9. S. M. Arfin, R. E. Cirullo, Arredondo-Vega, F. X. M. Smith, *Somatic Cell Genet.* **9**, 517 (1983).
10. Agarose blocks containing 4AF cells (10^6 cells per 80-μl block) were prepared and digested with Not I as described (*5*). Eight solid agarose blocks were loaded on a 20 by 20 cm 1% low melting point agarose gel (BRL, Ultra Pure), in 0.2 TBE (18 m*M* tris-borate, 0.4 m*M* EDTA, *p*H 8.3) and placed in an electrophoresis chamber (*3*). Preparative FIGE was run for 24 hours at 200 volts, 15°C, 45 mA. The voltage gradient was inverted periodically, with a time ratio of 3/1 and the longer time increasing linearly from 3 to 120 seconds. After running it was stained for 1 hour in 10 m*M* EDTA+ ethidium bromide (1 μg/ml), and destained for 1 hour in 10 m*M* EDTA, and photographed under 360-nm ultraviolet light (Fig. 1). One lane of the gel was blotted onto GeneScreen membranes. The other lanes were transferred to a millimeter grid, and sliced manually in 2.5-mm slices perpendicular to the running direction. From these gel slices (approximately 3 g) a piece corresponding to half of a lane was cut off, and the rest was stored at 4°C in 1 ml of 0.5*M* EDTA (*p*H 8.0). Alternatively, to avoid a bias due to uneven distribution of DNA, slices can be melted at 65°C in 100 m*M* NaCl and a small volume removed. The small gel pieces were melted at 95°C, brought to 0.3–0.5*M* NaOH by adding 2*M* NaOH stock, and left at 65°C for 10 minutes. This step denatures the DNA and hydrolyzes the agarose. They were then filtered through GeneScreen transfer membrane by means of a commercial microtiter filtration manifold (Schleicher & Schuell) that had been prewarmed to 37°C. Southern blots and dot blots were hybridized (*5*) to *met*D to identify slices containing the *met* Not I fragment (Fig. 1). Slices 14 and 15 were brought to 100 m*M* NaCl, melted at 65°C, and incubated overnight with 50 units of agarase (Calbiochem, deoxyribonuclease-free) per milliliter. High molecular weight DNA was recovered by phenol extraction and ethanol precipitation.
11. The gel-eluted DNA (estimated 1 μg) was resuspended in 80 μl TE (10 m*M* tris, 1 m*M* EDTA) of which 5 aliquots (10 μl each) were digested in a volume of 30 μl with 0.05, 0.017, 0.005, 0.0017, 0.0005 unit of Sau 3AI (Biolabs) for 30 minutes at 37°C. The reaction was stopped by EDTA and heat inactivation, and 1 μl was electrophoresed on a 0.3% agarose gel, blotted, and hybridized to radioactively labeled hamster DNA in order to evaluate the extent of the partial digests. The remaining 30 μl of DNA were then digested under optimal conditions (0.007 unit, 30 minutes, 100 μl, 37°C), and all suitable fractions (10 to 30 kbp) were phenol-extracted and precipitated. This DNA (estimated at 0.5 μg) was ligated in one reaction to 7 μg of EMBL3*Sam*–Bam HI–Eco RI (*12*) and 0.7 μg of EMBL6–Bam HI–Eco RI–Not I (*13*), in a volume of 170 μl. The large molar excess of vector DNA ensured high cloning efficiency from small amounts of DNA, and reduces the probability of coligation of insert fragments.
12. A.-M. Frischauf, N. E. Murray, H. Lehrach, *Methods Enzymol.*, in press; A.-M. Frischauf, H. Lehrach, A. Poustka, N. Murray, *J. Mol. Biol.* **170**, 827 (1983).
13. λ EMBL5A is derived from EMBL3A by replacing both Bam HI cloning sites by a Not I linker (*12*). An Eco RI digest of EMBL5A and EMBL3S was mixed, ligated, and plated on *E. coli* MC1061, a *sup*^{0} (nonpermissive) host (*20*). One bacteriophage containing a Bam HI cloning site at the left arm and a Not I site at the right arm was identified (EMBL6).
14. B. Seed, *Nucleic Acids Res.* **11**, 2427 (1983).
15. D. L. Van Dyke, M. J. Worsham, L. J. Fisher, L. Weiss, *Hum. Genet.* **73**, 130 (1986).
16. Human clones were identified by screening plaques with a human Alu repeat probe, which recognizes 1 in 50 plaques in the total library and a total of 120 plaques in the end-clone library.
17. S. Arfin and J. J. Wasmuth, personal communication.
18. F. Michiels *et al.*, unpublished data.
19. Agarose blocks of cell line 4AF were prepared and digested as described (*5*). For FIGE, blocks were loaded on a 1% agarose gel and electrophoresed as described (*10*), but for 30 hours at 180 volts, 35 mA, at 15°C. OFAGE was as described (*5*).
20. N. E. Murray, in *Lambda II*, R. W. Hendrix, J. W. Roberts, F. W. Stahl, R. A. Weisberg, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983), pp. 395–432.
21. L. Dente, M. Sollazzo, C. Baldari, G. Cesarini, R. Cortese, in *DNA Cloning*, D. M. Glover, Ed. (IRL Press, McLean, VA, 1986), vol. 1, pp. 101–106.
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Protein-DNA Interactions in Vivo Upstream of a Cell Cycle–Regulated Human H4 Histone Gene

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Cell cycle–dependent histone genes are transcribed at a basal level throughout the cell cycle, with a three- to fivefold increase during early S phase. Protein-DNA interactions in the 5′ promoter region of a cell cycle–regulated human H4 histone gene have been analyzed at single-nucleotide resolution in vivo. This region contains two sites, with four potential protein-binding domains, at which the DNA is protected from reaction with dimethyl sulfate in cells and from digestion with deoxyribonuclease I in nuclei. These protein-DNA interactions persist during all phases of the cell cycle and dissociate with 0.16 to 0.2*M* sodium chloride.

THE HUMAN HISTONE GENES CONstitute a multigene family organized as a series of clusters (*1, 2*). The expression of the cell cycle–dependent human histone genes is temporally and functionally coupled with DNA replication; control is mediated at both transcriptional and post-transcriptional levels (*3*). The cell cycle–dependent genes are transcribed throughout the cell cycle, but most interestingly exhibit a three- to fivefold increase in transcription during the initial 2 hours of S phase (*4, 5*). This increase is accompanied by modifications in chromatin structure (*6, 7*), including changes in deoxyribonuclease I (DNase I) and S1 nuclease–sensitive regions.

DNA-mediated transfection of human histone genes into cells of both primate and murine origin has demonstrated the existence of trans-acting regulatory factors (*8, 9*). For at least three cell cycle–dependent human histone genes, approximately 200 nucleotides of 5′ flanking sequence are sufficient to support specific initiation of transcription both in vitro (*10, 11*) and in vivo (*12*). In addition, more distal elements may influence the level of transcription (*12*). However, our understanding of proteins that interact with specific 5′ flanking sequences and influence the structure and transcription of human histone genes is still minimal.

To study in vivo protein-DNA interactions in the 5′ promoter of the cell cycle–regulated H4 histone gene, F0108, we used the genomic sequencing method of Church and Gilbert (*13*). Intact cells were treated with dimethyl sulfate (DMS), and the extent of methylation of each guanine residue was used to identify sites of specific protein-DNA interactions (*14*). DNA from nuclei treated with DNase I was also analyzed at single-nucleotide resolution (footprinting) (*15*), so that we were able to correlate data on chromatin structure with specific protein-DNA interactions.

We now report that the proximal 5′ promoter of this H4 gene contains two regions of DNA protected both from reaction with DMS in cells and from digestion with DNase I in nuclei. These protein-DNA interactions can be dissociated with 0.16 to 0.2*M* NaCl. Because these sites persist at all times during the cell cycle, they are thought to be important for at least the basal level transcription of this H4 histone gene.

The structure of the cell cycle–regulated F0108 H4 histone gene and the probes used for its analysis in vivo are shown (Fig. 1). The probes were designed to cover a region of 1000 nucleotides that includes the coding region. We initially focused on sites of protein-DNA interaction in vivo in the proximal 5′ flanking region of this gene. This region exhibits modifications in chromatin structure, which correlate with changes in the level of transcription (*6, 7*). In addition, deletion analysis of this region indicated that sequences between the translation start site and −120 bp were sufficient to support correct initiation of transcription (*12*).

Our initial studies in exponentially growing cells indicated strong protein-DNA contacts within the upstream proximal 200 nu-

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cleotides of this H4 histone gene. To examine these protein-DNA interactions at high resolution, we treated synchronized HeLa cells with DMS at various times during the cell cycle. DNA was isolated and digested with either Hinc II or Xmn I before analysis at single-nucleotide resolution by the genomic sequencing method. The upper strand of the 5′ flanking region was examined first by means of the 5′ upper Hinc II probe. Guanine residues at positions −69, −73, −74, −85, −86, −120, −123, −125, −126, −127, −129, −139, and −142 nucleotides from the translation start site exhibited substantial protection from DMS compared with the control plasmid DNA (Fig. 2A). Comparison of samples from various times during the cell cycle revealed no obvious differences in these footprints. The same DMS protection pattern was also observed in human HepG2 hepatoma cells.

Similar experiments at different times during the cell cycle were performed after DNase I treatment of HeLa cell nuclei. Figure 2B displays the presence of two DNase I–protected regions (footprints) on the upper strand of the proximal promoter, which directly coincide with the analogous DMS protection pattern. Such footprints were not observed with DNase I treatment of deproteinized DNA (Fig. 2B). By comparing the positions of guanine residues with the DNase I footprints, we were able to map these two protected sites between nucleotides −150 and −117 (site I) and positions −91 to −50 (site II). The sizes of the protected regions on the upper strand are approximately 33 nucleotides for site I and 41 nucleotides for site II. As with the DMS samples, no changes in the footprint patterns were apparent in the DNase I samples during the cell cycle. Furthermore, identical footprints were obtained from exponentially growing HeLa cell nuclei treated with DNase I (Fig. 2B).

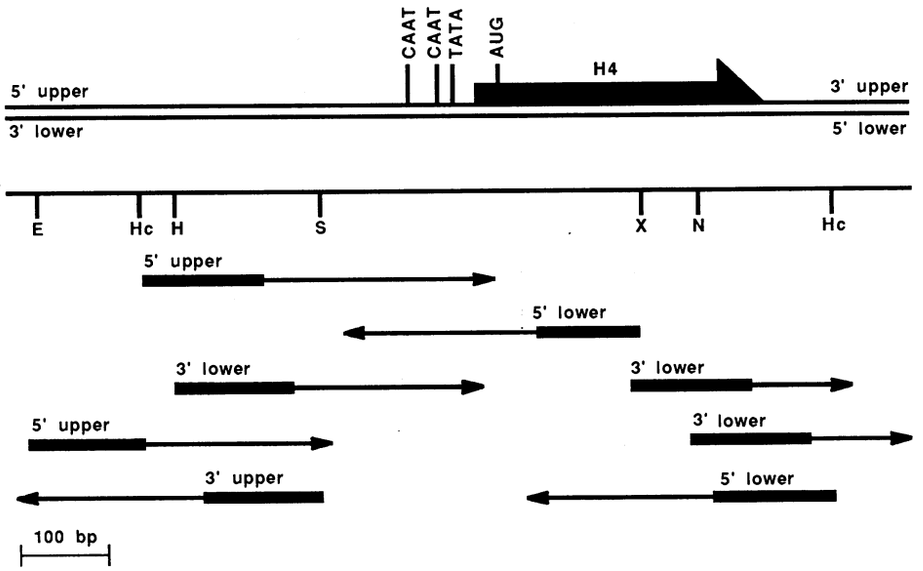

Fig. 1. F0108 human H4 histone gene and hybridization probes. (**Top**) F0108 human H4 histone gene, including flanking regions. The arrow marks direction and extent of transcription. Important elements in the promoter and the start of the coding region are shown. Restriction sites are: E, Eco RI; Hc, Hinc II; H, Hind III; S, Sma I; X, Xmn I; and N, Nco I. (**Bottom**) M13 probes used. The thick bar marks the M13 fragment, whereas the thin line shows the region scanned with each probe. One end of the probe always corresponds to an indicated restriction site.

To display protein-DNA interactions on the lower strand of the 5′ flanking region, we used the 5′ lower Xmn I probe (Fig. 2C). An examination of the region surrounding site I revealed no guanines with altered reactivity. However, there were four guanine residues at positions −70, −71, −76, and −80 nucleotides from the translation start site with decreased intensity compared with control DNA (Fig. 2C). These residues were in the same region (site II), which exhibited protein-DNA interactions on the upper strand. Again no differences were observed at different times of the cell cycle.

The DNase I protection pattern of the lower strand of the promoter region at various times during the HeLa cell cycle also demonstrated two sites of protein-DNA interaction (Fig. 2D). By comparison of the DNase I footprints with the guanine pattern

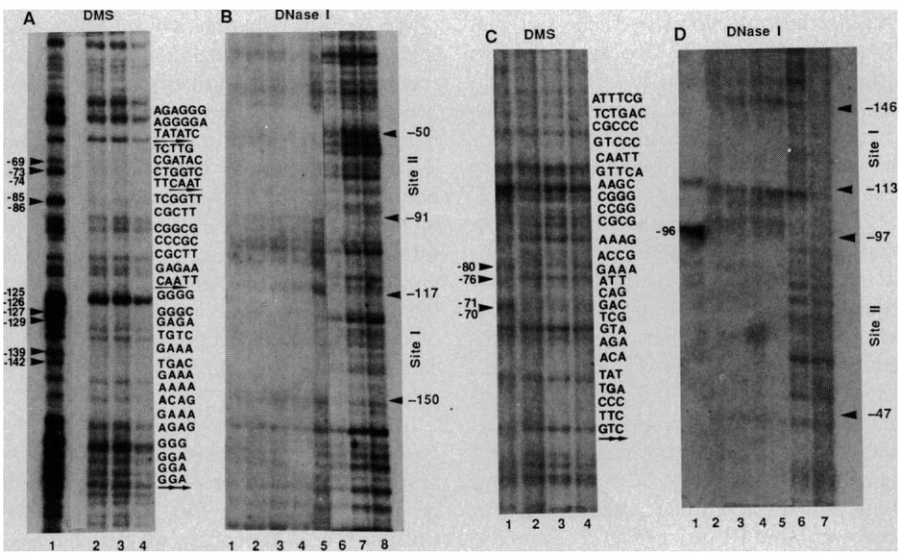

Fig. 2. DNA was isolated from DMS-treated cells or DNase I–digested nuclei, restricted with Hinc II (**A** and **B**) or Xmn I (**C** and **D**), and subjected to electrophoresis in a 6% sequencing gel (*13*). Approximately 220 nucleotides were run off the bottom of the gel. The 5′ upper Hinc II probe (A and B) or the 5′ lower Xmn I probe (C and D) was used for hybridization. The guanine pattern from DMS-treated cells: lane 1, control plasmid DNA (A) or deproteinized HeLa DNA (C) treated with DMS; lanes 2 to 4 of (A) and (C), DNA from early S phase, mid S phase, and mitotic plus G_1 phase cells, respectively. The sequence is displayed on the right. Arrowheads on the left indicate protected guanine residues. DNase I–digestion pattern from nuclei: lanes 1 to 4 of (B) and 2 to 5 of (D), DNA from nuclei of early S, mid S, mitotic plus G_1, and the subsequent S phase cells; lane 5 of (B), DNA from nuclei of exponentially growing cells; lane 1 of (D), size marker (noted at the left); lanes 6 to 8 of (B) and 6 and 7 of (D), deproteinized HeLa DNA digested with 100 to 400 pg of DNase I per microgram of DNA for 30 minutes at 20°C. Arrowheads on the right indicate protection from DNase I digestion. HeLa S3 cells were grown in suspension culture and were synchronized by double thymidine block (*6*). DMS treatment, extraction of DNA, and hybridization were done according to Church and Gilbert (*13*). DNase I treatment of nuclei was done according to Chrysogelos *et al* (*6*).

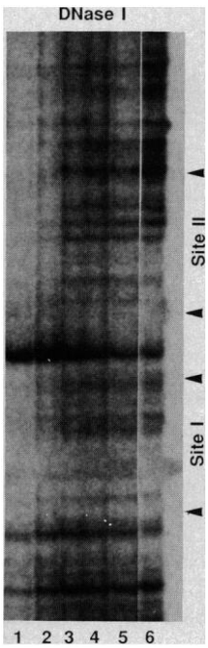

Fig. 3. DNase I pattern of salt-treated nuclei. Nuclei from early S phase cells were slowly adjusted to the desired NaCl concentration (0.12, 0.16, 0.2, 0.24, and 0.28*M* NaCl, lanes 1 to 5), incubated for 10 minutes at 37°C, washed once, and resuspended in 10 m*M* tris-HCl (*p*H 7.4), 10 m*M* NaCl, 3 m*M* $MgCl_2$. Digestion with DNase I (0.5 to 4 μg/ml) was for 10 minutes at 37°C, and the DNA was isolated (*6*), digested with Hind III, and hybridized with the 3′ lower Hind III probe. Lane 6, deproteinized HeLa DNA digested with DNase I. Sites of protection are indicated on the right.

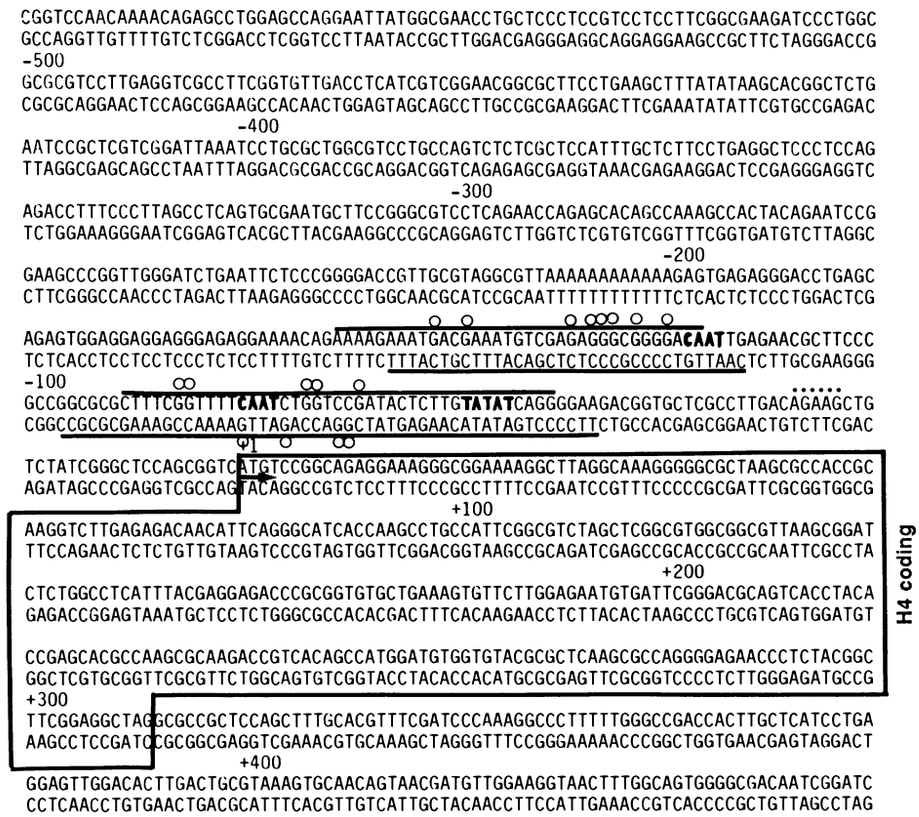

Fig. 4. Summary of protein-DNA interactions for the F0108 H4 histone gene. The histone H4 coding sequence is enclosed in solid lines. Protected guanine residues are marked with open circles. Sites I and II are marked by solid horizontal lines. The start of transcription is marked by a dotted line.

in the DMS samples and a restriction fragment marker, the boundaries of the footprints were defined. The two protected regions extended from nucleotides −146 to −113 (site I) and −97 to −47 (site II). These boundaries were confirmed by digesting the DNA samples with Hind III and by hybridizing with a 3′ lower Hind III probe. As with the upper strand of site I, the binding site on the lower strand covers 33 nucleotides with a four-nucleotide stagger at either end. Site II covers 50 nucleotides on the lower strand, and therefore the protected region is larger on the lower strand than on the upper strand.

Taken together, our results indicate that the footprints at sites I and II are due to binding of at least two different proteins. This conclusion follows from the fact that the sequences at these two sites have no significant homology. In addition, the guanine interactions at site I occurred on one strand only, whereas at site II protein contacts were observed with guanines on both strands.

We have so far assumed that the footprints originate from interactions of potential regulatory proteins with specific DNA sequences. To support this hypothesis, we examined the effects of increasing salt concentration on the pattern of DNase I protection at sites I and II. Isolated nuclei from early S phase cells were washed in 0.12 to 0.28*M* NaCl and then subjected to DNase I digestion. The sequence of the 5′ flanking region was displayed by hybridization with a 3′ lower Hind III probe (Fig. 3). After the nuclei were washed with 0.16*M* NaCl, a majority of the bands could be faintly seen within the footprint areas of the DNase I pattern. After the nuclei were washed with 0.2*M* NaCl, the bands reappeared within the footprints, indicative of the dissociation of protein-DNA complexes. There was no evidence for differential dissociation of protein or proteins from these sites. Identical results were obtained with nuclei from mitotic and G1 cells.

We and our co-workers have shown that there is a transcriptional component to the regulation of expression of the cell cycle–dependent human histone genes, which is reflected by a three- to fivefold enhanced level of transcription during the initial 2 hours of S phase (*4, 16*). Analysis of deletion mutants both in vivo and in vitro indicates three types of sequences contribute to the transcription of these genes: those that influence (i) site-specific initiation, (ii) levels of constitutive transcription, and (iii) the enhanced transcription at the onset of S phase.

In this study we have used genomic sequencing to investigate protein-DNA interactions in a cell cycle–dependent H4 histone gene in vivo. Within 150 nucleotides upstream of the ATG codon, we detected two sites at which specific guanine residues exhibited altered reactivity to DMS (site I, −150 to −117 nucleotides; site II, −91 to −50 nucleotides). The interaction of specific factors with these sites was confirmed by treating nuclei with DNase I, which yielded two footprints mapping precisely to the positions of the protected guanine residues. The same DNase I–treated nuclei contain a hypersensitive region, which extends over these sites (*6*), thereby correlating transcription-linked alterations in chromatin structure with specific protein-DNA interactions. Treating nuclei with increasing salt concentrations revealed that the loss of both footprints occurred at approximately 0.2*M* NaCl, indicating the dissociation of proteins which interact at these sites. Identical studies with synchronized human cells revealed that these protein-DNA interactions persist throughout the cell cycle.

We have also scanned flanking sequences from −600 to +500 nucleotides, using all of the probes depicted in Fig. 1. Sites I and II were the only regions where strong protein-DNA interactions could be detected (Fig. 4). However, deletion analysis has indicated that other upstream elements may influence the level of transcription of this gene (*12*); screening these regions should reveal subtle differences in the DMS and the DNase I patterns.

The comparison of nucleotide sequences

and the contacts with specific guanine residues within sites I and II lead us to predict that different proteins interact with these sites. Site II encompasses a TATA box and, unlike site I, contains interactions with guanine residues on both strands. The specific G contacts in site II can be visualized as part of a region with partial dyad symmetry (as shown below) with the insertion of a T·A base pair on the 5′ side of the dyad.

```
   o o       o
C T G G T C C G
G A C C A G G C
o         o o
```

In addition, the central sequence GGTCC has been shown to be specific for histone genes (*17*). The 5′ portion of site II includes two additional guanine contacts on the upper strand that are centered about the sequence *GCTTTCGGTTTTC*, containing an imperfect hexanucleotide repeat. These two other guanine contacts and the large size of the DNase I footprint indicate that more than one protein interacts at site II. A functional role for site II is suggested by the requirement for the entire site in order to support correctly initiated transcription when deletion mutants were assayed in vivo (*12*). Interestingly, only the 3′ segment of site II is necessary for properly initiated transcripts in whole cell extracts (*18*) or in nuclear extracts (*19*).

In the case of the 3′ portion of site I, the sequence around position −125 includes a region with similarity to the Sp1 decanucleotide consensus $\frac{G}{T}\frac{G}{A}GGCG\frac{G}{T}\frac{G}{A}\frac{G}{A}\frac{C}{T}$ (*20*). In addition the G contacts we detect in this region on the upper strand are similar to those for three tandem Sp1 binding sites in the HTLV-III retroviral promoter (*21*). However, we do not see the tandem arrangement of Sp1 sites characteristic of viral promoters in this H4 histone gene (*22*). In the 5′ portion of site I, we see two additional G contacts in a region containing a direct hexanucleotide repeat *GAAATGACGAAATG*; these contacts occur symmetrically around a central AC dinucleotide. A gel retardation assay provides evidence that sequences in the 5′ portion of site I are able to bind to a protein (HiNF-A) fractionated from HeLa nuclear extracts (*23*). The Taq I site at −133 is also highly accessible to restriction endonuclease digestion in nuclei, further evidence of the potential for interaction of two different proteins with site I. If the protein interacting with the 3′ portion of site I is Sp1, this would be the first demonstration of Sp1 binding in vivo. Our results are consistent with the potential for binding of at least four proteins to the two sites detected in vivo.

We have interpreted our results within the context of the types of sequences that most likely contribute to transcription of this cell cycle–regulated human H4 histone gene in vivo. Because these protein-DNA interactions persist throughout the cell cycle, we suggest that these complexes contribute to the basal level of transcription, which occurs during G_1, S, G_2, and mitosis (*16, 24*). These results do not provide a direct explanation for the enhanced transcription of this H4 histone gene in early S phase (*4, 16*). However, it is possible that these protein-DNA interactions, detected throughout the cell cycle, may serve as contact points for factors that are responsible for the cell cycle–specific fluctuations in transcription of this gene. Alternatively, specific protein-DNA interactions may be found in a more upstream or in a more downstream region than we have investigated.

REFERENCES AND NOTES

1. F. Sierra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1795 (1982).
2. N. Heintz, M. Zernik, R. G. Roeder, *Cell* **24**, 661 (1981).
3. G. S. Stein *et al.*, in *Recombinant DNA and Cell Proliferation*, G. S. Stein and J. L. Stein, Eds. (Academic Press, New York, 1984), pp. 107–143.
4. M. Plumb, J. Stein, G. Stein, *Nucleic Acids Res.* **11**, 2391 (1983).
5. M. Artishevsky, A. M. Delegeane, A. S. Lee, *Mol. Cell. Biol.* **4**, 2364 (1984).
6. S. Chrysogelos, D. E. Riley, G. Stein, J. Stein, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7535 (1985).
7. M. L. Moreno, S. A. Chrysogelos, G. S. Stein, J. L. Stein, *Biochemistry* **25**, 5364 (1986).
8. L. Green *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2315 (1986).
9. O. Capasso and N. Heintz, *ibid.* **82**, 5622 (1985).
10. H. L. Sive, N. Heintz, R. G. Roeder, *Mol. Cell. Biol.* **6**, 3329 (1986).
11. L. Dailey, S. M. Hanly, R. G. Roeder, N. Heintz, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7241 (1986).
12. P. Kroeger *et al.*, *ibid.*, in press.
13. G. M. Church and W. Gilbert, *ibid.* **81**, 1991 (1984).
14. A. Ephrussi, G. M. Church, S. Tonegawa, W. Gilbert, *Science* **227**, 134 (1985).
15. K. Zinn and T. Maniatis, *Cell* **45**, 611 (1986).
16. L. Baumbach, G. S. Stein, J. L. Stein, *Biochemistry*, in press.
17. C. C. Hentschel and M. L. Birnstiel, *Cell* **25**, 301 (1981).
18. F. Sierra, G. Stein, J. Stein, *Nucleic Acids Res.* **11**, 7069 (1983).
19. K. Wright, personal communication.
20. M. R. Briggs, J. T. Kadonaga, S. P. Bell, R. Tjian, *Science* **234**, 47 (1986).
21. K. A. Jones, J. T. Kadonaga, P. A. Luciw, R. Tjian, *ibid.* **232**, 755 (1986).
22. D. Gidoni *et al.*, *ibid.* **230**, 511 (1985).
23. A. J. van Wijnen, J. L. Stein, G. S. Stein, *Nucleic Acids Res.* **15**, 1679 (1987).
24. N. Heintz, H. L. Sive, R. G. Roeder, *Mol. Cell. Biol.* **3**, 539 (1983).

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The Dynamics of Free Calcium in Dendritic Spines in Response to Repetitive Synaptic Input

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Increased levels of intracellular calcium at either pre- or postsynaptic sites are thought to precede changes in synaptic strength. Thus, to induce long-term potentiation in the hippocampus, periods of intense synaptic stimulation would have to transiently raise the levels of cytosolic calcium at postsynaptic sites—dendritic spines in the majority of cases. Since direct experimental verification of this hypothesis is not possible at present, calcium levels have been studied by numerically solving the appropriate electro-diffusion equations for two different postsynaptic structures. Under the assumption that voltage-dependent calcium channels are present on dendritic spines, free intracellular calcium in spines can reach micromolar levels after as few as seven spikes in 20 milliseconds. Moreover, a short, but high-frequency, burst of presynaptic activity is more effective in raising levels of calcium and especially of the calcium-calmodulin complex than sustained low-frequency activity. This behavior is different from that seen at the soma of a typical vertebrate neuron.

AN INCREASE IN FREE, INTRACELLULAR calcium is believed to be the critical signal initiating the sequence of events leading to short- or long-term modifications of synaptic strength (*1, 2*). It has been proposed that the entry of calcium at the postsynaptic site triggers actin-myosin contractions in the spine neck (*3*), thereby inducing a change in the synaptic effectiveness (*4*). In other proposals, calcium activates a proteinase, which can increase the number of glutamate receptors (*2*), reduce

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