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A Sequence-Specific Single-Strand-Binding Protein for the Late-Coding Strand of the Simian Virus 40 Control Region

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We have purified a protein from uninfected monkey CV1 cells that binds specifically in vitro to the late-coding simian virus 40 DNA strand in the region of transcription control without any detectable binding to the complementary single strand. Nuclease protection experiments detected two binding sites in the 21-base-pair repeat region. The protein did not bind to this region in the double-stranded form, nor did it bind to RNA synthesized in vitro by using either DNA strand as a template. This protein, and perhaps other DNA single-strand-sequence-specific proteins, may play a role in the control of gene expression in higher organisms.

Dissection of eucaryotic genes has allowed the identification of DNA sequence elements that play a role in the control of gene expression, and many of them are thought to act at the level of transcription control by interacting with sequence-specific DNA-binding proteins. Purification of the factors that modulate gene expression by interacting with regulatory DNA sequences has thus become an important step towards a better understanding of gene regulation in higher organisms.

The control region of simian virus 40 (SV40) is a particularly good system for this kind of study, since it comprises, in a few hundred base pairs (bp), several DNA sequence elements that are important for viral gene transcription (for a review, see reference 23). Upstream from the gene for T antigen and the origin of replication, for example, is an AT-rich stretch similar to a TATA box; there are three direct repeats of a GC-rich, 21-bp sequence that also belong to the early promoter and to which the transcription factor Sp1 binds; and preceding the late genes is the enhancer sequence, which includes two 72-bp repeats and has the characteristic property of stimulating transcription in either orientation from a distance.

T antigen is the only virus-coded protein which interacts with the control region, and all other factors involved in transcription must necessarily arise from the host itself. Proteins from uninfected cells that bind to specific portions of this region include the well-characterized transcription factor Sp1, which binds to the sequence GGGCGG in the 21-bp repeats (5, 8–10, 14, 15, 19). A second transcription factor which also binds to the 21-bp repeat region has recently been identified (20). In addition, several research groups have shown, in different ways, the existence of factors that interact with the enhancer (1, 2, 4, 7, 18, 21, 22, 24, 27, 29, 30, 32–36, 40–42).

Here we report the identification of a protein that binds to the late-coding single strand at two specific sites in the 21-bp repeat region.

MATERIALS AND METHODS

Cell culture. African green monkey cells (CV1 line) were maintained as monolayers in Eagle minimal essential medium supplemented with penicillin and streptomycin and 10% newborn calf serum (Boehringer Mannheim Biochemicals). For nuclei preparations, cells were grown to confluence in 20 25-cm-square plates (Nunc), yielding approximately 10^9 cells.

DNA substrates. A plasmid (pMW1) containing the control region of SV40 with a single 72-bp repeat was constructed with SV40 DNA (strain 776): the *Hin*dIII fragment that encompasses the control region (map position [m.p.] 5171 to 1046) was inserted at the *Hin*dIII site of pBR322 and the 72-bp *Nsi*I fragment (SV40 m.p. 126 to 198) was deleted. Fragments obtained from this plasmid were used in most experiments; experiments performed with uncloned SV40 DNA gave identical results (data not shown).

DNA labeling was performed with either [γ-^{32}P]ATP (5,000 Ci/mmol; Amersham Corp.) and polynucleotide kinase or [α-^{32}P]dCTP (3,000 Ci/mmol) and the Klenow fragment of DNA polymerase. Following labeling, DNA fragments or single strands were purified by chloroform-isoamyl alcohol extraction, gel electrophoresis, electroelution, and ethanol precipitation.

For strand separation, DNA was denatured at 90°C for 2 min in 10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA, chilled in ice-cold water, adjusted to a concentration of 2% in glycerol, and immediately loaded on a 4% polyacrylamide gel similar to the gels used for fractionation of DNA-protein complexes (37; see below). In many cases, particularly in the case of the SV40 control region, strands of DNA fragments up to 250 bp could be separated on this type of gel. After electroelution of the single strands, their identities were determined by chemical sequencing. The slow-migrating strand of the SV40 control region was thus found to be the late strand (see Fig. 1 and 3).

RNA substrates. For in vitro synthesis of RNA substrates for protein binding, a plasmid containing a promoter for T7 RNA polymerase was used (pTZ19R; Pharmacia). The *Bgl*I-*Hpa*II fragment (SV40 m.p. 5235 to 346) of pMW1 was made blunt ended with T4 DNA polymerase and cloned in either orientation at the *Hin*dIII site of pTZ19R by using *Hin*dIII linkers (New England BioLabs, Inc.). Both plasmids were linearized and transcribed with T7 RNA polymerase (Boehringer kit) and [α-^{32}P]CTP (800 Ci/mmol; Amersham).

Nonradioactive CTP was added to the incubation mix in quantities such that the specific activities of both RNAs were approximately equal to the specific activity of the DNA used in DNA-protein-binding experiments.

The identities of the RNAs were confirmed by checking that their sizes depended in the expected way on the restriction site used for linearizing the plasmids. For the

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experiment shown in Fig. 5, the *Pst*I site in the plasmid polylinker was used, yielding RNA molecules 304 nucleotides long.

Following synthesis, the RNAs were purified by electrophoresis on a 4% polyacrylamide gel, followed by electroelution.

Purification of protein H16. Frozen nuclei from approximately 10^9 cells, purified as described elsewhere (37), were quickly thawed, pelleted, and suspended by gentle vortexing in 7 ml of cold 0.6 M NaCl–50 mM sodium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.5)–1 mM EDTA–1 mM dithiothreitol (DTT) containing the five proteinase inhibitors used during nuclei purification (phenylmethylsulfonyl fluoride, antipain, leupeptin, chymostatin, and pepstatin A). After 30 min at 0°C with occasional stirring, the suspension was centrifuged at 10,000 × *g* for 30 min at 4°C, and the pellet was discarded.

The 0.6 M NaCl nuclear extract was diluted sixfold with 50 mM sodium HEPES (pH 7.5)–1 mM EDTA–1 mM DTT. A light precipitate that formed upon dilution was discarded by centrifugation. The extract was applied to a 20-ml phosphocellulose column (P11; Whatman, Inc.) preequilibrated with 50 mM sodium HEPES (pH 7.5)–1 mM EDTA–1 mM DTT–75 mM NaCl. The column was washed with the same buffer and eluted with a linear gradient of 75 mM to 2 M NaCl in 50 mM sodium HEPES (pH 7.5)–1 mM EDTA–1 mM DTT.

Without the DNA-binding activities being assayed, four separate pools were made with the fractions eluting under 100 mM, between 100 and 250 mM, between 250 and 500 mM, and above 500 mM. Each pool was dialyzed against 25 mM Tris hydrochloride (pH 7.5)–1 mM DTT–30 mM NaCl and applied to a 1-ml fast-protein liquid chromatography mono Q column (Pharmacia) equilibrated in the same buffer. Elution was done with a 20-ml linear gradient of 30 mM to 1 M NaCl in 25 mM Tris hydrochloride (pH 7.5)–1 mM DTT. Fraction H16, which contained the protein described here, originated from the second phosphocellulose pool (100 to 250 mM NaCl) and eluted from the mono Q column at 250 mM NaCl.

Both columns were run at room temperature, with fractions immediately transferred to 0°C after collection. For long-term storage, fractions were adjusted to 15% glycerol and 1 mg bovine serum albumin per ml and kept at −70°C. For routine use, small aliquots were stored at −20°C in 50% glycerol and 1 mg of bovine serum albumin per ml.

Electrophoresis of DNA-protein complexes. For denaturation, DNA (or RNA) was heated at 90°C for 2 min in 10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA and cooled to room temperature just before use. Nonradioactive *Escherichia coli* DNA sonicated to an average length of 1 kilobase pair was used as competitor DNA.

DNA (5,000 cpm, about 0.1 ng) was incubated with proteins (1 μl) at room temperature (about 22°C) for 30 min in 25 μl of 50 mM NaCl–10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA–1 mM DTT–1 mg of bovine serum albumin per ml. Electrophoresis on a 4% polyacrylamide gel was done as described elsewhere (37). The gel was dried and autoradiographed.

Digestion of DNA-protein complexes with T4 DNA polymerase. The complex of protein H16 with 5'-end-labeled DNA was formed as described above but without the addition of competitor DNA. $MgCl_2$ was then added to 2 mM, and the mixture was digested at 37°C for 2 min with 0.3 U of T4 DNA polymerase (New England BioLabs). The reaction was stopped by the addition of EDTA.

RESULTS

Protein that binds specifically to one of the single strands of SV40 control region. Nuclear extracts were prepared from uninfected African green monkey CV1 cells, permissive for SV40, to search for proteins that bind specifically to the SV40 *Sty*I restriction fragment that extends from m.p. 37 to 333 (numbering as in reference 38) and encompasses both the enhancer and the 21-bp repeat region. The actual DNA fragment used was a cloned, 224-bp-long segment obtained by deleting one of the 72-bp repeats. After end labeling, this fragment was incubated with protein extracts in the presence of various amounts of nonradioactive competitor *E. coli* DNA, and the DNA-protein complexes formed were detected by polyacrylamide gel electrophoresis and autoradiography. Since this technique detects abundant nonspecific proteins as well as the specific ones, the sequence specificity of the proteins was assessed by comparing their binding to a labeled fragment from pBR322 (37).

Initial assays performed with crude nuclear extracts failed to reveal any protein but Sp1. However, since rare proteins may be masked in the assay by abundant specific proteins, such as Sp1, or even by nonspecific proteins, we fractionated the nuclear extracts. We first loaded a crude extract on a phosphocellulose column and arbitrarily made four pools with the eluted fractions without assaying their DNA-

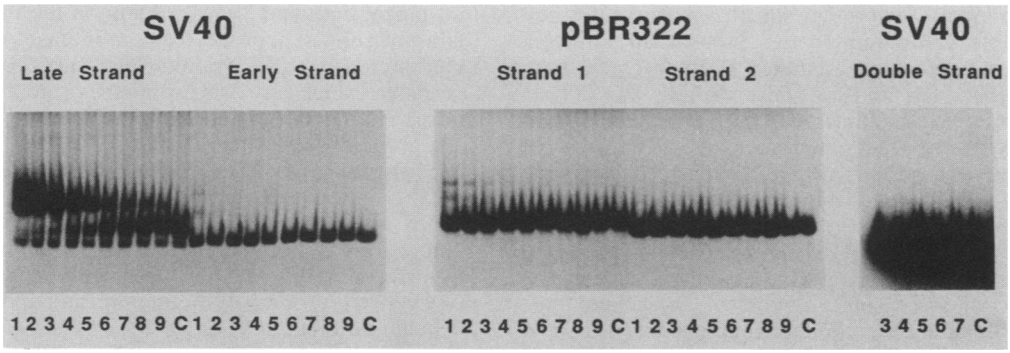

FIG. 1. Interaction of proteins from fraction H16 with DNA. Polyacrylamide gel electrophoresis was used to assay the binding of proteins contained in fraction H16 to the following five 5'-end-labeled DNA fragments: both single strands of the 224-bp *Sty*I fragment from the SV40 control region (the late strand is slightly contaminated with the early strand; see late strand, lane C), both single strands of the 185-bp *Eco*RI-*Eco*RV fragment from pBR322, and the same *Sty*I fragment from SV40 as described above but double stranded. Incubation of the labeled DNA (about 0.1 ng) with fraction H16 was done in the presence of increasing amounts of denatured competitor DNA (2, 4, 8, 15, 30, 60, 125, 250, and 500 ng in lanes 1 through 9, respectively). Lanes C, Control, free DNA, no protein added.

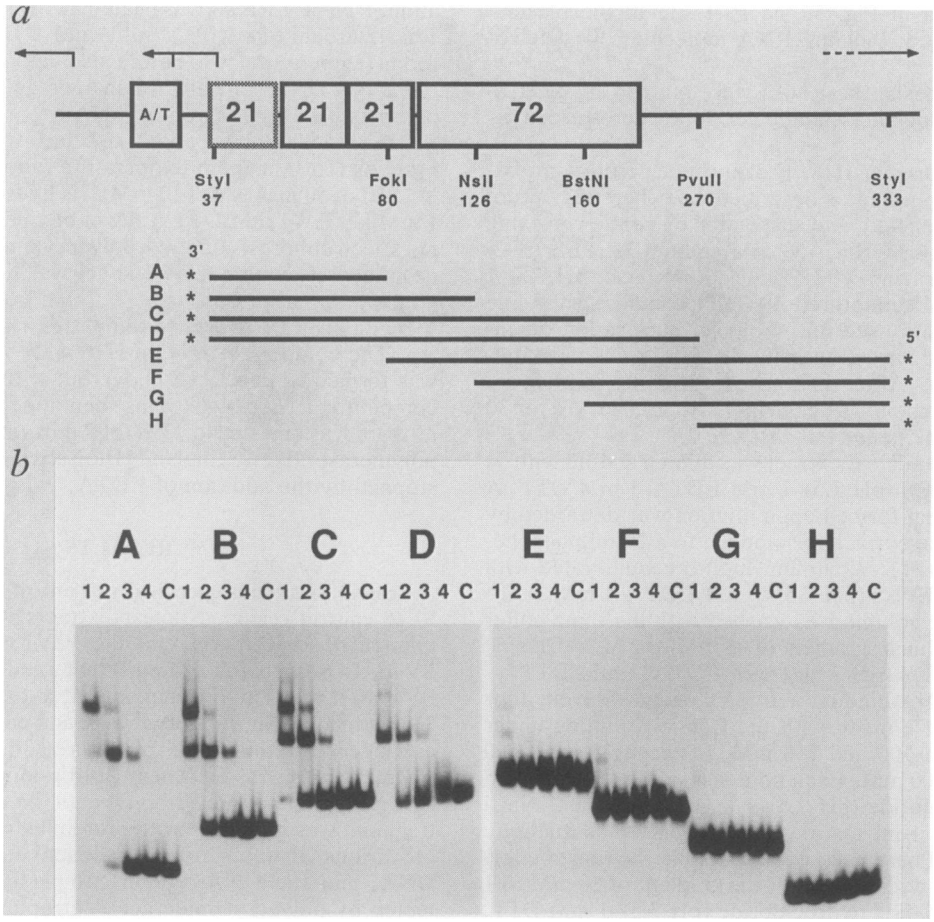

FIG. 2. Binding of protein H16 to single-stranded subfragments of the SV40 control region. (a) Schematic map of the control region of SV40 with a deletion of one of the 72-bp repeats. Arrows represent the sites of initiation of transcription (from left to right: early-early, late-early, and late transcripts). The restriction sites and the DNA fragments (A through H) used for the experiment shown in panel b are indicated, with the position of the ^{32}P end label either 5′ or 3′(*). Numbers indicate base pairs. (b) The late strands of the eight end-labeled fragments (A through H), schematically shown in panel a, were purified, mixed with protein H16 in the presence of increasing amounts of denatured competitor DNA (2, 8, 30, and 125 ng in lanes 1 through 4, respectively), and electrophoresed on a polyacrylamide gel. Equal molar amounts of labeled DNA were used. Lanes C, Control, free DNA, no protein added.

binding activities (see Materials and Methods). Subsequent fractionation of each pool on the fast-protein liquid chromatography anion exchange mono Q column gave us some 40 fractions that were individually assayed. In addition to Sp1, we were able to identify two other specific protein activities that were capable of binding to the SV40 control region (unpublished data). We used a labeled DNA fragment that was slightly denatured, and we observed in one of the fractions (fraction H16) a third binding activity that was specific for only one of the single strands.

Subsequently, both strands of the SV40 DNA fragment and those of the pBR322 control fragment were purified from their complementary counterparts and used individually in binding assays with fraction H16 (Fig. 1). The labeled single strands were incubated with a given amount of fraction H16 in the presence of increasing amounts of nonradioactive, sonicated, heat-denatured *E. coli* DNA (lanes 1 to 9). A band of slower mobility was observed only with the SV40 late strand. In contrast, few band migration differences were observed with the SV40 early strand and the pBR322 strands. Note that the small amount of early strand that contaminated the late strand in Fig. 1 remained uncomplexed even at the lowest amount of competitor used, whereas the late strand was completely shifted to the complex. We interpret the retarded band as a protein-DNA complex, since it was proteinase K sensitive and RNase A resistant; its formation was indifferent to the presence of 2 mM magnesium and 1 mM calcium in the incubation buffer (data not shown). The decrease in intensity of the complex upon increasing the amount of competitor DNA is not surprising, since any DNA-binding protein is expected to find some cognate sequences in the genome of *E. coli*. Such sites are probably rare or weak, however, since we have tested the protein with several single-stranded fragments of pBR322, covering about 80% of the plasmid, without observing any interaction (data not shown).

Incubation of fraction H16 with the double-stranded SV40 DNA fragment did not show any bands with retarded migration even at low competitor amounts and upon overexposure of the autoradiogram (Fig. 1).

Localization of binding sites with smaller restriction fragments. To determine more precisely where protein H16 binds, we prepared eight subfragments of the initial *Sty*I fragment (Fig. 2a, fragments labeled A through H). The late strands were individually purified and used for binding assays. Fraction H16 strongly bound to fragments A, B, C,

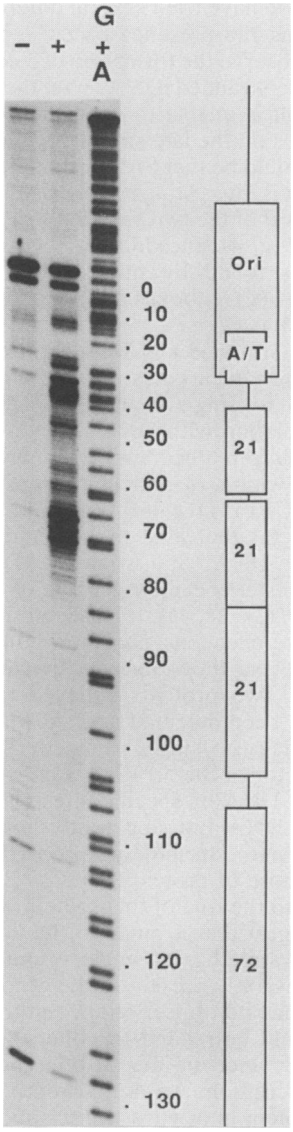

FIG. 3. T4 DNA polymerase digestion of protein H16-DNA complexes. The late strand of SV40 (fragment *Bst*NI, m.p. 5092 through 160) was 5′ end labeled at position 160 and digested by the 3′ exonuclease activity of T4 DNA polymerase in the absence (−) or presence (+) of protein H16. DNA was extracted and electrophoresed on an 8% sequencing gel with a G+A chemical sequencing reaction. The origin of replication, 21-bp repeats, and 72-bp repeat are shown on the right. Numbers to the right of the gel indicate SV40 map positions.

and D but did not bind to fragments E, F, G, and H (Fig. 2b). For fragments E through H, retarded bands of different mobilities and much lower intensities than those observed for A, B, C, and D may indicate the presence of a contaminating binding protein, perhaps not sequence specific. We conclude that protein H16 binds specifically to the late strand of SV40 in the 21-bp repeat region (see Fig. 2a). The appearance of two retarded bands may represent fragments with either one or both of the two binding sites (see below) occupied by the protein.

Binding sites localization by nuclease digestion. For footprinting experiments with a single-strand-binding protein, most enzymes are unsuitable: DNase I does not efficiently digest single-stranded DNA; micrococcal nuclease has a strong sequence specificity; DNase II, S1 nuclease, and mung bean nuclease do not work at the neutral pH required for formation of a stable protein H16-DNA complex. In addition, dimethyl sulfate interference experiments gave negative results. Therefore, we used the single-strand 3′→5′ exonuclease activity of T4 DNA polymerase. Figure 3 shows the result of T4 polymerase digestion of a 5′-end-labeled late-strand fragment that encompasses the 21-bp repeats in the absence or the presence of fraction H16. There were two regions in which the progression of the enzyme was strongly slowed only when H16 was present: the first one, just preceding the first of the 21-bp repeats, is around nucleotide 38; the second one, in the second 21-bp repeat, is around nucleotide 70 (Fig. 4). Immediately 5′ to the regions in which the enzyme pauses, i.e. at the putative binding sites of protein H16, the sequence 5′-CCGCCCC-3′ was found. However, a third CCGCCCC motif present in the third 21-bp repeat did not seem to bind protein H16. This suggests that the sequence recognized is not strictly limited to CCGCCCC and that neighboring bases on its 5′ side are also important determinants of the specificity. In any case, precise knowledge of the nucleotide sequences and of the DNA structure recognized by the protein will require an analysis of its binding sites on a large set of different DNA fragments.

Failure of protein H16 to bind to RNA. To exclude the possibility that H16 is a sequence-specific RNA-binding protein that also binds DNA in vitro when presented with homologous single-stranded DNA sequences, we investigated its RNA-binding properties. The SV40 control region was cloned in either orientation in plasmid pTZ19R, next to a promoter for T7 RNA polymerase, and RNA was synthesized in vitro with either the early strand or the late strand as the template. Figure 5 shows the interaction of protein H16 with both RNAs and with the late strand of DNA in the presence of various amounts of denatured competitor DNA. No complex was observed with RNA, while the same band

FIG. 4. DNA sequence of the 21-bp repeat region. The upper strand is the early strand; the lower strand is the late strand. The six Sp1-binding sites are indicated by rectangles above the sequence, with shading intensity increasing with Sp1 affinity. The two regions in which T4 DNA polymerase digestion pauses in the presence of protein H16 are represented by dark rectangles below the sequence. Numbers below the sequence indicate nucleotide positions.

pattern as described above (Fig. 1) was observed with the late strand of DNA.

DISCUSSION

We have purified a single-strand-binding protein that binds in vitro to specific regions in the 21-bp repeat sequence of the late strand of the control region of SV40. It does not bind to the complementary single strand, the double strand, or RNA synthesized with either DNA strand as a template.

A conservative estimate of the affinity of the protein for the late strand relative to that for the early strand can be obtained from the data presented in Fig. 1 by applying the equations of equilibria (12) to homologous lanes. With regard to lanes 4, for example, the ratio of bound DNA to free DNA is about 5 for the late strand and less than 0.5% for the early strand, as determined by quantitation of the bands on the autoradiogram. This leads to an estimate of 3 orders of magnitude for the ratio of specific to nonspecific affinities. However, it should be stressed that the protein is not pure and that the faint bands observed with the early strand might be due to a contaminating protein. This value is therefore a minimum estimate, and the actual value might be much higher. Purifying the protein to homogeneity will allow us to determine precisely this ratio, as well as the absolute affinity constants.

The transcription factor Sp1 also binds to SV40 DNA in this region. However, these two proteins are distinct, as judged by their different chromatographic behaviors (unpublished data) and, most convincingly, by the fact that protein H16 does not bind to double-stranded DNA whereas Sp1 does. Although we have tried several different conditions for binding, including the presence of Zn^{2+} or ATP, we have been unable to observe the formation of a complex of protein H16 with double-stranded DNA. We therefore think that protein H16 itself is unable to open the DNA double helix and make its sites on the late strand accessible. An interesting possibility would be that Sp1 participates in this process. In this respect, it is interesting to note that both H16 sites are located at the level of the two weakest Sp1 sites on the DNA sequence (15) (Fig. 4). In addition, the first H16 site near position 45 was found by mutagenesis to be the most important of the six GC boxes for transcription initiation at the early-early sites (3).

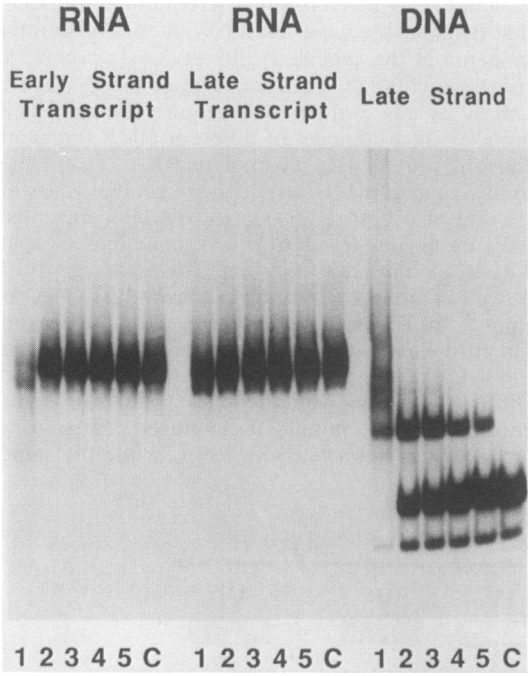

FIG. 5. Interaction of protein H16 with RNA. ^{32}P-labeled RNA, synthesized in vitro from a T7 polymerase promoter by using either the late strand or the early strand of the SV40 control region as the template (see Materials and Methods), was mixed with protein H16 in the presence of increasing amounts of denatured competitor DNA (0, 1, 2, 4, and 8 ng, lanes 1 through 5, respectively) and electrophoresed on a 4% polyacrylamide gel. The same experiment was done with late-strand DNA. Lanes C, No protein added.

Several single-stranded DNA-binding proteins have been isolated in higher eucaryotes. Many of those that bind reasonably well to single-stranded DNA-cellulose apparently have no relation with DNA in vivo (e.g., dehydrogenases, a protocollagen precursor, serum proteins involved in complement activation, α_1-antichymotrypsin; see references in 43). Proteins UP1 from calf thymus and HDP-1 from mice are related proteins that bind to single-stranded DNA, destabilize duplex DNA, and stimulate DNA polymerase, similar to proteins encoded by gene 32 of bacteriophage T4 and *ssb* of *E. coli* (17, 28, 43). It has been suggested that they derive from heterogeneous nuclear ribonucleoproteins by proteolysis (25), and they have no known sequence specificity. Recently, two proteins believed to be involved in replication have been detected and shown to possess some sequence specificity to single-stranded DNA (13, 39). The transcription factor of the 5S RNA genes, TFIIIA, binds to 5S RNA, to 5S DNA at specific sites (11, 26, 31), and to single-stranded DNA but apparently not in a sequence-specific manner (16). Its binding properties are thus very different from those of protein H16.

With respect to the role of this protein, we have no direct evidence to suggest that it binds to the late strand in vivo. However, short stretches of single-stranded DNA are supposed to be transiently present in the genome, for example, during replication and transcription, and the possible role of protein H16 could be related to either of these processes. Given the importance of the 21-bp repeat region in the control of transcription, we are currently investigating the effect of the protein in in vitro transcription experiments.

In 1971, F. Crick published a model for the chromosomes of higher organisms in which "the recognition sites needed for control purposes are mainly unpaired single-stranded stretches of double-stranded DNA" (6). Indeed, single-stranded DNA presents more structural flexibility, as well as a greater number of contact points for specific protein-DNA interactions. Achieving the high sequence specificity necessary to properly control gene activity in higher organisms may thus be made easier by the use of such interactions.

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LITERATURE CITED

1. **Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin.** 1987. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated *trans*-acting factor. Cell **49**:729–739.
2. **Azorin, F., and A. Rich.** 1985. Isolation of Z-DNA binding

proteins from SV40 minichromosomes: evidence for binding to the viral control region. Cell **41**:365–374.

3. **Barrera-Saldana, H., K. Takahashi, M. Vigneron, A. Wildeman, I. Davidson, and P. Chambon.** 1985. All six GC-motifs of the SV40 early upstream element contribute to promoter activity *in vivo* and *in vitro*. EMBO J. **4**:3839–3849.
4. **Bohmann, D., W. Keller, T. Dale, H. R. Schöler, G. Tebb, and I. W. Mattaj.** 1987. A transcription factor which binds to the enhancers of SV40, immunoglobulin heavy chain and U2 snRNA genes. Nature (London) **325**:268–272.
5. **Briggs, M. R., J. T. Kadonaga, S. P. Bell, and R. Tjian.** 1986. Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. Science **234**:47–52.
6. **Crick, F.** 1971. General model for the chromosomes of higher organisms. Nature (London) **234**:25–27.
7. **Davidson, I., C. Fromental, P. Augereau, A. Wildeman, M. Zenke, and P. Chambon.** 1986. Cell-type specific protein binding to the enhancer of simian virus 40 in nuclear extracts. Nature (London) **323**:544–548.
8. **Dynan, W. S., and R. Tjian.** 1983. Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. Cell **32**:669–680.
9. **Dynan, W. S., and R. Tjian.** 1983. The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. Cell **35**:79–87.
10. **Dynan, W. S., and R. Tjian.** 1985. Control of eukaryotic mRNA synthesis by sequence-specific DNA binding proteins. Nature (London) **316**:774–778.
11. **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.
12. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
13. **Fry, M., F. W. Perrino, A. Levy, and L. A. Loeb.** 1988. Factor D is a selective single-stranded oligodeoxythymidine binding protein. Nucleic Acids Res. **16**:199–211.
14. **Gidoni, D., W. S. Dynan, and R. Tjian.** 1984. Multiple specific contacts between a mammalian transcription factor and its cognate promoters. Nature (London) **312**:409–413.
15. **Gidoni, D., J. T. Kadonaga, H. Barrera-Saldaña, K. Takahashi, P. Chambon, and R. Tjian.** 1985. Bidirectional SV40 transcription mediated by tandem Sp1 binding interactions. Science **230**: 511–517.
16. **Hanas, J. S., D. F. Bogenhagen, and C.-W. Wu.** 1984. Binding of Xenopus transcription factor A to 5S RNA and to single stranded DNA. Nucleic Acids Res. **12**:2745–2758.
17. **Herrick, G., and B. Alberts.** 1976. Purification and physical characterization of nucleic acid helix-unwinding proteins from calf thymus. J. Biol. Chem. **251**:2124–2132.
18. **Johnson, P. F., W. H. Landschulz, B. J. Graves, and S. L. McKnight.** 1987. Identification of a rat liver nuclear protein that binds to the enhancer core element of three animal viruses. Genes Dev. **1**:133–146.
19. **Kadonaga, J. T., K. R. Carner, F. R. Masiarz, and R. Tjian.** 1987. Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. Cell **51**:1079–1090.
20. **Kim, C. H., C. Heath, A. Bertuch, and U. Hansen.** 1987. Specific stimulation of simian virus 40 late transcription *in vitro* by a cellular factor binding the simian virus 40 21-base-pair repeat promoter element. Proc. Natl. Acad. Sci. USA **84**:6025–6029.
21. **Lee, W., A. Haslinger, M. Karin, and R. Tjian.** 1987. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. Nature (London) **325**:368–372.
22. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell **49**:741–752.
23. **McKnight, S., and R. Tjian.** 1986. Transcriptional selectivity of viral genes in mammalian cells. Cell **46**:795–805.
24. **Mitchell, P. J., C. Wang, and R. Tjian.** 1987. Positive and negative regulation of transcription *in vitro*: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. Cell **50**:847–861.
25. **Pandolfo, M., O. Valentini, G. Biamonti, C. Morandi, and S. Riva.** 1985. Single stranded DNA binding proteins derive from hnRNP proteins by proteolysis in mammalian cells. Nucleic Acids Res. **13**:6577–6590.
26. **Pelham, H. R. B., and D. D. Brown.** 1980. A specific transcription factor that can bind either the 5S RNA gene or 5S RNA. Proc. Natl. Acad. Sci. USA **77**:4170–4174.
27. **Piette, J., and M. Yaniv.** 1987. Two different factors bind to the a-domain of the polyoma virus enhancer, one of which also interacts with the SV40 and c-*fos* enhancers. EMBO J. **6**:1331–1337.
28. **Planck, S. R., and S. H. Wilson.** 1980. Studies on the structure of mouse helix-destabilizing protein-1. DNA binding and controlled proteolysis with trypsin. J. Biol. Chem. **255**:11547–11566.
29. **Robbins, P. D., D. C. Rio, and M. R. Botchan.** 1986. *trans*-Activation of the simian virus 40 enhancer. Mol. Cell. Biol. **6**:1283–1295.
30. **Rosales, R., M. Vigneron, M. Macchi, I. Davidson, J. H. Xiao, and P. Chambon.** 1987. *In vitro* binding of cell-specific and ubiquitous nuclear proteins to the octamer motif of the SV40 enhancer and related motifs present in other promoters and enhancers. EMBO J. **6**:3015–3025.
31. **Sakonju, S., D. D. Brown, D. Engelke, S.-Y. Ng, B. S. Shastry, and R. G. Roeder.** 1981. The binding of a transcription factor to deletion mutants of a 5S ribosomal RNA gene. Cell **23**:665–669.
32. **Sassone-Corsi, P., A. Wildeman, and P. Chambon.** 1985. A *trans*-acting factor is responsible for the simian virus 40 enhancer activity in vitro. Nature (London) **313**:458–463.
33. **Scholer, H., A. Haslinger, A. Heguy, H. Holtgreve, and M. Karin.** 1986. *In vivo* competition between a metallothionein regulatory element and the SV40 enhancer. Science **232**:76–80.
34. **Schöler, H. R., and P. Gruss.** 1984. Specific interaction between enhancer-containing molecules and cellular components. Cell **36**:403–411.
35. **Schöler, H. R., and P. Gruss.** 1985. Cell type-specific transcriptional enhancement *in vitro* requires the presence of *trans*-acting factors. EMBO J. **4**:3005–3013.
36. **Sergeant, A., D. Bohmann, H. Zentgraf, H. Weiher, and W. Keller.** 1984. A transcription enhancer acts in vitro over distances of hundreds of base-pairs on both circular and linear templates but not on chromatin-reconstituted DNA. J. Mol. Biol. **180**:577–600.
37. **Strauss, F., and A. Varshavsky.** 1984. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome. Cell **37**:889–901.
38. **Tooze, J.** 1982. DNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Traut, W., and E. Fanning.** 1988. Sequence-specific interactions between a cellular DNA-binding protein and the simian virus 40 origin of DNA replication. Mol. Cell. Biol. **8**:903–911.
40. **Wang, X.-F., and K. Calame.** 1986. SV40 enhancer-binding factors are required at the establishment but not the maintenance step of enhancer-dependent transcriptional activation. Cell **47**:241–247.
41. **Wildeman, A. G., P. Sassone-Corsi, T. Grundström, M. Zenke, and P. Chambon.** 1984. Stimulation of *in vitro* transcription from the SV40 early promoter by the enhancer involves a specific *trans*-acting factor. EMBO J. **3**:3129–3133.
42. **Wildeman, A. G., M. Zenke, C. Schatz, M. Wintzerith, T. Grundström, H. Matthes, K. Takahashi, and P. Chambon.** 1986. Specific protein binding to the simian virus 40 enhancer in vitro. Mol. Cell. Biol. **6**:2098–2105.
43. **Williams, K. R., K. L. Stone, M. B. LoPresti, B. M. Merril, and S. R. Planck.** 1985. Amino acid sequence of the UP1 calf thymus helix-destabilizing protein and its homology to an analogous protein from mouse myeloma. Proc. Natl. Acad. Sci. USA **82**: 5666–5670.