Proc. Natl. Acad. Sci. USA
Vol. 77, No. 3, pp. 1432–1436, March 1980
Biochemistry

Identification of regulatory sequences in the prelude sequences of an H2A histone gene by the study of specific deletion mutants *in vivo*

(DNA manipulation/*Xenopus* oocyte injection/surrogate genetics/H2A mRNA synthesis/nuclease S1 mapping)

R. GROSSCHEDL AND M. L. BIRNSTIEL

Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland

Communicated by A. Frey-Wyssling, December 26, 1979

ABSTRACT **Conserved DNA sequence elements of putative regulatory functions were deleted from the prelude region of a sea urchin H2A histone gene. For this, the wild-type H2A gene of the 6-kilobase histone DNA repeat unit was replaced by various mutant H2A genes by cloning. The effects of the manipulation on H2A mRNA synthesis were studied by injection of the mutant DNAs into centrifuged *Xenopus* oocytes. The unmanipulated H2B gene residing within the same repeat unit provided a suitable internal control for these studies. Deletion of the T-A-T-A-A-A-T-A motif, once thought to be the functional equivalent of the bacterial Pribnow box, did not abolish transcription of the gene; instead, a number of novel mRNA 5′ termini were generated. We argue that the T-A-T-A-A-A-T-A motif is a specificity element, a selector of eukaryotic gene transcription. Deletion of the "cap-sequence," 5′ pyrimidine-C-A-T-T-C-purine 3′ and most of the mRNA leader sequence did not abolish transcription but created yet another mRNA 5′ terminus. In contrast to these deletions, which are both down-mutations, deletion of H2A gene-specific conserved DNA sequences upstream from the T-A-T-A-A-A-T-A motif enhanced mRNA synthesis. A hypothesis for the function of these DNA sequences as eukaryotic promoter elements is discussed.**

Active genes in eukaryotes reside in chromatin of distinct molecular structure, and consequently chromatin conformation may play a major role in the control of gene expression and cell differentiation (cf. ref. 1). Perhaps a more simple aspect of gene regulation is the control of initiation and termination of transcription of the active gene. We consider the investigation of these processes as the first necessary step in unravelling the mechanisms involved in differentiation. The histone genes provide an ideal material for such studies because they form a multigene family whose expression is developmentally controlled. In the sea urchin *Psammechinus miliaris* the genes coding for the five histone proteins alternate with spacer DNA and are contained in the DNA repeat unit of approximately 6 kilobase (kb) pairs. A histone DNA repeat, termed h22, has been cloned; most of its nucleotide sequence has been determined (2, 3); and the gene arrangement, direction of transcription, and developmental stage at which this gene repeat is expressed have been determined (4–6).

The DNA sequences encoding the histone mRNA 5′ and 3′ termini are of particular interest in that they mark gene boundaries. The 5′ termini of the histone mRNAs were shown to be located near or within a pentameric consensus sequence 5′ pyrimidine-C-A-T-T-C-purine 3′ called "cap-sequence" (7), 25 base pairs (bp) downstream from a conserved decameric DNA sequence 5′ G-T-A-T-A-A-A-T-A-G 3′ referred to here as the "Hogness box" (8). Nine nucleotides upstream there is a pentameric consensus sequence 5′ G-A-T-C-C 3′ (3). In addition to these conserved DNA sequences, which are common to all known histone genes, there is a 30-nucleotide-long DNA block upstream of the histone H2A structural gene that has been conserved in distantly related histone gene repeats (3). In this paper this block is referred to as the "H2A gene specific conserved sequence."

Because the classical genetical approach cannot be used for the elucidation of the regulatory elements controlling these histone genes, we sought to identify them by surrogate genetics techniques (9) involving DNA sequence manipulation followed by oocyte injection of the altered sequences. Here we show that biochemical manipulation of the DNA sequences preceding the H2A gene has marked effects on both the rate of transcription and the nature of the H2A mRNA made, when the manipulated histone gene repeat units are presented to the living oocyte nucleus of *Xenopus laevis* for interpretation.

MATERIALS AND METHODS

Enzymes and Reagents. Phage T4 polynucleotide kinase was obtained from P-L Biochemicals, and Klenow DNA polymerase, calf intestine alkaline phosphatase, and *Eco*RI restriction endonuclease were purchased from Boehringer. Other restriction enzymes were obtained from New England BioLabs. T4 DNA ligase was obtained from New England BioLabs and from Miles.

Construction of Specific Deletion Mutants. Because of the complexity of restriction sites in the 6-kb h22 DNA unit, the construction of specific deletion mutants was subdivided into three steps. *Step 1*: h22 DNA was cleaved with *Xho* I and subsequently with *Taq* I (see Fig. 1). The two *Taq* I fragments linked to the *Xho* I restriction site were isolated and ligated via their *Taq* I ends to plasmid pBR322 DNA that was linearized with *Cla* I. After transfection of *Escherichia coli* HB101 cells, colonies hybridizing with nick-translated h22 DNA were further characterized by restriction analysis (data not shown). The resulting clone pBRH2A-3 contains a single H2A gene with the *Taq* I site at position −184 of the H2A gene residing near the *Eco*RI site of pBR322 DNA. *Step 2*: For the deletion of the B segment in the prelude of the H2A gene, pBRH2A-3 DNA was cleaved with *Hpa* II. After separation of the fragments on 1.5% agarose gels the 1.3-kb fragment and the mixture of two coincident 0.54-kb DNA fragments were isolated and purified. The staggered ends of the latter fragments were filled in with Klenow DNA polymerase as described (10). The now flush-ended 0.54-kb *Hpa* II fragments were digested with *Eco*RI and the 80-bp *Eco*RI/*Hpa* II (flush-end) fragment was selected by electrophoresis on a 6% polyacrylamide gel. The 1.3-kb fragment (see above) was cleaved with *Xho* I. Because of the presence of two *Hin*cII sites at positions −74 and −15 (the second also being a *Hpa* I site; Fig. 1) a partial *Hin*cII digest was made to obtain the desired *Hin*cII (position −74)/*Xho* I fragment. After ligation of the 80-bp *Eco*RI/*Hpa* II fragment and the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase; bp, base pairs.

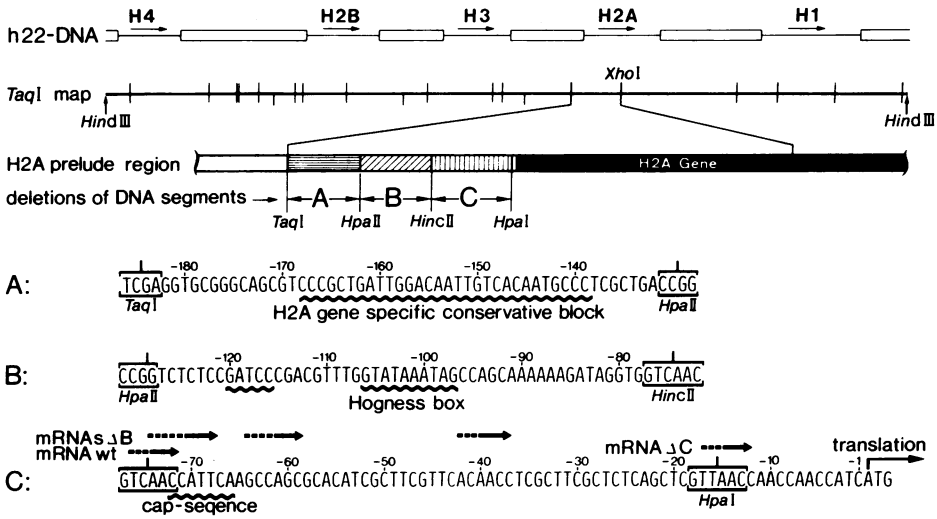

FIG. 1. Structure of the 6-kb repeat unit h22 of sea urchin histone DNA. The structural map of the prelude region of the H2A gene is shown in the context of the h22 DNA. Conserved sequence motifs (3) are underlined. Sites for the restriction enzymes defining the boundaries of segments A, B, and C are indicated, and the DNA sequences are denoted (cf. ref. 2). The approximate map positions of the 5′ termini of the H2A mRNAs are indicated. wt, Wild type.

263-bp *Hin*cII/*Xho* I fragment, the ligated fragments were cleaved with *Hpa* II, *Eco*RI, and *Xho* I and the reaction products were separated on a 6% polyacrylamide gel. The fusion product of the 263-bp and the 80-bp fragment in the correct orientation was identified, isolated, purified, and inserted into an *Eco*RI- and *Xho* I-cleaved pBRH2A-3 plasmid, from which the 0.4-kb *Eco*RI/*Xho* I fragment had been removed. Deletion clone pBRH2A-ΔC was produced in an analogous manner by fusion of the *Hin*cII site (−74) and the *Hpa* I site (−15). *Step 3*: For the construction of the 6-kb h22 ΔB clone, pBRH2A-ΔB DNA was digested with *Eco*RI and *Xho* I to reclaim the manipulated DNA section. After isolation of the 343-bp DNA fragment, this *Eco*RI/*Xho* I fragment was further cleaved with *Taq* I and the reaction products were separated on a 6% polyacrylamide gel. The desired 320-bp *Taq* I/*Xho* I fragment was isolated, purified, and used as component *c* (see below). A component *a* was prepared that consisted of the left portion of the h22 DNA upstream of the *Taq* I site (−184; see Fig. 1). It was obtained by digestion of h22 DNA with *Xho* I, followed by a partial cleavage with *Taq* I. Component *b* represents the right hand portion of the h22 DNA downstream of the *Xho* I restriction site already linked via the terminal h22 *Hin*dIII site to pBR322 DNA as a cloning vector. Components *a*, *b*, and *c* were ligated and, after transfection, ampicillin-resistant colonies were grown and analyzed by restriction endonuclease analysis. The deletion mutant clones pBRh22-ΔC and pBRh22-ΔA were constructed in a similar way but using the *Taq* I/*Xho* I fragment of pBRH2A-ΔC and the *Hpa* II/*Xho* I fragment of pBRH2A-3, respectively, as component *c*. The containment conditions used for the construction and amplification of the recombinant plasmids were those specified by the National Institutes of Health Guidelines (P2, EK1).

Preparation of Circularized h22 DNAs and Determination of Nucleotide Sequence. Purified 6-kb DNA was circularized by ligation at a DNA concentration of 0.75 μg/ml with T4 DNA ligase at 0.03 unit/ml as described (10). The 5′ end-labeling of DNA fragments and the chemical partial cleavage reactions were performed as described (11).

DNA Injection and RNA Analysis. Four nanograms of 6-kb DNA circles was injected together with 0.2 or 0.5 μCi (1 Ci = 3.7×10^{10} becquerels) of [α-^{32}P]GTP (Amersham) per oocyte. The incubation time was usually 16–20 hr. The oocytes were homogenized and the RNA was extracted (12, 13).

Nuclease S1 Mapping of the H2A mRNA 5′ Termini. For the S1 mapping described by Berk and Sharp (14) the *Taq* I/*Xho* I fragments of the h22 wild-type DNA and the DNA of the deletion mutants h22 ΔB and h22 ΔC were labeled at their *Xho* I sites and hybridized to 5–14S RNA obtained from injected oocytes.

RESULTS

Experimental Design. In evolutionary terms, the DNA sequences flanking the histone structural genes consist of a series of conserved sequences hyphenated by divergent sequences (3). Fortuitously, known restriction sites fall into the nonconserved areas of the H2A gene prelude and can be used to remove the conserved sequence motifs from the histone DNA as intact units. Deletions created by excision and removal of short restriction endonuclease fragments yield mutants of known sequence. Furthermore, short internal sections of any area containing restriction sites can be easily removed. Finally, the availability of short restriction fragments of known function makes possible the manufacture of inversion mutants and the reconstruction of functional artificial gene units from regulatory elements of defined function.

Because we have shown previously that the H2B and the H2A genes are both faithfully expressed when the histone gene repeat h22 is injected as covalently circular DNA into the oocyte nucleus of *X. laevis* (12, 13), we have a functional *in vivo* system to test the expression of the DNAs that have been modified in the above ways. Because we wished to measure both quantitative and qualitative effects of the sequence deletions on the expression of the H2A gene surrounded by its natural genetic background, the manipulated H2A genes were reintroduced into the 6-kb unit in the correct context and without the addition of any foreign DNA (see Fig. 1). This procedure enabled us to compare the expression of the manipulated H2A gene with that of the wild-type H2B gene residing within the same molecule. Because the histone DNA was injected into the oocyte as a covalently circular 6-kb DNA unit, free of any plasmid DNA, difficulties owing to the read-through of polymerases from the foreign vector DNA obviously could not arise.

Construction of Deletions Mutants. The structural map and sequence of the prelude and spacer regions of the H2A gene are shown in Fig. 1. Small deletions of 55, 54, and 59 bases were produced by removing DNA segments delimited by the re-

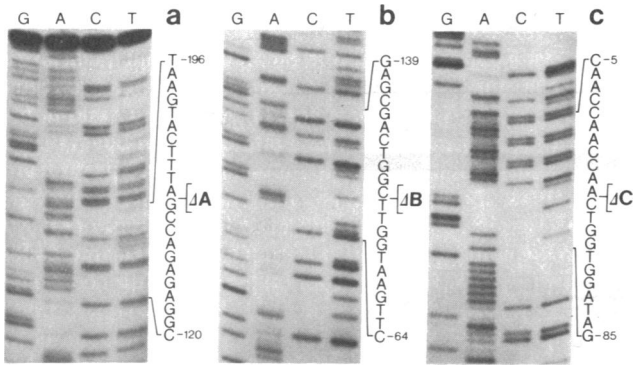

FIG. 2. Sequence gels showing the boundaries of the deletions A, B, and C. The nucleotide sequences of h22 ΔA (**a**) and h22 ΔB (**b**) were determined from the *Hpa* I restriction site (−15; Fig. 1). For the sequence analysis of h22 ΔC the *Hpa* II restriction site at −130 was end-labeled (**c**). The positions of the deleted sections are indicated. The numbering of the nucleotides is that of the wild-type sequence and corresponds to that given in Fig. 1.

striction sites *Taq* I, *Hpa* II, *Hin*cII, and *Hpa* I. In this way the H2A gene-specific conserved 30-nucleotide block of DNA could be removed (deletion A; sequence −184 to −130). A sequence containing the pentameric conserved sequence G-A-T-C-C and the Hogness box (deletion B; −128 to −75) and a third DNA segment that contains the cap-sequence and most of the DNA template for the mRNA leader (deletion C; −74 to −16) could be removed from the H2A gene. The deletions of the DNA segments A, B, and C were confirmed by nucleotide sequencing as shown in Fig. 2.

Expression of Manipulated Histone Gene Repeats in the Frog Oocytes. All modified and wild-type h22 DNAs were freed of all vector DNA, circularized, and injected into the nuclei of centrifuged oocytes together with labeled GTP (12, 13). The gel pattern of total RNA produced after injection of wild-type and mutant DNA is shown in Fig. 3. Newly synthesized endogenous *Xenopus* RNA species were found mainly near the top of the gel. Their mobilities remained unaffected by our manipulations. Further down on the gel prominent H2A and H2B histone mRNAs of the sea urchin are clearly seen for injected wild-type h22 DNA (Fig. 3, slot b). After deletion of the cap-sequence and most of the mRNA leader template

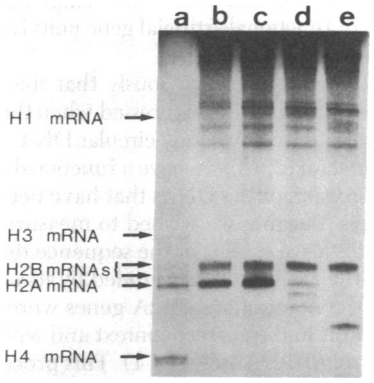

FIG. 3. Gel analysis of total RNA obtained from oocytes injected with wild-type and deletion mutant h22 DNAs together with [α-^{32}P]GTP. In contrast to previous publications (12, 13), the histone DNA transcripts were not selected by hybridization to h22 DNA. Slot **a**, ^{3}H-labeled "9S RNA" from cleaving sea urchin embryos. Note the polymorphism of the natural H2B mRNAs. The H2B mRNA produced by h22 comigrates with the slower component (cf. ref. 13). Slot **b**, RNA from oocytes injected with h22 wild-type DNA. Slot **c**, RNA from h22 ΔA DNA. Slot **d**, RNA from h22 ΔB DNA. Slot **e**, RNA from h22 ΔC DNA. The amount of RNA in each slot corresponds to a sample of 0.5–1 oocyte. Exposure to x-ray film was for 8 hr.

(deletion C) a shorter, novel H2A mRNA was produced (Fig. 3, slot **e**), which hybridized quantitatively to recombinants containing the H2A single gene alone (results not shown). By comparison with the control H2B mRNA in the same slot, it can be estimated that the incorporation of radioactivity into this novel H2A mRNA was reduced to about 1/4 (see Table 1). After injection of the deletion B DNA, three size-classes of H2A mRNAs were obtained (Fig. 3, slot **d**), two of them clearly smaller than wild-type H2A mRNA. All of them hybridized to the H2A single gene clone (data not shown). The radioactivity summed over these three classes of RNA species amounted to about 1/5 of the H2B control value (for calculation see Table 1). Deletion of the conserved H2A gene-specific block further upstream (deletion A) enhanced synthesis of the H2A mRNA, by a factor of about 2 (Table 1), and did not alter the electrophoretic mobility of the mRNA.

Mapping of the 5′ Ends of the H2A mRNAs. Because the migration pattern of the H2A mRNAs derived from the deletion mutants was changed, we wished to define these novel RNA species by mapping their 5′ ends onto their cognate template DNA. As a control, RNA from cleavage-stage sea urchins was hybridized to a DNA fragment that was obtained from the wild-type h22 DNA and was delimited on the left by the *Taq* I restriction site (−184; Fig. 1) and on the right by the 5′-^{32}P-labeled *Xho* I restriction site. S1 trimming of the hybrids generated labeled DNA fragments that, when compared to the nucleotide sequence ladder derived from the appropriate DNA, comigrated with nucleotides −75 and −77, respectively, in accordance with previous results (ref. 7; see Fig. 4 **a** and **d**). The H2A mRNA synthesized in the *Xenopus* oocyte nucleus after injection of sea urchin wild-type h22 histone DNA mapped to the same position (Fig. 4**b**). Hence, the *Xenopus* oocyte produces H2A mRNA with faithful 5′ ends.

In interpreting the S1 protection experiments, a correction of one base relative to the DNA sequence has to be made (cf. ref. 7). Therefore, the H2A mRNA 5′ ends map at positions −74 and −76 of the template sequence. Furthermore, S1 cleavage commonly leaves an overhang of one to five nucleotides, usually seen as a series of closely staggered DNA bands (7). Both the sea urchin H2A mRNA produced *in vivo* and the H2A mRNA produced in the frog oocyte from the wild-type h22 DNA yielded two protected DNA bands, spaced two nucleotides apart. Because no intervening band could be seen this pattern may reflect a genuine heterogeneity of 5′ termini. The positions (uncorrected) of the termini of the S1-protected DNA fragments are indicated in Fig. 4 **d** and **f**. For the mapping of the H2A mRNAs derived by transcription of deletion B and deletion C DNA, the cognate templates were used. In the case of the deletion B mutant an obvious heterogeneity of the H2A mRNA 5′ termini can be observed (Fig. 4**c**). Two features of this heterogeneity should be pointed out. The 5′ ends of the

Table 1. H2A mRNA synthesis for injected wild-type and mutant DNAs

Injected DNA	H2A mRNA/H2B mRNA
Wild-type	1.0
h22 ΔA	1.9
h22 ΔB	0.22
h22 ΔC	0.24

The autoradiogram shown in Fig. 3 (slots b–e) was traced densitometrically. The densitogram was calibrated against H2B mRNA as an internal control that is unaffected by the deletions in the prelude of the H2A histone gene. The values shown have been standardized by setting the ratio of H2A mRNA/H2B mRNA for the wild-type h22 DNA as 1. The ratios obtained proved to be highly reproducible.

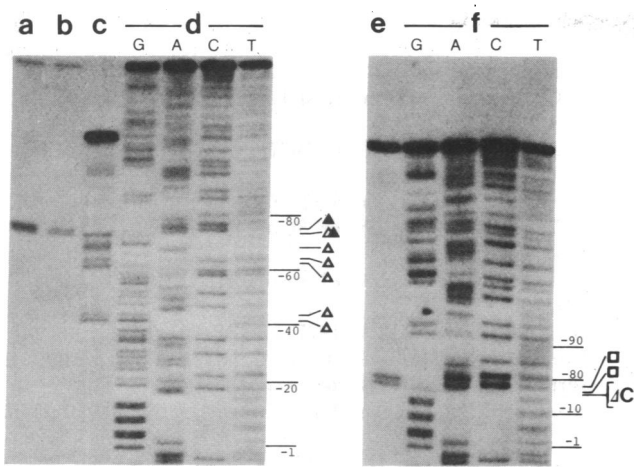

FIG. 4. S1 mapping of the 5′ termini of H2A mRNAs. Histone mRNAs from the sea urchin and from injected *Xenopus* oocytes were hybridized to their cognate DNAs, ^{32}P-labeled at the *Xho* I site (see Fig. 1). The S1-protected DNA fragments were prepared (7, 14) and electrophoresed with the sequence ladder of the wild-type h22 DNA (slots **d**) and h22 ΔC DNA (slots **f**). The numbering of the sequences is that shown in Fig. 1. In slots **a**–**c** and **e** the RNA used for the S1 protection experiment was sea urchin histone mRNA, wild-type histone h22 RNA, h22 ΔB RNA, and h22 ΔC RNA, respectively. Symbols at the side of the sequence ladders denote the uncorrected 5′-terminus map positions: ▲ of H2A mRNA of the sea urchin and the wild-type h22 oocyte transcripts; △ of h22 ΔB; and □ of h22 ΔC transcripts.

H2A mRNAs from the deletion B mutant DNA fall into three major subclasses, which presumably correspond to the three mRNAs seen in Fig. 3, slot **d**. The 5′ ends of the first subclass map to positions −74 and −70 with a distribution of closely spaced protected DNA fragments around position −70, which might indicate a microheterogeneity of the H2A mRNA 5′ termini. The second and third subclass of the 5′ ends consists of rather discrete components mapping at position −65, −61 and −42, −40, respectively. An intriguing feature is that the novel predominant 5′ termini are confined within the DNA segment −74 to −40. As seen in Fig. 4**e**, the map positions of the 5′ termini of H2A mRNA derived from deletion C DNA resemble the wild-type situation in that no striking heterogeneity is found. The 5′ terminus of this RNA maps on the cognate DNA at positions −17 and −15.

DISCUSSION

In tRNA and 5S RNA genes, both of which are transcribed by RNA polymerase III, most or all of the prelude regions can be removed and yet these genes can be transcribed rapidly within the oocytes or in oocyte extracts (10, 15, 16). Significantly, the prelude regions to these genes show little or no conservation during evolution (17–19). This is in marked contrast to the genes served by RNA polymerase II, in which there is conservation of sequences in the prelude regions (3). Increasingly, it is suspected that these conserved sequences play a role in the regulation of gene expression.

The T-A-T-A-A-A-T-A sequence motif, the so-called Hogness box, has been most prominent in this discussion, and its similarity to the bacterial Pribnow box has been noted (cf. ref. 8). It may be significant for the function of the T-A-T-A-A-A-T-A signal that it maps at a more or less constant distance from the 5′ end of mRNAs (8, 20). Because the T-A-T-A-A-A-T-A sequence is not found in the prelude of some viral genes (cf. ref. 20), the generation of the 5′ ends of mRNAs is obviously not inextricably correlated with the presence of this sequence motif. However, as has been pointed out, those viral wild-type genes that lack the Hogness box generate mRNAs with 5′-terminal sequence heterogeneity *in vivo* (20).

In the case of the deletion B mutant three relatively discrete size classes of mRNAs are recovered. This indicates that the DNA sequence encoding the mRNA leader contains secondary and apparently redundant signals for the synthesis of 5′ termini of the mRNA. These signals are usually repressed in the wild-type, because these novel 5′ termini cannot be detected in either oocyte transcripts of wild-type h22 DNA or RNA isolated from sea urchin embryos (see Fig. 4 **a**–**c**). Thus, deletion of segment B creates a situation similar to that observed in normal viral genomes: in the absence of the T-A-T-A-A-A-T-A motif mRNA is still produced but shows 5′ terminal heterogeneity. From these considerations, we propose that fragment B as a whole, or because of the conservative sequences it contains, is most likely instrumental in selecting a unique 5′ mRNA terminus out of several possible sites. It should be noted that, in clones in which the cap-sequence has been deleted, a single species of H2A mRNA is still synthesized. By contrast, the absence of a similar "cap-site" of a late simian virus 40 gene in the naturally occuring deletion mutant A1 1811 caused the appearance of several, presumably interrelated, cap structures (21). The basic difference in the response to a cap-site deletion in these two cases may arise from the fact that the simian virus 40 late gene does not have a T-A-T-A-A-A-T-A motif, whereas such a sequence is left unchanged in the deletion mutant C.

A common feature of deletion mutants A, B, and C is that the rate of transcription is changed by a relatively small factor. Reproducibly, deletion A enhances transcription of the H2A gene and is therefore an up-mutation (Table 1). Its function in the sea urchin could be the modulation of the rate of H2A gene transcription. Both B and C deletions are down-mutations by a factor of 5 and also have specific effects on the mRNA structures, as discussed above. The strongest (factor 15) down-mutation that we have observed to date (unpublished results) was created by deletion of a 300-bp section of the A+T-rich spacer DNA preceding the H2A gene-specific conserved sequence. The question remains open whether this DNA segment is polar in function and contains important sequence motifs.

There is increasing evidence that the histone genes are transcribed monocistronically in both *Drosophila* (22) and humans (23). The same appears to be true for the sea urchin, because our attempts to detect *in vivo* transcripts by using specific spacer DNA probes have failed (7). Sea urchin histone mRNAs are capped (24, 25). RNA capping and initiation of transcription are closely related phenomena. This has led to the "cap–promoter hypothesis" (26), in which promotion and capping are thought to be compulsorily coupled with one another. From this one would expect that removal of the sequence coding for the normal mRNA 5′ terminus would simultaneously suppress the synthesis of H2A mRNA. In fact, in the case of the deletion C mutant the H2A mRNA synthesis is reduced but not abolished. It may be relevant that even under these circumstances the 5′ end of the novel mRNA maps about 24 nucleotides downstream of the T-A-T-A-A-A-T-A sequence.

The major conclusion from our results is that the T-A-T-A-A-A-T-A motif in the prelude of the H2A histone gene cannot be the direct functional equivalent of the Pribnow box because deletion of the latter in a bacterial gene abolishes all transcription (27). It could, of course, still be that the T-A-T-A-A-A-T-A motif is evolutionarily related to the Pribnow box, but that its prime function is modified in that it no longer acts as a promoter for transcription but rather serves to guide the RNA polymerase into a correct initiation frame. If one accepts this hypothesis, then the T-A-T-A-A-A-T-A motif has become an

element specifying the correct 5′ end of the mRNA and is now a *selector* element for the transcription of eukaryotic genes. The simplest interpretation of our results is that sections A, B, and C represent a modulator, a selector, and an initiator segment, respectively. Together these three sections form a relatively long stretch of more than 150 bp. However, this DNA can still be imagined to interact directly with RNA polymerase II, because the enzyme is complex and contains many subunits. Possibly, a more subtle mechanism is at work and at least some of the changes we observed are a consequence of alteration at the level of chromatin assemblage and structure. If this were true, a eukaryotic promoter would then have to be viewed as a three-dimensional, rather than a linear, chromosomal structure.

Restriction enzyme *Cla* I was a kind gift of Dr. H. Mayer (Braunschweig, West Germany). We thank Dr. M. Chipchase for critical reading of the manuscript and Mrs. S. Oberholzer for expert preparation of the manuscript. We are grateful to Mr. F. Ochsenbein for photography and skillful drawings. We thank Mrs. V. Kurer for preparing recombinant plasmid DNA. This work was supported by the State of Zürich and the Swiss Nationalfonds, Grant 3.257.077.

1. Felsenfeld, G. (1978) *Nature (London)* **271,** 115–122.
2. Schaffner, W., Kunz, G., Daetwyler, H., Telford, J., Smith, H. O. & Birnstiel, M. L. (1978) *Cell* **14,** 655–671.
3. Busslinger, M., Portmann, R., Irminger, J. C. & Birnstiel, M. L. (1980) *Nucleic Acids Res.*, in press.
4. Gross, K. W., Probst, E., Schaffner, W. & Birnstiel, M. L. (1976) *Cell* **8,** 455–469.
5. Schaffner, W., Gross, K. W., Telford, J. & Birnstiel, M. L. (1976) *Cell* **8,** 471–478.
6. Gross, K. W., Schaffner, W., Telford, J. & Birnstiel, M. L. (1976) *Cell* **8,** 479–484.
7. Hentschel, C., Irminger, J. C., Bucher, P. & Birnstiel, M. L. (1980) *Nature (London)*, in press.
8. Gannon, F., O'Hare, K., Perrin, F., Le Pennec, J. P., Benoist, C., Cochet, M., Breathnach, R., Royal, A., Garapin, A., Cami, B. & Chambon, P. (1979) *Nature (London)* **278,** 428–434.
9. Birnstiel, M. L. & Chipchase, M. (1977) *Trends Biochem. Sci.* **2,** 149–152.
10. Telford, J., Kressmann, A., Koski, R. A., Grosschedl, R., Müller, F., Clarkson, S. G. & Birnstiel, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2590–2594.
11. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
12. Kressmann, A., Clarkson, S. G., Telford, J. & Birnstiel, M. L. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 1077–1082.
13. Probst, E., Kressmann, A. & Birnstiel, M. L. (1979) *J. Mol. Biol.* **134,** 709–732.
14. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
15. Kressmann, A., Hofstetter, H., Di Capua, E., Grosschedl, R. & Birnstiel, M. L. (1979) *Nucleic Acids Res.* **7,** 1749–1763.
16. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. (1980) *Cell* **19,** 13–25.
17. Korn, L. J. & Brown, D. D. (1978) *Cell* **15,** 1145–1156.
18. Müller, F. & Clarkson, S. G. (1980) *Cell* **19,** 345–353.
19. Goodman, H. M., Olson, M. V. & Hall, B. D. (1978) *Proc. Natl. Acad. Sci, USA* **74,** 5453–5457.
20. Baker, C. C., Herisse, J., Courtois, G., Galibert, F. & Ziff, E. (1979) *Cell* **18,** 569–580.
21. Haegeman, G., Heuverswyn, H., Gheysen, D. & Fiers, W. (1979) *J. Virol.* **31,** 484–493.
22. Lifton, R. P., Goldberg, M. L., Karp, R. W. & Hogness, D. S. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 1047–1051.
23. Hackett, P. B., Traub, P. & Gallwitz, D. (1978) *J. Mol. Biol.* **126,** 619–635.
24. Surrey, S. & Nemer, M. (1976) *Cell* **9,** 589–595.
25. Faust, M., Millward, S., Duchastel, A. & Fromson, D. (1976) *Cell* **7,** 597–604.
26. Ziff, E. B. & Evans, R. M. (1978) *Cell* **15,** 1463–1475.
27. Miller, J. H. (1978) in *The Operon*, eds. Miller, J. H. & Reznikoff, W. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 66.