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Regulation of human ribosomal RNA transcription

(RNA polymerase I/cloned mutant template/virus–host interactions/simian virus 40 tumor antigen)

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ABSTRACT **We have used a cell-free polymerase I transcription system derived from HeLa cells to study the regulation of human rRNA synthesis. Analysis of deletion mutants spanning the start site of transcription at nucleotide +1 indicates that the control region affecting initiation of human rRNA synthesis is contained within sequences from nucleotides −158 to +18. This promoter region can be subdivided into (*i*) a central segment of approximately 40 base pairs that is required for transcription and (*ii*) flanking sequences that influence the efficiency of transcription *in vitro*. We have examined the *in vitro* transcriptional activity of the human extract under various conditions that are thought to modulate rRNA synthesis *in vivo*. Cell-free extracts prepared from HeLa cells infected with adenovirus 2 synthesize human rRNA at levels greatly decreased relative to uninfected cell extracts. By contrast, *in vitro* transcription of human rRNA is stimulated 2- to 3-fold by the addition of purified simian virus 40 large tumor antigen to the transcription reaction. Moreover, a mutant tumor antigen known to be defective for rRNA activation *in vivo* is incapable of stimulating rRNA synthesis *in vitro*. The ability to detect these different regulatory phenomena *in vitro* provides us with an experimental basis for investigating the molecular mechanisms that control rRNA synthesis.**

In order to respond to changes in the environment, cells must be able to regulate the expression of genes that affect their metabolic and physiological state. Therefore, it is not surprising that the levels of abundant and metabolically important gene products such as the rRNAs are modulated according to the growth state of the cell. Although the regulation of rRNA synthesis in mammalian cells has been well documented, the molecular basis of transcriptional control remains poorly understood. A detailed biochemical analysis of the mechanisms governing rRNA transcription in eukaryotes has awaited the development of *in vitro* transcription systems that allow efficient *de novo* initiation of rRNA synthesis by RNA polymerase I (pol I). Recently, a number of such *in vitro* systems have been described (1–5). With the availability of these *in vitro* transcription systems, it is now possible to study certain aspects of rRNA transcription and regulation.

A detailed analysis of ribosomal transcriptional regulation will require the identification of both the proteins and the DNA sequences involved in this process. Control sequences that govern transcriptional initiation can be identified by mutagenesis of the DNA flanking the initiation site and testing the ability of the mutant DNAs to function as templates in an *in vitro* transcription assay. This approach has provided valuable information on the sequence requirements of RNA polymerase II and III control regions, but has not been rigorously applied to the regulatory regions that control transcription by pol I. As a first step toward studying the regulation of rRNA transcription in human cells, we have made a series of deletions in the region surrounding the RNA start site and have identified the location of the pol I promoter region.

We were also interested in the modulation of rRNA synthesis in response to changes in the physiological state of the cell. rRNA transcriptional control is most clearly demonstrated in the stringent response to amino acid starvation in bacterial cells (6). There is also ample documentation for regulation of rRNA synthesis in the nucleolus of animal cells under various physiological states (7, 8). For example, the growth state of tissue culture cells correlates well with the levels of rRNA transcription (9, 10). Cells that are quiescent due to nutrient starvation or serum deprivation exhibit a greatly reduced level of rRNA transcription by pol I relative to actively growing cells (9). It has also been observed that virus infection can have a dramatic effect on the levels of rRNA transcription. Early after infection of host cells by simian virus 40 (SV40) or polyoma virus, there is activation of rRNA transcription (11–13). By contrast, there appears to be a shutdown of rRNA transcription late after infection of human cells by adenovirus 2 (14, 15).

In this report, we focus on how viral infection and specific viral gene products can modulate the transcriptional activity of pol I in extracts prepared from HeLa cells. Our findings provide a basis for studying specific regulatory events that modulate the expression of rRNA under various physiologic and metabolic states.

MATERIALS AND METHODS

Cells and Viruses. Suspension cultures of HeLa cells and CV-1 monkey cells were grown as described (5, 16). SV40 strain 776 and the SV40 mutant dl 1151 (17) were propagated on monkey cells as described (16, 18).

Recombinant DNA. The plasmid prHu4 contains a 1.3-kilobase *Sal* I fragment of the human rDNA repeat inserted in both orientations into the *Sal* I site of pBR322. The Δ5′ mutants were constructed by *Eco*RI digestion of prHu4a (transcription proceeding counterclockwise) followed by BAL 31 nuclease treatment. The Δ3′ series mutants were generated by cleaving prHu4b (transcription proceeding clockwise) with *Bst*EII and then digesting with nuclease BAL 31. Both sets of linear DNAs were treated with *Pvu* II which creates a blunt end at a unique site within the bacterial sequences and thus fixes one end point for each deletion mutant. These molecules were recircularized with bacteriophage T4 DNA ligase and used to transform competent *Escherichia coli* 294. Ampicillin-resistant colonies were chosen randomly and the plasmid DNAs were purified as described (19, 20). The size of deletions was confirmed by direct chemical sequence determination techniques (21).

Synthesis and Analysis of *in Vitro* RNAs. HeLa cells were grown to a density of 4–6 × 10^5 cells per ml in suspension at

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Abbreviations: SV40, simian virus 40; pol I, RNA polymerase I; T antigen, tumor antigen; bp, base pair(s).

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37°C. Cells infected with adenovirus 2 were harvested at 19 hr after infection (22). Whole-cell transcription extracts were prepared as described by Sugden and Keller (23). Transcription reactions were performed as described (5). In transcription experiments in which tumor antigen (T antigen) was used, purified T antigen was diluted to a concentration of 10 μg/ml in 20 mM Hepes, pH 7.9/0.1 mM EDTA/2 mM dithiothreitol/17% (vol/vol) glycerol containing bovine serum albumin at 10 μg/ml and was added to the reaction mixture; transcription was initiated by adding the nucleotide triphosphates, including the labeled GTP.

RNA polymerase assays were performed as described by Schwartz *et al.* (24).

Purification of T Antigen and Related Proteins. Monkey cells (CV1) infected with SV40 or HeLa cells infected with adenovirus–SV40 hybrid viruses were used as sources of SV40 T antigen. The D2 protein produced in Ad2$^+$D2-infected HeLa cells was purified as described by Tjian (25). Wild-type T antigen from HeLa cells infected with AdSV284 and mutant T antigen from CV-1 monkey cells lytically infected with dl 1151 (17) were purified by immunoaffinity chromatography.

RESULTS AND DISCUSSION

Mapping the Transcriptional Control Region. In order to map the regulatory sequences that govern rRNA transcription initiation, a series of deletion mutants was generated in the region flanking the initiation site and the ability of the mutant templates to function in the *in vitro* transcription assay was tested. Deletions originating both upstream and downstream from the RNA start site were introduced into the rDNA to give a series of overlapping 5′ and 3′ deletion mutants. From a large assortment of these mutants, 10 5′ and 6 3′ deletion mutants were chosen for further characterization. The DNA sequence for each of these 16 mutants was determined in order to identify the precise end points of the deletion.

DNA templates derived from these mutants were used to direct *in vitro* pol I transcription. Two types of assays were used to measure the relative efficiency of transcription directed by the mutant templates. In one case, only the mutant DNA was added to the *in vitro* reaction and thus the level of RNA synthesis reflected the efficiency with which the mutant DNA served as template (Fig. 1*A*). In the second assay, the *in vitro* reaction was supplemented with an equal molar amount of both mutant DNA and wild-type DNA, each truncated with a different restriction enzyme to generate runoff RNA products of distinguishable sizes (Fig. 1*B*). This mixing assay allows us to measure the ability of mutant template to compete with the wild-type DNA for transcription factors.

Mutants Δ5′ −234 and −158 and Δ3′ +71, +22, and +18 directed transcription of runoff products at levels indistinguishable from those of full-length wild-type templates derived from prHu5 in both the single-template assay and the mixing experiment. By contrast, mutants Δ5′ −132, −109, −83, −55, and −52 and Δ3′ +16 and +7 showed a decreased level of transcription. It is interesting to note that the Δ5′ deletion mutants were only 20% as efficient in competing with wild-type DNA in the mixing assay but were 45% as efficient in directing transcription alone. The Δ3′ +16 and +7 deletion mutants exhibited a reduction in template efficiency of approximately 50–60% in both types of assays. Mutant Δ5′ −26 directed transcription at a level approximately 5% that of wild-type whereas Δ5′ −17 and Δ3′ −8 were essentially incapable of directing any detectable levels of RNA synthesis in the *in vitro* reactions. Finally, truncation of wild-type template DNA by cleavage with *Hin*cII at nucleotide −166 rendered the DNA incapable of directing transcription in either assay (Fig. 1*A*, lane 11; Fig. 1*B*, lane 11). Based on the size of the runoff transcripts, RNA synthesis directed by mutant DNA templates all appear to initiate at the same site as transcripts synthesized from wild-type rDNA, although mapping the 5′ termini of the RNA at nucleotide resolution is still not complete.

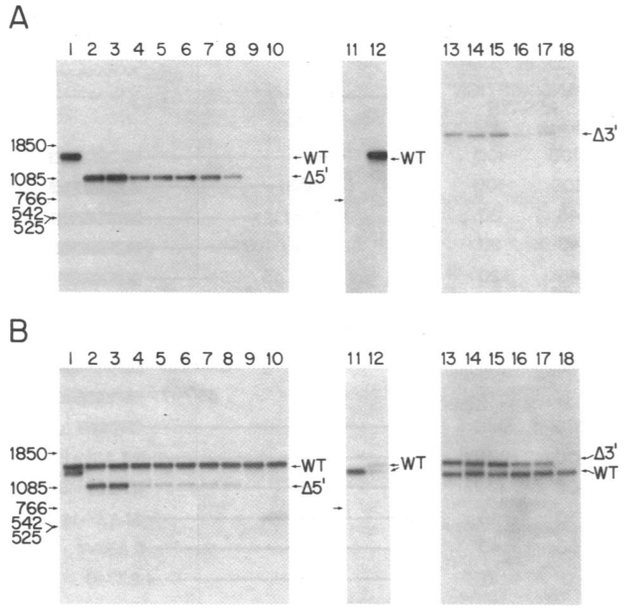

FIG. 1. Analysis of *in vitro* transcripts directed by promoter deletion mutants. (*A*) The transcripts derived from wild-type and mutant Δ5′ −234, −158, −132, −109, −83, −55, −52, −26, and −17 templates cleaved by *Bam*HI are shown in lanes 1–10, respectively. Transcription products from prHu3 cleaved with *Hin*cII and *Bam*HI are shown in lanes 11 and 12, respectively. Transcription derived from mutants Δ3′ +71, +22, +18, +16, +7, and −8 templates produced by cleavage with *Pst* I are shown in lanes 13–18, respectively. Arrows denote the predicted size of the runoff RNA from the wild-type (WT) and mutant templates. (*B*) Truncated mutant and wild-type templates were simultaneously added in equimolar amounts in a mixing assay. Lanes 1 and 12 exhibit the results of mixing two wild-type templates. Transcripts from the mixing assays using mutants Δ5′ −234, −158, −132, −109, −83, −55, −52, −26, and −17 are shown in lanes 2–10 (lower bands), respectively, and those using mutants Δ3′ +71, +22, +18, +16, +7, and −8 are in lanes 13–18 (upper bands), respectively. Lane 11 exhibits the competition between an *Xho* I-cleaved wild-type template and wild-type template cleaved with *Hin*cII. Nucleotide numbers to the left refer to standards.

These findings indicate that sequences necessary to promote transcription of the human rRNA are contained within nucleotides −158 to +18 (Fig. 2). Recent reports suggest that a similar region of rDNA in *Xenopus laevis*, mouse, and *Drosophila melanogaster* contains the control sequences for transcription by pol I (26–28). Our deletion analysis reveals that the putative human pol I promoter sequence can be divided into three domains. A central region within nucleotides −26 to +7 appears to contain sequences that are essential for transcriptional initiation. Flanking this core domain is a stretch of sequences between nucleotides −158 and −26 that appears to play some role in modulating the efficiency of transcription *in vitro* but is not absolutely required. The third domain in the control region includes a short stretch of DNA located at the start of the transcribed sequences (nucleotides +7 to +18).

Unlike the region upstream from the start site, the transcribed spacer DNA contains a 16-base-pair (bp) sequence extending from +1 to +18 that is highly conserved among the rDNA sequences of man, rhesus monkey, mouse, and rat (refs. 4 and 29; unpublished data). Strikingly, the third domain in the

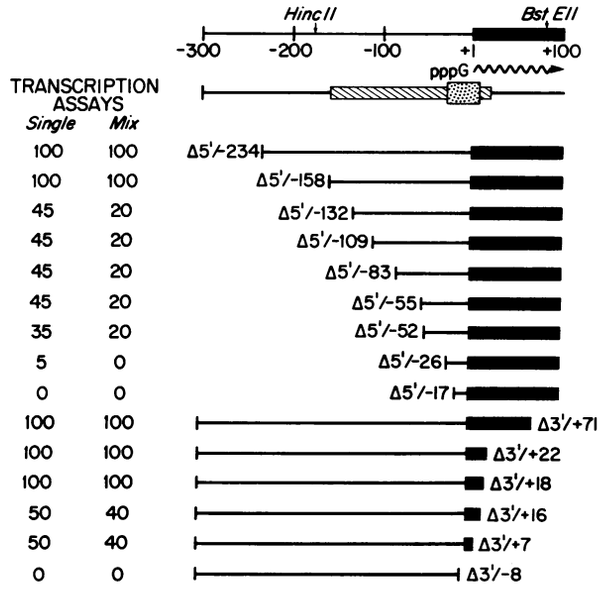

FIG. 2. Diagram of deletion mutants defining the ribosomal promoter region. The 5′ flanking DNA is represented as lines; the transcribed DNA is shown as solid boxes. The two columns at the left indicate the *in vitro* transcription efficiencies of the mutants alone (Single) or mixed with wild type (Mix) as determined by scanning densitometry of the runoff transcripts in Fig. 1. In the diagram at the top, the stippled box indicates the minimal sequences necessary for directing transcription of rDNA; the hatched boxes indicate the 5′ and 3′ flanking regions that have an effect on the efficiency of rRNA transcription by pol I.

human pol I regulatory region is contained entirely within this region of homology. Like the 5′ flanking domain, this region appears to have some effect on the level of transcription *in vitro*. The mutational analysis thus suggests a functional role for this conserved sequence in the initiation of rRNA synthesis.

Inhibition of rRNA Synthesis by Adenovirus 2 Infection. Changes in host rRNA transcription as a consequence of viral infection have been observed for many types of animal viruses, such as adenovirus 2 (14, 15), herpes simplex virus (30–32), poxvirus (33, 34), and poliovirus (35). Most of these viral infections cause a significant inhibition of rRNA synthesis in the host cell. In HeLa cells infected with adenovirus 2, the synthesis of mature 18S and 28S rRNA has been shown to be dramatically reduced late in the infectious cycle (14, 36). Some earlier studies also suggested that the synthesis of 45S nuclear ribosomal precursor RNA is inhibited by adenovirus 2 infection (15). The mechanism of this virus-induced shutoff of host rRNA synthesis is not known.

Our *in vitro* pol I transcription system derived from HeLa cells has allowed us to begin investigating the molecular basis of this phenomenon. We compared the efficiency of pol I transcription in cell-free extracts prepared from either uninfected or adenovirus 2-infected HeLa cells. Promoter-dependent transcription from a cloned human ribosomal template was measured by using the *in vitro* runoff assay. The cell-free extract prepared from adenovirus 2-infected HeLa cells was approximately 1/20th as active in directing transcription initiating from the human rRNA promoter as the extract prepared from uninfected HeLa cells (Fig. 3*A*). This differential transcriptional activity between infected and uninfected cell extracts is not due merely to a difference in RNA polymerase concentrations because both extracts contained approximately the same amounts of pol I activity when assayed with a nonspecific template such as sonicated calf thymus DNA in the presence of α-amanitin (Fig. 4). Moreover, inhibition of transcription appears

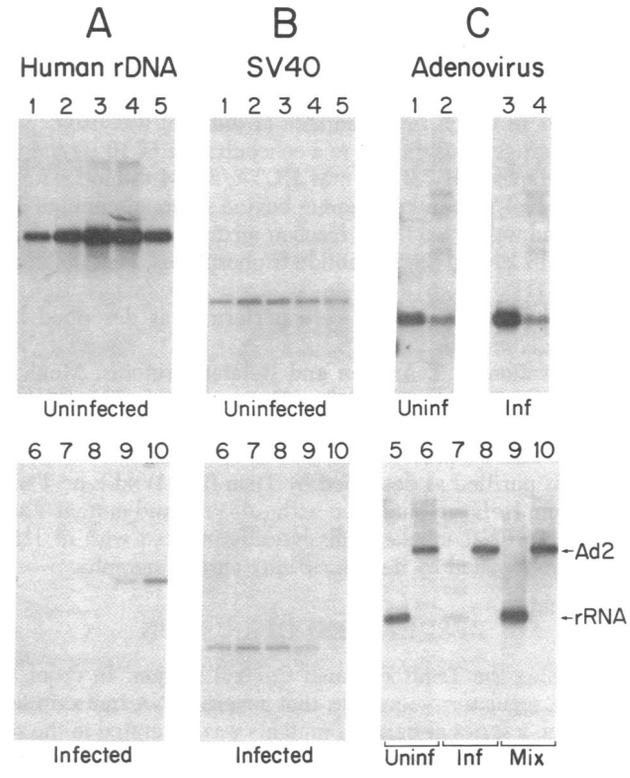

FIG. 3. Specific transcription by pol I and II in extracts prepared from HeLa cells and adenovirus 2-infected HeLa cells. (*A*) Transcription reactions containing human rDNA were supplemented with 250 μg (lanes 1 and 6), 200 μg (2 and 7), 150 μg (3 and 8), 100 μg (4 and 9), or 50 μg (5 and 10) of extract from uninfected (1–5) or adenovirus 2-infected (6–10) HeLa cells. (*B*) Polymerase II runoff transcription directed by template DNA containing the SV40 early promoter (pSV01) was carried out in the presence of 800 μg (lanes 1 and 6), 700 μg (2 and 7), 600 μg (3 and 8), 500 μg (4 and 9), or 300 μg (5 and 10) of extract from uninfected (1–5) or adenovirus-infected (6–10) HeLa cells. (*C*) Polymerase II runoff transcription directed by template DNA containing the adenovirus 2 major late promoter (pALE) was carried out in the presence of 600 μg (lanes 1 and 3) and 300 μg (2 and 4) of extract prepared from uninfected (1 and 2) or infected HeLa cells (3 and 4). Transcription reaction mixtures containing either pol I template (lanes 5, 7, and 9) or pol II template (6, 8, and 10) were used to direct RNA synthesis by uninfected HeLa cell extracts (5 and 6), adenovirus 2-infected HeLa cell extract (7 and 8), or a mixture of equal amounts of uninfected and infected transcription extract (9 and 10). The polymerase I template DNA was prHu3 cleaved with *Bam*HI; the polymerase II template DNA was pALE cleaved with *Eco*RI. Arrows denote the predicted size of the runoff RNA.

to be specific for rRNA synthesis because the polymerase II activity, measured in both the specific and nonspecific assays, was similar in the infected and uninfected cell extracts (Fig. 3*B*, lanes 1–10; Fig. 3*C*, lanes 1–4; Fig. 4). Although the amount of RNA synthesized from SV40 templates was slightly reduced in the extract from infected cells (Fig. 3*B*, lanes 1–10), the amount of RNA transcribed from the adenovirus template (containing the major late promoter) actually was greater in the infected extract than in the uninfected extract (Fig. 3*C*, lanes 1–4). A similar observation was made previously (37).

These *in vitro* results are in good agreement with the accepted view that adenovirus 2 somehow can modulate transcription of both host rRNA and viral mRNA late in the infectious cycle. These findings also provide direct evidence that the regulation of rRNA synthesis in adenovirus-infected cells is at least partly mediated at the level of transcriptional initiation. The reduced level of rRNA transcription observed *in vitro* could

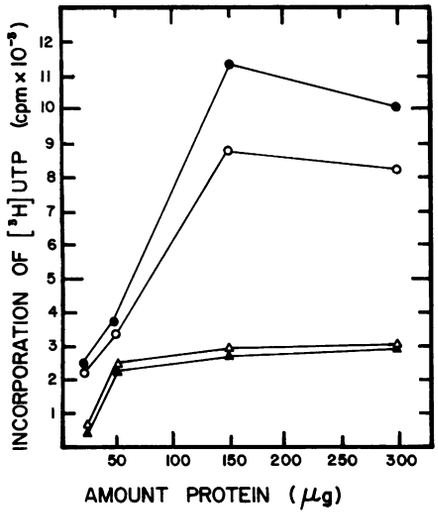

FIG. 4. Transcription by pol I and II from calf thymus DNA in extracts prepared from HeLa cells and adenovirus 2-infected HeLa cells. Reactions were carried out by adding calf thymus DNA to infected and uninfected HeLa cell extracts. The incorporation of [^{3}H]UTP into acid-insoluble RNA chains is plotted as a function of the amount of protein in reactions containing extract from uninfected cells (open symbols) or extract from adenovirus 2-infected HeLa cells (solid symbols). Reaction mixtures containing α-amanitin (100 μg/ml) were used to measure the pol I activity in the extracts (○, ●). The level of pol II was determined by calculating the difference in incorporation between reactions performed in the presence of α-amanitin (1 μg/ml) and absence of α-amanitin (△, ▲).

be the result of specific repressors induced or encoded by adenovirus. However, in an experiment in which equal amounts of extract prepared from infected and uninfected cells were mixed, we observed that rRNA transcription occurred at a level comparable to that of the uninfected extract alone (Fig. 3C, lane 9). This result suggests an alternative possibility in which a host rRNA transcription factor has become depleted or specifically inactivated by adenovirus infection, possibly as the result of viral shutoff of host cell protein synthesis (38). These preliminary studies do not allow us to distinguish between a positive or negative transcriptional regulatory mechanism, but our findings do provide a rational approach for isolating specific cellular or viral transcription factors that modulate polymerase activity on rDNA templates.

Effect of SV40 T Antigen on rRNA Transcription. Although viral infection in many cases leads to an inhibition of rRNA synthesis, there are some viruses, such as polyoma and SV40, that actually stimulate host rRNA synthesis upon infection. The activation of rRNA transcription by SV40 has been extensively investigated, and some recent findings provide compelling evidence that this response is due to the product of the viral A gene, large T antigen (12, 39, 40). For example, it was shown that specific mutations mapping within coordinates 0.28–0.39 in the A gene structural sequences (41) render the virus incapable of activating rRNA transcription (42). As a first step in developing a system for studying this phenomenon, we wanted to devise an assay for measuring the effects of T antigen on rRNA synthesis *in vitro*.

Addition of homogeneous T antigen or D2 protein to the *in vitro* transcription system stimulated rRNA synthesis 2- to 3-fold (Fig. 5, lanes 1–8). Under similar conditions, an equivalent amount of SV40 T antigen has no effect on the transcription from the adenovirus 2 major late promoter by RNA polymerase II (43, 44). At the standard concentrations of HeLa cell extract in the assay, maximal stimulation consistently occurred when

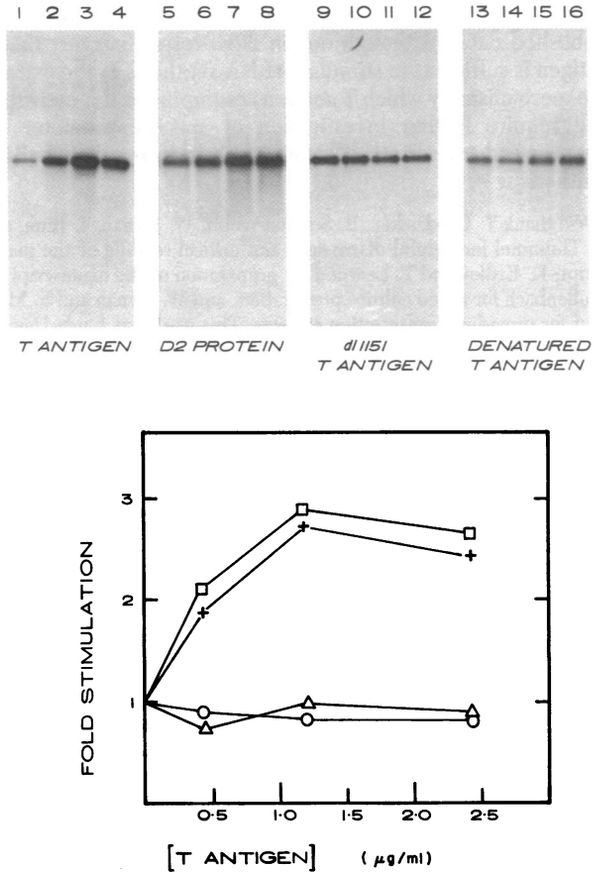

FIG. 5. Effect of SV40 T antigen on *in vitro* RNA synthesis. (*Upper*) Reactions were carried out by adding purified T antigen to a mixture of template DNA and uninfected HeLa cell extract. Runoff transcription was directed by prHu3 DNA (200 ng) cleaved with *Bam*HI. Reactions were performed in the presence of wild-type T antigen (lanes 1–4), D2 protein (5–8), mutant dl 1151 T antigen (9–12), or heat-denatured T antigen (13–16). The transcription reactions contained either no added T antigen protein (lanes 1, 5, 9, and 13), 0.4 μg (2, 6, 10, and 14), 1.2 μg (3, 7, 11, and 15), or 2.4 μg (4, 8, 12, and 16) of added T antigen or T antigen-related protein. (*Lower*) Incorporation of [α-^{32}P]GTP into rRNA was quantitated by densitometric scanning of autoradiograms that were exposed without the use of intensifier screen and normalized to the value obtained for the reaction lacking T antigen. The data presented represent the mean values obtained from at least five independent experiments. □, Wild type SV40 T antigen; +, D2 protein; ○, dl 1151 T antigen; △, heat-denatured T antigen.

0.5–1.5 μg of T antigen was added per ml. Transcription of rRNA was not affected when heat-denatured T antigen was added (Fig. 5, lanes 13–16). More importantly, no activation of rRNA transcription was observed when the *in vitro* assay was supplemented with a mutant T antigen that was shown to be defective for activating silent rRNA genes *in vivo* (Fig. 5, lanes 9–12).

These findings indicate that purified T antigen alone is sufficient to stimulate the rates of rRNA synthesis in a whole-cell extract prepared from uninfected cells. Although the degree of transcriptional activation *in vitro* is not very high (only 2- to 3-fold), it should be noted that *in vivo* the increased rate of rRNA transcription induced by SV40 or polyoma infection also is only about 2-fold (11, 13). Our results suggest that T antigen may act in a direct manner to modulate rRNA transcription. The well-characterized DNA binding properties of T antigen (25) prompted us to test the ability of T antigen to interact specifically with sequences on the rDNA template. Our preliminary studies suggest that there are no specific binding sites for T antigen in the

region containing the transcriptional control sequences (unpublished data). Although our *in vitro* results suggest that T antigen is sufficient to stimulate rRNA synthesis in the extract, the mechanism by which T antigen accomplishes this activation will require further investigation of specific promoter sequences and transcription factors involved in regulating rRNA synthesis.

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1. Miller, K. & Sollner-Webb, B. (1981) *Cell* **27,** 165–174.
2. Mishima, Y., Yamamoto, O., Kominami, R. & Muramatsu, M. (1981) *Nucleic Acids Res.* **9,** 6773–6785.
3. Grummt, I. (1981) *Nucleic Acids Res.* **9,** 6093–6102.
4. Miesfeld, R. & Arnheim, N. (1982) *Nucleic Acids Res.* **10,** 3933–3949.
5. Learned, R. M. & Tjian, R. (1982) *J. Mol. Appl. Genet.* **1,** 575–584.
6. Gallant, J. & Lazzarini, R. A. (1976) in *Protein Synthesis: A Series of Advances*, ed. McCartney, E. H. (Dekker, New York), Vol. 2, pp. 309–359.
7. Barry, J. & Gorski, J. (1971) *Biochemistry* **10,** 2384–2390.
8. Nowell, P. C. (1960) *Cancer Res.* **20,** 462–466.
9. Grummt, I., Smith, V. & Grummt, F. (1976) *Cell* **7,** 439–465.
10. Hershko, A., Mamont, P., Shields, R. & Tomkins, G. M. (1971) *Nature (London) New Biol.* **232,** 206–211.
11. Salomon, C., Turler, H. & Weil, R. (1977) *Nucleic Acids Res.* **4,** 1483–1503.
12. Soprano, K., Dev, V., Croce, C. & Baserga, R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3885–3889.
13. May, P., May, E. & Borde, J. (1976) *Exp. Cell Res.* **100,** 433–436.
14. Raskas, H., Thomas, D. & Green, M. (1970) *Virology* **40,** 893–902.
15. Hodge, L. (1970) *Bacteriol. Proc.* **173,** 156 (abstr.).
16. Todaro, G. & Takemoto, K. (1969) *Proc. Natl. Acad. Sci. USA* **62,** 1031–1037.
17. Pipas, J., Peden, K. & Nathans, D. (1983) *Mol. Cell. Biol.* **3,** 203–213.
18. Clark, R., Peden, K., Pipas, J., Nathans, D. & Tjian, R. (1983) *Mol. Cell. Biol.* **3,** 220–228.
19. Holmes, D. S. & Quigley, M. (1981) *Anal. Biochem.* **114,** 193–197.
20. Hirt, B. (1981) *J. Mol. Biol.* **26,** 365–369.
21. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–580.
22. Petterson, U. & Sambrook, J. (1973) *J. Mol. Biol.* **73,** 125–130.
23. Sugden, B. & Keller, W. (1973) *J. Biol. Chem.* **248,** 3777–3788.
24. Schwartz, L. B., Sklar, V. E. F., Jaehning, J. A., Weinmann, R. & Roeder, R. G. (1974) *J. Biol. Chem.* **249,** 5889–5897.
25. Tjian, R. (1978) *Cell* **13,** 165–179.
26. Grummt, I. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6908–6911.
27. Reeder, R. H., Wilkinson, J., Bakken, A., Morgan, G., Busby, S. J., Roan, J. & Sollner-Webb, B. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
28. Kohorn, B. & Rae, P. M. (1982) *Nucleic Acids Res.* **10,** 6879–6886.
29. Financsek, L., Mizumoto, K., Mishima, Y. & Muramatsu, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3092–3096.
30. Hay, J., Koteles, G., Kerr, H. & Subak-Sharpe, H. (1965) *Biochem. J.* **94,** 5P.
31. Flanagan, J. (1967) *J. Virol.* **1,** 583–590.
32. Wagner, E. & Roizman, B. (1969) *J. Virol.* **4,** 36–57.
33. Salzman, N., Shatkin, A. & Sebring, E. (1964) *J. Mol. Biol.* **8,** 405–416.
34. Ewton, D. & Hodes, M. (1967) *Virology* **33,** 77–83.
35. Darnell, J. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27,** 49–158.
36. Philipson, L., Petterson, U., Lindberg, U., Tibbets, C., Vennstrom, B. & Persson, T. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39,** 447–456.
37. Fire, A., Baker, C. C., Manley, J. L., Ziff, E. B. & Sharp, P. A. (1981) *J. Virol.* **40,** 703–719.
38. Ginsberg, H. S., Bello, L. J. & Levine, A. J. (1967) in *Molecular Biology of Viruses*, eds. Colter, J. S. & Paranolych, W. (Academic, New York), p. 547–562.
39. Baserga, R., Ide, T. & Whelly, S. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 685–691.
40. Soprano, K., Jonak, G., Galanti, N., Floros, J. & Baserga, R. (1981) *Virology* **109,** 127–136.
41. Tooze, J. (1980) *DNA Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 2nd Ed.
42. Soprano, K., Galanti, G., Jonak, G., Pipas, J., Peden, K. & Baserga, R. (1983) *Mol. Cell. Biol.* **3,** 214–219.
43. Rio, D., Robbins, A., Myers, R. & Tjian, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5706–5710.
44. Myers, R., Rio, D., Robbins, A. & Tjian, R. (1981) *Cell* **25,** 373–384.