 105-bp late H2b promoter fragment competes with L1 H2b gene for the stimulatory factor. (A) The 0.45 to 1 M salt extract was injected into oocytes as described in Materials and Methods. The pSpL-1 plasmid bearing the late H2b gene and the *Bam*HI 105-bp fragment were subsequently coinjected into the oocyte nuclei. Transcripts derived from the injected gene were quantitated by primer extension. Sources of RNA were as follows: lane 1, sea urchin gastrula; lanes 2, 4, and 6, oocytes injected with 1:0, 1:1, and 1:5 ratios of plasmid to competitor DNA, respectively; lanes 3, 5, and 7, oocytes injected with the gastrula nuclear extract and subsequently with DNA as in lanes 2, 4, and 6, respectively. Arrow, L1 late H2b mRNA primer extension product. (B) Control experiment showing competition by linearized pSV2CAT DNA with the L1 H2b gene for the stimulatory factor. Lane 1, Sea urchin gastrula; lanes 2 and 4, oocytes injected with the L1 H2b DNA; lanes 3 and 5, oocytes injected with the gastrula nuclear extract and subsequently with L1 H2b and pSV2CAT DNA in 1:1 and 1:9 molar ratios as in lanes 2 and 4. Note that different groups of oocytes were used for the experiments shown in panels A and B, probably accounting for the difference in the stimulation of L1 H2b transcript levels. (C) Quantitation of L1 late H2b transcript stimulation. We scanned the autoradiograms shown in panels A and B and calculated the degree of stimulation of late H2b mRNA levels as a function of the number of moles of competitor *Bam*HI 105-bp fragment (upper panel) or pSV2CAT (lower panel) DNA.

promoter fragment only reduced the signal to about 75% of maximum (Fig. 9A, lane 13). These results suggest that the factor binds to both early and late H2b histone genes more tightly than to the *tk* gene and indicate that the factor may bind to the late H2b gene more tightly than to the early H2b gene, though the difference in affinity for the early versus the late genes is relatively modest.

Abundance of *Bam*HI 105-bp DNA-binding factor decreases on a per genome basis during sea urchin development. If the *Bam*HI 105-bp binding factor was involved in the temporal regulation of early or late H2b gene expression, its intracellular concentration (or activity) should change during development in concert with the temporal changes in early or late H2b gene activity. To test this possibility, we measured the relative amounts of *Bam*HI 105-bp binding factor in embryos at different stages of development. Since it proved difficult to prepare nuclei from embryos at different developmental stages in reproducible and quantitative yields, we extracted factors from whole embryos. We found that the properties of the binding activity in whole-cell extracts were indistinguishable from those of the activity in nuclear extracts in the gel retardation assay (data not shown).

The activity of the *Bam*HI binding factor was measured in whole-cell extracts prepared from sea urchin embryos at different developmental stages by the mobility shift assay as described above. The level of this factor, expressed on a per embryo basis, was lowest in the unfertilized egg, increased through the midblastula stage, leveled off, and then increased again in mesenchyme blastula and gastrula embryos (Fig. 10A). When expressed on a per cell basis, the level of the factor was highest in the unfertilized egg, declined through cleavage and blastulation, and then began to increase at the last blastula stage (Fig. 10B).

DISCUSSION

We showed that a chromatin extract from sea urchin gastrulas stimulated early and late H2b histone gene expression in *Xenopus* oocytes. Although our data do not enable us to distinguish unambiguously transcriptional and posttranscriptional mechanisms for this stimulation, we believe that a transcriptional mechanism is the more likely. The half-lives of both early and late H2b mRNAs are approximately 3 h in oocytes (36). In our experiments, oocytes were typically incubated for 3 to 5 h after DNA injection. If the chromatin extract lengthened the early and late H2b mRNA half-life to the maximum extent possible, from 3 h to infinity, the amount of H2b mRNA accumulating during the experiment would increase only about twofold. Since in many experiments the extract stimulated both early and late H2b transcript levels much more than twofold the stimulatory effect is probably largely transcriptional.

The H2b-stimulatory factor probably is not a general transcription factor since neither the herpesvirus thymidine kinase gene nor the sea urchin Spec 1 gene responded to the extract in injected oocytes, while late H2b transcript levels were stimulated 20- to 30-fold (Fig. 3). The specificity of the activity is also supported by the observation that non-H2b DNA fragments compete poorly for the binding of factor to the *Bam*HI 105-bp fragment, the probable site of action of

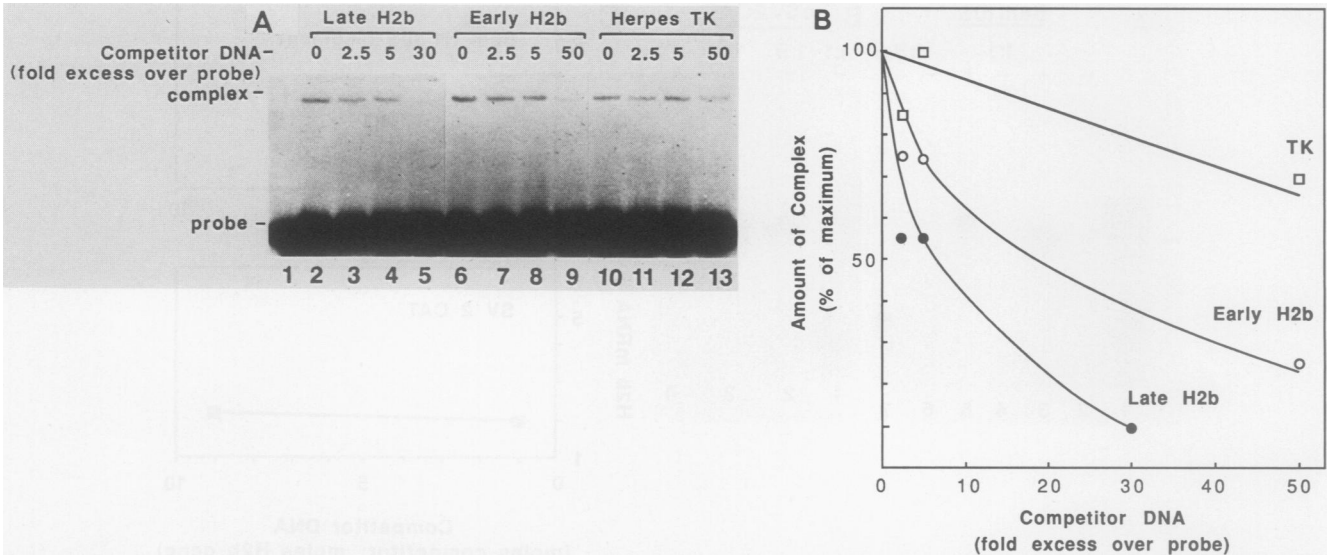


FIG. 9. Competition of early H2b, late H2b, and *tk* DNA sequences (Herpes TK) for *Bam*HI 105-bp fragment binding activity. The *Bam*HI 105-bp late H2b promoter fragment was labeled at its 3′ terminus with [^{32}P]dATP and T4 DNA polymerase. Labeled fragment (2 ng) was incubated with 1 μg of 0.45 to 1 M salt extract, 1 μg of poly(dI-dC), and a competitor fragment derived from the promoter regions of the late H2b, early H2b, or *tk* gene. The competitor fragments are described in Materials and Methods. DNA-protein complexes were resolved on a low-salt acrylamide gel and visualized by autoradiography. (A) Competition of late H2b and early H2b histone gene DNAs and *tk* promoter fragments for *Bam*HI 105-bp fragment binding activity. Lanes 1 to 5, Competition with 0, 2.5-, 5-, and 30-fold molar excess of the late H2b gene fragment; lanes 6 to 9, competition with 0, 2.5-, 5-, and 50-fold molar excess of the early gene fragment; lanes 10 to 13, competition with 0, 2.5-, 5-, and 50-fold molar excess of the *tk* gene fragment. (B) Graphic representation of panel A.

the stimulatory activity. Whether the factor is specific for H2b histone genes or affects more than one histone gene type remains to be determined.

The results of competition experiments performed under conditions of limiting factor suggest that the same factor stimulates both early and late H2b gene expression. A portion of the stimulatory activity may, however, be specific for the L1 late H2b gene, since this is not entirely eliminated by competition with a 20-fold excess of the early histone repeat unit.

When equimolar amounts of early and late H2b genes were coinjected into oocytes, the factor simulated late H2b transcript levels three to four times more than early transcript levels. This selectivity was observed whether the genes were injected on separate plasmids or closely linked on the same plasmid. Thus, the tandem repeat structure of the early histone genes does not appear to be important for the differential response of early and late H2b genes to the factor. Also, the DNA sequences required for the stimulatory effect must lie within the 1.8-kb early H2b fragment (Fig. 1). This fragment includes only about 600 bp of 5′-flanking DNA sequence.

Taken together, deletion analysis (Fig. 7) and fragment competition in the oocyte (Fig. 8) indicate that at least some

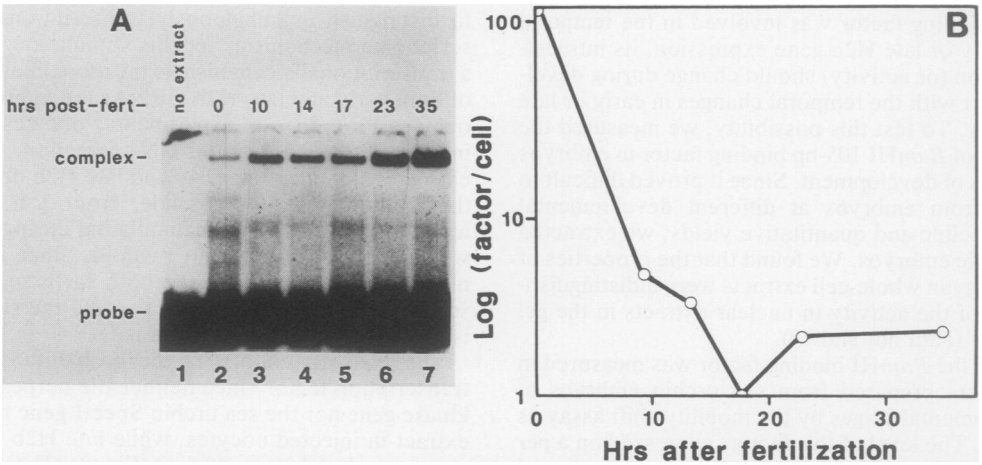


FIG. 10. Developmental profile of *Bam*HI binding factor activity. Protein was extracted from whole embryos as described in Materials and Methods and analyzed for *Bam*HI 105-bp fragment binding activity by the mobility shift assay (Fig. 9). (A) Autoradiogram of gel showing binding activity in different stages of embryogenesis: 10 h, blastula; 35 h, gastrula. Lane 1, Probe alone; lane 2, egg extract; lanes 3 to 7, extracts from various stages of sea urchin development. (B) Graph showing relative factor concentration per cell during development.

of the sequences required for the enhancement of L1 H2b transcript levels are located on a 105-bp *Bam*HI fragment that spans a region of the L1 H2b gene between −43 and +62. A gel retardation assay showed that a factor(s) present in the 0.45 to 1 M NaCl extract bound specifically to this fragment (Fig. 9), whereas neither the 0.45 M nor the 2 M salt extract contained detectable binding activity (26). This DNA-binding protein is a likely candidate for the transcription-stimulatory factor.

The 105-bp *Bam*HI fragment contains the TATA box and a closely associated upstream sequence of 26 bp together referred to as the TATA element (Maxson et al., in press). In addition, it contains the 28-bp mRNA leader sequence and 34 bp of protein-coding sequence. Although we do not know where on this fragment the factor(s) binds, the TATA element is a likely site. Comparisons of 5′-flanking regions of several sea urchin histone genes showed that the TATA element, having the consensus sequence TTGGATCCC GGCGNNTGATATAAATA, is the only conserved sequence motif in this region (Maxson et al., in press). Interestingly, this sequence differs in 4 of 26 positions between early and L1 late H2b genes. These differences could cause a factor to bind to the two genes with different affinities and thus lead to the differential stimulation of early and late H2b transcription.

Gel retardation experiments showed that the *Bam*HI 105-bp factor binds to both early and late H2b promoters substantially more tightly than to the *tk* promoter. This is consistent with lack of response of the *tk* gene to the factor in *Xenopus* oocytes and supports the view that the *Bam*HI 105-bp factor is not a general transcription factor. The gel retardation data further suggest that the *Bam*HI 105-bp factor binds more tightly to the late H2b promoter than to its early counterpart, although we stress that a quantitatively rigorous analysis is required to confirm this result.

The differential activity of the factor on early and late H2b genes is consistent with a model proposed to explain 5S rRNA gene regulation by the transcription factor TfIIIA in developing *Xenopus* embryos. Like the sea urchin histone gene family, the *Xenopus* 5S gene family consists of two subfamilies differing greatly in gene copy oogenesis and early development. The somatic subfamily is reiterated at a much lower frequency and is expressed throughout development and in the adult. The TfIIIA factor binds with a fourfold-higher affinity to the somatic gene subfamily than to the oocyte gene subfamily. This difference in affinity, together with a decline in the intranuclear concentration of TfIIIA during development, has been proposed as an explanation for the inactivation of the oocyte 5S genes in late-stage embryos (5, 37). In support of such a model, we found that the stimulatory factor acts selectively on the late H2b genes. Moreover, the amount of *Bam*HI 105-bp binding factor activity decreased during development on a per nucleus basis, like the concentration of TfIIIA during *Xenopus* development.

On the other hand, the 5S model predicts that late H2b genes, like somatic 5S genes, should be transcribed at a constant rate throughout development, which is not the case. Transcription rate measurements in vivo and in isolated nuclei have documented a three- to fivefold increase in the rate of late histone gene transcription in late blastula sea urchin embryos (23; Ito et al., submitted), and this increase cannot be accounted for by a factor behaving like TfIIIA. Thus, although the 5S model does provide an explanation for the inactivation of early histone genes in late-stage embryos, it is too simple to explain as well the activation of late histone genes. Rather, the temporal regulation of early and late histone gene expression is better explained by a two-factor model in which one factor functions like TfIIIA to reduce early gene expression in late-stage embryos and a second factor stimulates late gene expression in late-stage embryos.

Mous et al. (30) showed that a 0.3 M salt extract of sea urchin (*Psammechinus miliaris*) chromatin stimulates expression of early H2b histone genes in *Xenopus* oocytes. (Late histone genes were not tested.) Several differences in the experimental approach used by these workers, including the species of sea urchin and the stage at which the extracts were prepared, make it impossible to know now whether the activity they detected is the same as the one we describe. Perhaps the strongest evidence that the two activities are not identical is that they elute from chromatin at significantly different salt concentrations (0.3 versus 0.45 M). On the other hand, they seem to require similar regions of the genes for the enhancement of gene expression. Regardless of whether these activities prove to be identical, we emphasize that our observations are unique in two respects. (i) We showed that the sea urchin nuclear extract stimulates expression of both early and late H2b genes, the late H2b gene to a greater extent than its early counterpart; (ii) a DNA fragment that competes for the stimulatory factor in the oocyte also bind specifically to a protein in the 0.45 to 1 M salt extract. This latter observation may provide this basis of a simple assay for the stimulatory factor that may ultimately enable us to purify it.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants DH 18582 to R.M. and GM 34103 to L.D. E. from the National Institutes of Health and grant NP584 to L.D.E. from the American Cancer Society. M.I. and F.L. were supported by a fellowship from the California Foundation for Biomedical Research, and S.B. was supported by a Rosalie B. Hite Foundation fellowship.

We thank Fred Wilt for a critical review of this manuscript.

LITERATURE CITED

1. **Berk, A., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. Cell **12**:721–732.
2. **Bogenhagen, D., S. Sakonju, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. II. The 3′ border of the region. Cell **19**:27–35.
3. **Bonner, W.** 1975. Protein migration into nuclei. I. Frog oocyte nuclei accumulate microinjected histones, allow entry to small proteins, and exclude larger proteins. J. Cell Biol. **64**:421–430.
4. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Brown, D. D.** 1984. The role of stable complexes that repress and activate eukaryotic genes. Cell **37**:359–365.
6. **Childs, G., C. Nocente-McGrath, T. Lieber, C. Holt, and J. Knowles.** 1982. Sea urchin (*Lytechinus pictus*) late stage histone H3 and H4 genes: characterization and mapping of a clustered but non-tandemly linked multigene family. Cell **31**:383–393.
7. **Cohen, L., K. M. Newrock, A. Zweidler.** 1975. Stage specific switches in histone synthesis during embryogenesis of the sea urchin. Science **190**:194–197.
8. **Engelke, D. R., S. Y. Ng, B. S. Shastry, and R. G. Roeder.** 1980. Specific interaction of a purified transcription factor with an internal control region of 5S RNA genes. Cell **19**:717–728.
9. **Etkin, L. D.** 1982. Analysis of the mechanisms involved in gene regulation and cell differentiation by microinjection of purified genes and somatic cell nuclei into amphibian oocytes and eggs. Differentiation **21**:149–159.

10. **Etkin, L. D., and M. A. DiBerardino.** 1983. Expression of nuclei and purified genes microinjected into oocytes and eggs, p. 127–156. *In* N. Maclean, S. Gregory, and R. A. Flavell (ed.), Eukaryotic genes, their structure, activity and regulation. Butterworths, London.
11. **Etkin, L. D., and R. E. Maxson.** 1980. The synthesis of authentic sea urchin transcriptional and translational products by sea urchin histone genes injected into *Xenopus laevis* oocytes. Dev. Biol. **75**:13–25.
12. **Etkin, L., B. Pearman, M. Roberts, and S. Bektesh.** 1984. Replication, integration, and expression of exogenous DNA injected into fertilized eggs of *Xenopus laevis*. Differentiation **26**:194–202.
13. **Fried, M., and D. Crothers.** 1980. Equilibria and kinetics of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
14. **Gargiulo, G., F. Razvi, and A. Worcel.** 1984. Assembly of transcriptionally active chromatin in *Xenopus* oocytes requires specific DNA binding factors. Cell **38**:511–521.
15. **Graves, B. J., P. F. Johnson, and S. L. McKnight.** 1986. Homologous recognition of a promoter domain common to the MSVLtr and HSVtk gene. Cell **44**:565–576.
16. **Grosschedl, R., and M. L. Birnstiel.** 1980. Identification of regulatory sequences in the prelude sequences of an H2A histone gene by the study of specific deletion mutants *in vitro*. Proc. Natl. Acad. Sci. USA **77**:1432–1436.
17. **Halsell, S., M. Ito, and R. Maxson.** 1987. Differential expression of early and late embryonic histone genes in adult tissues of the sea urchin *Strongylocentrotus purpuratus*. Dev. Biol. **119**:268–274.
18. **Hardin, S., C. D. Carpenter, D. E. Hardin, A. M. Bruskin, and W. H. Klein.** 1985. Structure of the Spec 1 gene encoding a major calcium-binding protein in the embryonic ectoderm of the sea urchin, *Strongylocentrotus purpuratus*. J. Mol. Biol. **186**: 243–255.
19. **Harvey, R. P., A. J. Robbins, and J. R. E. Wells.** 1982. Independently evolving chicken H2b genes: identification of ubiquitous H2b-specific 5′ element. Nucleic Acids Res. **10**: 7851–7863.
20. **Hinegardner, R. T.** 1967. Echinoderms, p. 139–155. *In* F. Wilt and N. Wessels (ed.), Methods in developmental biology. Crowell-Collier, New York.
21. **Kaumeyer, J. F., and E. S. Weinberg.** 1986. Sequence, organization and expression of late embryonic H3 and H4 histone genes from the sea urchin, *Strongylocentrotus purpuratus*. Nucleic Acids Res. **14**:4557–4576.
22. **Kedes, L. H.** 1979. Histone genes and histone messengers. Annu. Rev. Biochem. **48**:837–870.
23. **Knowles, J., and G. Childs.** 1984. Temporal expression of late histone messenger RNA in the sea urchin *Lytechinus pictus*. Proc. Natl. Acad. Sci. USA **81**:2411–2415.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Mauron, A., L. Kedes, B. Hough-Evans, and E. H. Davidson.** 1982. Accumulation of individual histone mRNAs during embryogenesis of the sea urchin *Strongylocentrotus purpuratus*. Dev. Biol. **94**:425–434.
26. **Maxson, R. E., M. Ito, S. Balcells, M. Thayer, and L. Etkin.** 1987. Stimulation of sea urchin early and late histone gene expression in *Xenopus* oocytes by a gastrula nuclear extract, p. 253–265. *In* R. Firtel and E. Davidson (ed.), Molecular approaches to development. Alan R. Liss, Inc., New York.
27. **Maxson, R. E., T. J. Mohun, G. Gormezano, G. Childs, and L. H. Kedes.** 1983. Distinct organizations and patterns of expression of early and late histone gene sets in the sea urchin. Nature (London) **301**:120–125.
28. **Maxson, R. E., and F. H. Wilt.** 1981. The rate of synthesis of histone mRNA during development of sea urchin embryos (*Strongylocentrotus purpuratus*). Dev. Biol. **83**:380–386.
29. **Mohun, T. J., R. E. Maxson, G. Gormezano, and L. Kedes.** 1985. Differential regulation of individual late histone genes during development of the sea urchin *Strongylocentrotus purpuratus*. Dev. Biol. **108**:491–502.
30. **Mous, J., H. Stunnenberg, O. Georgiev, and M. Birnstiel.** 1985. Stimulation of sea urchin H2B histone gene transcription by a chromatin-associated protein fraction depends on gene sequences downstream of the transcription start site. Mol. Cell. Biol. **5**:2765–2769.
31. **Overton, C., and E. Weinberg.** 1978. Length and sequence heterogeneity of the histone gene repeat unit of the sea urchin. Cell **14**:247–257.
32. **Ruderman, J. V., and P. R. Gross.** 1974. Histones and histone synthesis in sea urchin development. Dev. Biol. **36**:286–298.
33. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.
34. **Stunnenberg, H., and M. Birnstiel.** 1982. Bioassay for components regulating eukaryotic gene expression: a chromosomal factor involved in the generation of histone mRNA 3′ termini. Proc. Natl. Acad. Sci. USA **79**:6201–6204.
35. **Weinberg, E. S., M. B. Hendericks, K. Hemminki, P. E. Kuwabara, and L. A. Farrelly.** 1983. Timing and rates of synthesis of early histone mRNA in the embryo of *Strongylocentrotus purpuratus*. Dev. Biol. **98**:117–129.
36. **Woodland, H. R., and F. H. Wilt.** 1980. The functional stability of sea urchin histone mRNA injected into oocytes of *Xenopus laevis*. Dev. Biol. **75**:199–211.
37. **Wormington, W. M., D. F. Bogenhagen, E. Jordan, and D. Brown.** 1981. A quantitative assay for *Xenopus* 5S RNA gene transcription *in vitro*. Cell **24**:809–817.