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Biochemistry

Spacer DNA sequences upstream of the T-A-T-A-A-A-T-A sequence are essential for promotion of H2A histone gene transcription *in vivo*

(DNA manipulation/*Xenopus* oocyte injection/surrogate genetics/H2A histone mRNA synthesis/eukaryotic promoter)

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ABSTRACT **The control region of a sea urchin H2A histone gene may be functionally dissected into at least three DNA segments, which we have termed modulator, selector, and initiator elements. While the initiator and in particular the selector containing the T-A-T-A-A-A-T-A sequence are specificity elements that dictate the generation of faithful 5′ ends to H2A mRNA, the modulators control the rate at which these specificity elements operate [Grosschedl, R. & Birnstiel, M. L. (1980) *Proc. Natl. Acad. Sci.* USA 77, 1432–1436]. By functional tests of *in vitro* mutated histone DNA in the *Xenopus* oocyte we have now discovered that the segment E of the A+T-rich spacer DNA lying at a considerable distance upstream of the conservative T-A-T-A-A-A-T-A sequence is a strong modulator element of H2A gene transcription. Deletion of this element creates a 15- to 20-fold H2A-specific down mutation. Segment E by itself cannot elicit initiation of transcription except in coordination with the prelude sequence of the H2A gene. The nucleotide sequence of the relevant spacer element showing modulator activity has been determined and found to contain a pattern of T and A runs as well as a series of inverted repeats. Additional pre-H2A spacer mutants, including a spacer inversion mutant, have been constructed *in vitro,* that, when injected into the oocyte nucleus, modulate the expression of the H2A gene by an overall factor as large as 100. Other factors controlling promoter activity are discussed.**

In prokaryotes, the role of regulatory proteins and their interaction with specific DNA sequences in transcription is being elucidated by the process of classifying the various phenotypes produced by mutation of the regulatory sequences and the genes coding for regulator proteins. This classical genetical approach is complemented by well-defined *in vitro* transcriptional systems and protein-binding studies. In principle, this method can also be used for animal viruses, which readily express their genetic information in the host cell (cf. ref. 1). Study of the control of transcription of eukaryotic *chromosomal* genes has been facilitated by the development of surrogate genetics techniques (2, 3) in which *in vitro* sequence manipulation of the cloned genes is followed by functional tests of the mutants within the living cell (4–6).

We previously used this approach to study the transcriptional control of a H2A histone gene of the sea urchin *Psammechinus miliaris* (6). Short sections [54–59 base pairs (bp)] were deleted from the H2A 5′ mRNA leader coding sequence and sequences preceding it. Transcriptional changes in the expression of the modified genes injected into the nucleus of centrifuged *Xenopus* oocytes (cf. ref. 7) were then examined. From the various mRNA "phenotypes" produced we concluded that RNA polymerase II promoters contain both specificity and rate-determining elements that were termed, in order of their occurrence on the DNA: modulator, selector, and initiator elements (6). One such modulator, or rate-determining element, was delineated as being coincident with the previously described H2A gene-specific conserved sequence, which contains a tandem repeat of the C-A-A-T motif (8) also found in the prelude of many other mRNA genes. We now present evidence of a second modulator element, which resides in segment E in the proximal half of the pre-H2A spacer DNA. Our results suggest that the presence of this region of spacer is crucial for the regulated expression of the sea urchin H2A gene, because manipulations in this region can alter the level of expression of the H2A gene in the oocyte by a factor of as much as 100. Although this spacer DNA is essential for normal transcription, it is inactive alone and can elicit transcription only when combined with appropriate specificity elements—i.e., the selector and initiator segment of the H2A gene unit.

METHODS AND MATERIALS

Enzymes and Reagents. Restriction enzymes were purchased from New England BioLabs. Phage T4 DNA ligase was obtained from Miles. Calf intestine alkaline phosphatase was purchased from Boehringer, and T4 polynucleotide kinase was purchased from P-L Biochemicals.

Construction of Spacer Mutant Clones. The strategy for the construction of spacer mutants was similar to that described (6). The containment conditions were P2 and EK1 according to the National Institutes of Health guidelines.

Spacer deletion mutants h22ΔD and h22ΔE. Three DNA components were prepared. Component **a** consisted of the left-hand portion of the h22 DNA up to a *Taq* I site, in the case of deletion D near amino acid codon 105 of the H3 gene (9), in the case of deletion E near nucleotide position −524 in the pre-H2A spacer (see Fig. 1). These components were prepared by a partial cleavage of the 4-kilobase (kb) *Hin*dIII/*Xho* I h22 DNA fragment with *Taq* I. Component **b** represented the right-hand portion of h22 DNA—i.e., the 2-kb *Xho* I/*Hin*dIII fragment, which was already linked through the *Hin*dIII end to *Hin*dIII-cleaved pBR322 plasmid DNA. Component **c** was the 375-bp *Taq* I/Xho I fragment containing the 5′ coding portion of the H2A gene and was prepared from the 0.4-kb *Eco*RI/*Xho* I fragment of pBR-H2A-3 DNA (see ref. 6). Components **a**, **b**, and **c**, were ligated and, after transfection of *Escherichia coli* HB101 cells, ampicillin-resistant colonies were grown and analyzed by restriction nuclease analysis.

Spacer inversion mutant h22iE. Component **a** extended up to the *Taq* I site at position −490. Component **b** was the same as above, whereas instead of component **c** a component **c*** was prepared. For this the 970-bp *Hpa* II fragment of h22 DNA (9), delimited on the right by the *Hpa* II site at position −130 in the pre-H2A spacer (see Fig. 1), was isolated and digested with *Taq* I. The 306-bp *Taq* I/*Taq* I fragment (−490 to −184) was isolated from a 6% polyacrylamide gel, purified, and ligated to the 375-bp *Taq* I/*Xho* I fragment containing the left-hand portion of the H2A gene (see above). The desired 680-bp liga-

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Abbreviations: kb, kilobase (pairs); bp, base pair(s).

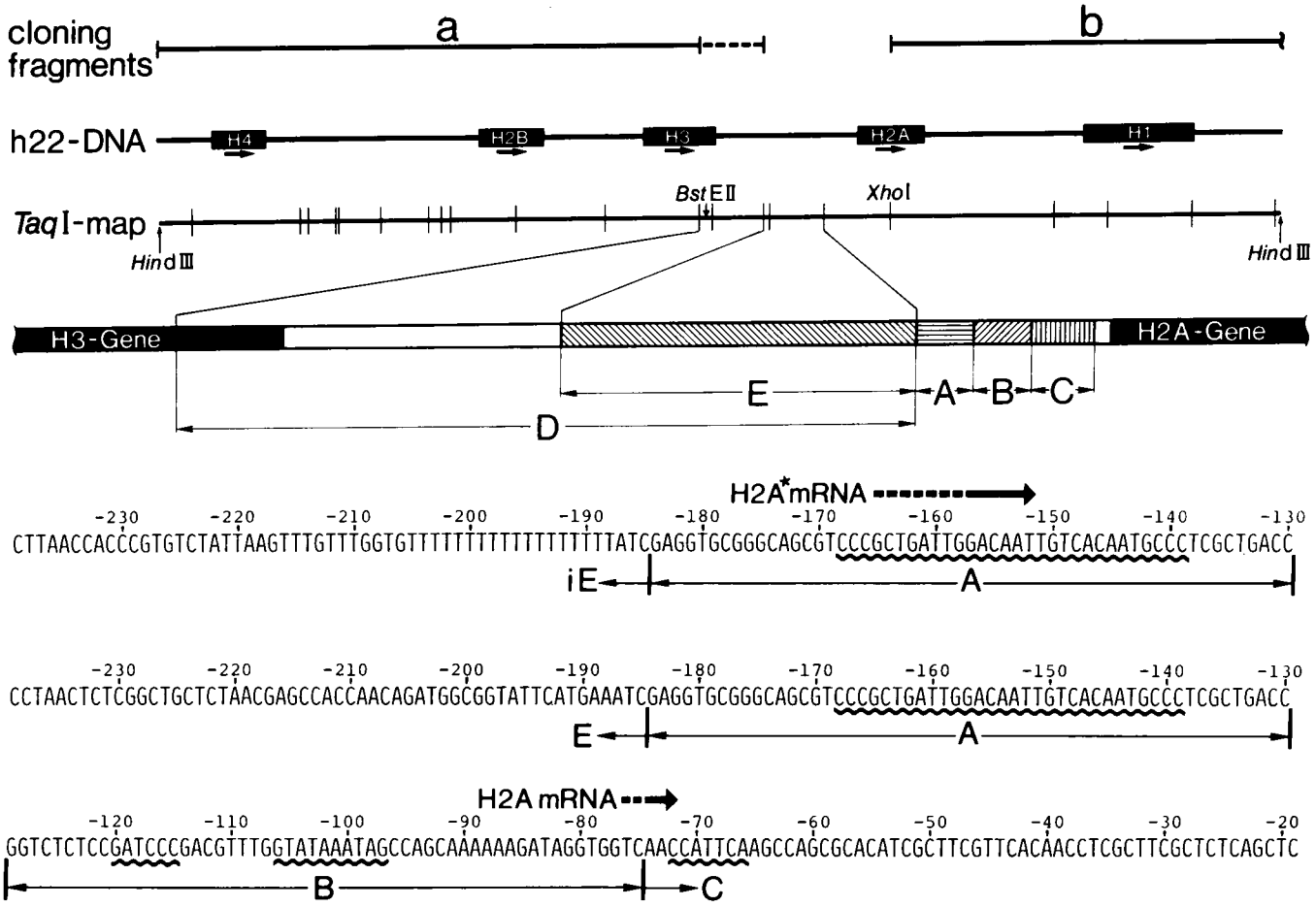

FIG. 1. Structure of the 6-kb histone gene repeat unit h22 of sea urchin DNA. The structural map of the H3-H2A spacer is shown in context of the h22 DNA. The boundaries of manipulated DNA segments within the spacer are indicated. The DNA sequences of the right-hand portion of inverted E segment fused onto the A segment are denoted in the first sequence line. The DNA sequences of wild-type DNA, including the right-hand portion of segment E and segments A, B, and C (6) are denoted in sequence lines 2 and 3 (cf. ref. 9). Conserved sequence motifs in the H2A gene prelude (segments A, B, and C) are underlined (8). The approximate map positions of the H2A mRNA and H2A* mRNA 5′ termini are indicated.

tion products, containing segment E in both orientations ligated to the *Taq* I end of the 375-bp *Taq* I/*Xho* I fragment, were identified on a 1.5% agarose gel, isolated, purified and used as component **c***. After ligation of components **a**, **b**, and **c***, transfection, and selection (see above), the inversion E mutant clone was identified by restriction analysis of the isolated 1-kb *Bst*EII/*Xho* I fragments of several clones.

Determination of Nucleotide Sequence. DNA sequencing and preparation of the "G-ladder" were performed by the Maxam and Gilbert technique (10) and used to verify all mutants.

DNA Injection and RNA Analysis. Injection of 4 ng vector-freed 6-kb circles (for preparation of DNA circles see refs. 6 and 11) together with 0.2 or 0.5 μCi (1 Ci = 3.7×10^{10} becquerels) of [α-^{32}P]GTP (Amersham) per oocyte was performed as described (3, 7, 11). The extracted total RNA was used for electrophoresis or purified on CsCl step gradients for nuclease S1 mapping.

Nuclease S1 Mapping of H2A mRNA. For the S1 mapping of mRNA 5′ termini according to Berk and Sharp (12) the 400-bp *Eco*RI/*Xho* I fragment of pBR-H2A-3 DNA and the 1-kb *Bst*EII/*Xho* I fragment of h22iE DNA were 5′ end-labeled at their *Xho* I sites. The molecules were hybridized to total purified RNA obtained from injected oocytes. For the detailed mapping of the H2A* mRNA (see text) 5′ terminus the 0.7-kb *Hin*f/*Hpa* I fragment of h22iE-DNA 5′-end-labeled at the *Hpa* I site at position −16 of the H2A gene was used.

RESULTS

Expression of Two pre-H2A Spacer Deletion Mutants in the Frog Oocyte. The cloned 6-kb histone DNA h22 repeat unit of the sea urchin *P. miliaris* contains the five histone genes alternating with spacer DNA (9, 13). Its structural features and expression in the oocyte of *Xenopus laevis* have been extensively described (3, 7–10, 14, 15). Specifically, we have shown that after injection of covalently circular h22 DNA, freed from all vector DNA, into the cell nucleus of centrifuged *Xenopus* oocytes, predominantly the H2A and the H2B genes are transcribed and yield faithful mRNA transcripts. Because both of these genes are expressed, one of them, the H2B gene, was used as an internal control for the quantitation, by densitometry of autoradiograms, of the transcription of the manipulated H2A gene (6). The validity of using the H2B gene as an internal control has more recently been corroborated. By dividing the wild-type h22 DNA into halves, one containing the H2A gene, the other the H2B gene, we were able to show that these two genes are not cotranscribed and hence provide independent controls for one another (unpublished observation).

An interesting feature of the deletion mutants described earlier (6) was that removal of the DNA segments containing the T-A-T-A-A-A-T-A and "cap box" elicited both qualitative and quantitative changes in transcription but that they did not abolish expression of the H2A gene. This finding suggested that additional information for promotion of the H2A gene lay elsewhere. Additionally, the finding that deletion of the up-

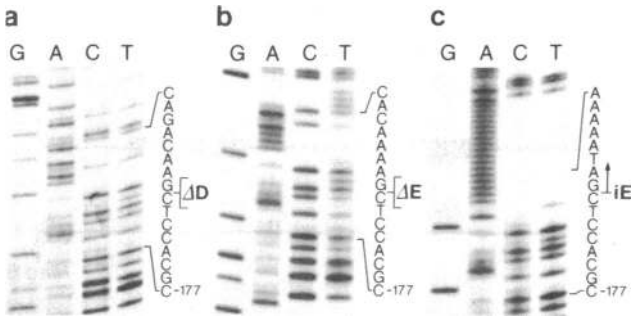

FIG. 2. Sequencing gels showing the boundaries of the deletions D and E as well as the correct fusion of the inverted segment E onto segment A. The nucleotide sequences of h22ΔD (*a*), h22ΔE (*b*), and h22iE (*c*) were all determined from the 5′-end-labeled *Hpa* II restriction sites at position −130. The positions of the deleted segments as well as the right breakpoint of the inversion are indicated. The numbering of nucleotides is that of wild-type DNA (see Fig. 1).

stream palindromic H2A-gene-specific conserved sequence (8) actually accelerates transcription encouraged us to search for promoting activities still further upstream within the spacer DNA. To this end, a deletion mutant of h22 DNA was constructed in which all DNA intervening between the H3 and the H2A gene was removed. This was effected by fusion of a *Taq* I site near amino acid codon 105 of the H3 gene with a *Taq* I site at position −184 in the pre-H2A spacer (see Fig. 1). This left all of the H2A prelude sequences intact up to and including the H2A-gene-specific conserved sequence. The nucleotide sequence of this deletion D mutant was determined to verify the deletion (Fig. 2*a*) and was injected into the frog oocytes. Total unfractionated RNA was isolated and the labeled histone mRNA was assayed by autoradiography of electropherograms (6). Under these circumstances H2A mRNA was synthesized at 1/15 to 1/20 the rate by comparison with both the H2B mRNA in the same slot (Fig. 3, slot a) and the H2A mRNA obtained by injection of wild-type h22 DNA (Fig. 3, slot d; for calculation see ref. 6).

Using a modification of the Berk and Sharp S1 transcript mapping procedure (12), Hentschel *et al.* (15) were able to show

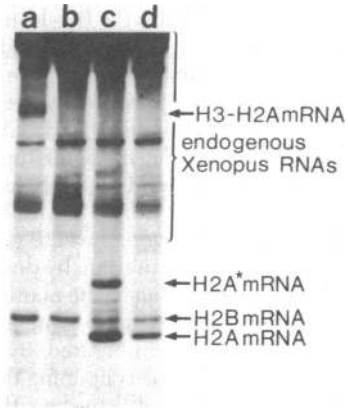

FIG. 3. Autoradiogram of gel electrophoresis of total RNA obtained from oocytes injected with wild-type and mutant h22 DNA together with α-^{32}P-labeled GTP. Because in this instance the histone-DNA-specific RNA was not selected by hybridization to h22 cloned DNA (10), labeled endogenous *Xenopus* transcripts are present and appear as strong bands in the upper sections of the gel slots. RNAs were from: slot a, oocytes injected with h22ΔD DNA; slot b, h22ΔE DNA; slot c, h22iE DNA; slot d, h22 wild-type DNA. The positions of histone mRNAs are indicated. The amount of the RNA in each slot corresponds to a sample of two oocytes.

that, in the oocyte, 5′ ends of the H3 transcripts are generated with a frequency similar to that of the 5′ ends of H2B and H2A mRNAs. No faithful and distinct H3 mRNA is found because many of the H3 transcripts terminate at random within the spacer DNA following the H3 gene, with a minority ending within the H2A gene terminus (15). Because in the deletion D mutant both the H3 gene terminus and all pre-H2A spacer DNA sequences have been deleted, a large incidence of fused H3-H2A mRNA of distinct molecular weight would be anticipated. Indeed, such molecules can be seen as a prominent band near the top of the analytical RNA gel of Fig. 3, slot a. The nature of this fused metabolically stable mRNA has been identified by the "inverse S1 mapping procedure" (16) and shown to include the 5′ end of H3 mRNA linked to the 3′ terminus of the H2A mRNA (results not shown). The major change in the deletion D mutant is, however, that virtually all H2A gene expression is abolished, thus implicating a direct involvement of spacer DNA in H2A gene transcription. A possible formal criticism of the experiment is that initiation of H2A gene transcription was inhibited not because the spacer has been deleted but because of read-through of polymerases from the truncated H3 gene. To refute this possibility and to define the 5′ boundary of the modulating spacer element more closely, we constructed a smaller spacer deletion mutant in which the breakpoint to the right remained unchanged at the *Taq* I site, position −184, but in which the deletion extended to about the middle of the A+T-rich spacer (position −524), well clear of the 3′ terminus of the H3 gene (see Fig. 1). Correct joining of the fragments was verified by DNA sequence determination (Fig. 2*b*). Injection of this deletion E mutant into oocytes resulted in a similar specific reduction of H2A gene expression (to about 1/15 to 1/20) when compared to the h22 wild-type levels (Fig. 3, slot b). This experiment clearly demonstrates that the spacer DNA from position −524 to −184 either as a whole, or because of specific sequences lying within it, is a strong modulator of H2A gene expression.

Expression of a Spacer Inversion Mutant in the Frog Oocyte. The finding that spacer DNA served as a rate-regulating element in H2A gene transcription was unexpected. The molecular mechanisms involved are at present unknown, but we have considered at least two hypotheses; (*i*) the spacer acts by virtue of its A+T content alone and (*ii*) the spacer contains specific sequences that are recognition sites for interaction with regulatory proteins or polymerases. In contrast to A+T content alone, such sites might well show a polarity of function with respect to the DNA sequence. A simple test to distinguish between these two hypotheses is possible by examining the effects of inversion of the relevant spacer segment E (Fig. 1). In this way, the overall A+T content of the spacer is maintained, but the polarity of the spacer is reversed. Proof of the correct construction of such a mutant, h22iE, was obtained by DNA sequence determination (Fig. 2*c*). The DNA of the inversion E mutant was circularized and injected into oocytes. If the A+T content alone were important, one would expect a level of H2A mRNA synthesis similar to that of the wild-type h22 DNA, whereas if the polarity were a dominant factor only residual levels of H2A mRNA synthesis would be anticipated.

Neither of these predictions came true. Unexpectedly, H2A mRNA was produced at 3–4 times greater rate than in comparable experiments with wild-type h22 DNA. Moreover, in addition to the normal H2A mRNA, a prominent more slowly migrating H2A* mRNA (Fig. 3, slot c) was synthesized *de novo*. The 5′ ends of both the H2A and H2A* mRNAs were mapped by using both the inverse S1 mapping technique (ref. 16, results not shown) and the Berk and Sharp procedure (refs. 12 and 14; see Fig. 4). These experiments demonstrate conclusively that

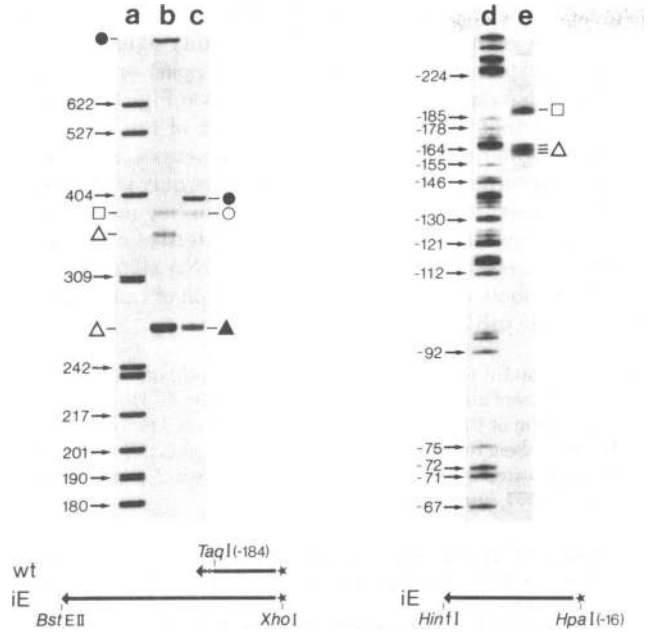

FIG. 4. S1 mapping of the 5′ termini of H2A mRNAs. Total purified RNAs obtained from injected oocytes were hybridized to cognate DNA fragments labeled at their *Xho* I sites and trimmed with nuclease S1 (12, 14). The S1-protected DNA fragments were run in parallel with *Hpa* II-cleaved pBR322 DNA as size marker (slot a). In slot b RNA from h22iE DNA and in slot c RNA from wild-type DNA was used. Note that for the S1 mapping of the wild-type H2A mRNA 5′ end a chimeric DNA probe was used with pBR322 sequences fused at the *Taq* I site (−184; see Fig. 1). Therefore all transcripts initiating upstream of this *Taq* I site give rise to a common S1 protected DNA fragment of 375 nucleotides, which is symbolized by ○. The 5′ termini map positions of H2A mRNAs are denoted by ▲ for wild-type h22 transcripts and △ for h22iE RNAs. ● denotes the renatured DNA fragments. □ marks a DNA band that most probably arose from S1 cutting in the pure A+T region of the H2A spacer between positions −186 and −205 of h22iE DNA, because no RNA of the corresponding size can be detected in the gel electrophoresis of labeled RNA obtained from oocytes injected with h22iE DNA (compare Fig. 3c). Slot d shows the "G-ladder" obtained from the *Hinf*/*Hpa* I fragment 5′-end-labeled at the *Hpa* I site (−16; see Fig. 1). The numbering of the "G-ladder" is that of the wild-type sequence (Fig. 1). The same *Hinf*/*Hpa* I fragment was used for the S1 mapping of the H2A and H2A* mRNA 5′ termini shown in slot e. The uncorrected map positions (6, 14) of the 5′ termini of h22iE transcripts are indicated by △. □ denotes the S1 artefact (see above). Note that the 50-bp hybrid of H2A mRNA and the DNA fragment was unstable and therefore did not give rise to an S1-protected DNA fragment.

the 5′ terminus of the H2A* mRNA maps downstream of the inversion break point near position −157 to −165, whereas the H2A mRNA terminates at position −74 to −76, as do H2A mRNAs of h22 DNA and of sea urchin-derived RNA. Thus an additional, normally cryptic, H2A mRNA start is revealed by the spacer inversion mutation. Interestingly, the very same cryptic initiation point for transcription has recently also been detected in transcripts of the deletion B mutant, in which the selector element is missing and consequently the initiation of transcription has become inexact (6).

Inversion of spacer DNA clearly leads to an increase in the production of genuine H2A mRNA which, together with the new H2A* mRNA, results in an overall increase of the H2A gene expression of 4- to 5-fold. Because we have noted above that the deletion of the same spacer element reduces the rate of transcription of the H2A gene by a factor of 1/15 to 1/20, we conclude that the spacer can modulate transcription of the structural gene downstream of it by a factor of 60–100.

DISCUSSION

In eukaryotic genes such as the histone genes of the sea urchin that are transcribed by polymerase II (11, 17), several conservative sequence elements are found in spacer regions adjacent to the 5′ and 3′ boundaries of the structural genes (8, 18); these elements have been proposed as regulatory sequences involved in controlling the initiation and termination of transcription (8, 14, 18). Deletion of these conserved sequence motifs from the H2A prelude sequences have been shown to have definite quantitative or qualitative effects on transcription but a total elimination of all H2A mRNA synthesis was not observed (6). By contrast we noted in preliminary experiments that removal of the pre-H2A spacer drastically reduced H2A mRNA synthesis (6). We have now investigated the role of this DNA segment and have found it to be a powerful modulator of transcription. Deletion of this segment produces the closest approximation to a "null allele" obtained so far.

It would be interesting to compare our results with those obtained by standard genetical procedures. However, assuming our results can be extended to the living organism, it may be very difficult to isolate organisms with the required phenotypes, because even large deletions of key sequences may not lead to a complete eradication of gene expression upon which most classical methods for mutant selection are based. The surrogate genetics approach, alternatively, has allowed us to distinguish between three types of regulatory elements of the sea urchin H2A histone gene that we wish to discuss and compare to one another.

Modulators. These segments modulate the rate of synthesis of structurally unaltered, faithful mRNAs. We have discovered two such modulators in segments A and E. A very unusual feature of segment E is that it is located far upstream of the 5′ terminus of the messenger coding sequences and yet it still exerts a strong modulation of transcription. Inspection of the DNA sequences of segment E reveals a pattern of A and T runs as well as a number of inverted repeats, mainly in the region −345 to −184. By inversion of segment E we have shown that it can act on H2A gene transcription in both orientations, and this is consistent with the presence of palindromic structures within it. The question arose whether the spacer segment E, by itself, would be capable of promoting transcription when linked to sequences other than the T-A-T-A-A-A-T-A and "cap box." Such a topological situation exists within the wild-type h22 DNA, where the strong modulator E faces the other way and, if active, would give rise to transcripts of antisense strand RNA from the 3′ terminus of the H3 gene. To test this concept a DNA probe, 5′-end-labeled at the *Bst*EII site within the H3 gene and extending to the right up to the restriction site *Xho* I (see Fig. 1), was prepared; this probe permitted mapping of any antisense strand RNA initiating within the spacer DNA and transcribing leftwards into the H3 gene. No significant protected DNA fragments were obtained (results not shown) under conditions in which control experiments with RNA derived from h22iE DNA gave very strong signals as shown in Fig. 4, slot b. It may therefore be concluded that the modulating spacer segment can act to a significant extent only when presented with appropriate sequences downstream of it, normally with the selector and initiator sequences of the H2A gene. It is possible that it plays a major role in the developmental regulation of specific histone gene variants.

Selector. As discussed earlier, the DNA segment B containing the conserved T-A-T-A-A-A-T-A box is a specificity element that selects a unique or at least predominant 5′ mRNA terminus *in vivo* (6). The T-A-T-A-A-A-T-A box is an absolute requirement of *in vitro* transcription of mRNA genes (19) and may be related in evolution to the bacterial Pribnow box (6). Because deletion of the Hogness box does not abolish expression of the

H2A gene (6) or of the early simian virus 40 gene (20) *in vivo*, we feel it inappropriate to equate it with a promoter.

Common to selector and modulator(s) is that they both influence reactions further downstream on the DNA. Once the selector is removed, there is clearly a loss of information for faithful initiation and a reduction in the rate of transcription. By contrast, deletions of modulators create up mutations (deletion A) or down mutations (deletion D and E) without modifying the 5′ end of the H2A mRNA.

Initiator Element. There is increasing evidence that in most cases the capped 5′ ends of mRNAs are generated by initiation of transcription rather than by processing of a high molecular weight precursor (21, 22). The initiation sites of many wild-type mRNAs have now been aligned with the corresponding DNA sequences with reasonable accuracy (cf. ref. 8). On the whole, the sequences around these sites do not appear to be universally conserved and even within gene families homology extends only for a few nucleotides. However, initiation of transcription in prokaryotic and eukaryotic mRNA genes preferentially occurs in a sequence pyrimidine-purine-pyrimidine, with C-A-pyrimidine at a fixed distance from the Pribnow box or the Hogness T-A-T-A-A-A-T-A box, respectively, being a particularly favored base combination (14, 23). In the case of sea urchin histone genes somewhat more homology exists in that a conserved heptameric sequence can be recognized near the 5′ ends of histone mRNAs (8, 14), and this has been named "cap box."

A striking feature of the T-A-T-A-A-A-T-A deletion is that in this mutant three additional specific initiation sites are revealed, which are all clustered in a region of 60 bp around the normal initiation site, and all the sites are used to generate mRNA with similar frequency (6). Clearly, there is a redundancy of information for the initiation of transcription in the prelude region of the H2A gene. The fact that, once the DNA segment containing the T-A-T-A-A-A-T-A box is restored, the activity of the normal initiation site is enhanced and that of all others abolished points to a hierarchical relationship between the selector and initiator elements. The principle of a predominant selector is also seen at work in the case of the deletion C mutant, in which the normal initiation site has been removed. Initiation occurs at yet another site, some 24 bp downstream of the T-A-T-A-A-A-T-A sequence (6), as is the case for many cellular genes (24–26). Identical results have been obtained recently in related simian virus 40 mutants (27).

Organization of the Promoter Sequences at a Higher Level. The centrifuged oocyte system provides a rapid means of testing the biological effects of sequence manipulations within the living cell nucleus (for technical details, see ref. 7). Like many other covalently circular DNAs (28), h22 DNA injected into the frog nucleus first becomes completely relaxed and within 1 hr can be recovered as superhelical DNA (unpublished results). Supercoiling of the DNA is not a trivial feature of gene expression, because relaxed linear DNA yields no faithful H2B mRNA in the oocyte and the synthesis of the H2A mRNA is markedly inhibited (11). Although the point remains to be rigorously established, it appears likely that the genes injected into the oocyte nucleus are assembled into active chromatin (28) and thus contrast with genes assayed in *in vitro* transcriptional systems. Conceivably this difference may account for the fact that the selector but no modulator element(s) have as yet been detected in *in vitro* systems (19). Such chromatin assemblage may prove to be of paramount importance, because recently there has been increasing evidence of a close phase relationship between nucleosomes and DNA sequences (29). In this context it would be interesting to determine whether the segment E sequences reside in a chromatin structure with unusual properties and whether this segment is instrumental in phasing nucleosomes. Another important function of chromatin assemblage may be that regulatory signals for the H2A gene expression that seem to lie far apart on linearly stretched DNA, such as sections E and C (see Fig. 1), can be brought together by appropriate packaging of the DNA for interaction with regulatory proteins or polymerases. The study of the higher-order structure of the promoter may well reveal that the primary structure of the DNA (including modulators and selector and initiator elements), the pattern of chromatin assemblage, and the superhelicality of the DNA all interrelate and all contribute information for the initiation of transcription of eukaryotic mRNA genes.

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