

Stable Transcription Complex on a Class III Gene in a Minichromosome

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We have constructed recombinant simian virus 40 molecules containing *Xenopus* 5S RNA and tRNA genes. Recombinant minichromosomes containing these genes were isolated to study the interaction of RNA polymerase III transcription factors with these model chromatin templates. Minichromosomes containing a tRNA^{Met} gene can be isolated in a stable complex with transcription factors (IIIB and IIIC) and are active in vitro templates for purified RNA polymerase III. In contrast, minichromosomes containing a 5S RNA gene are refractory to transcription by purified RNA polymerase III in either the absence or the presence of other factors.

Fractionation of soluble transcription systems has indicated that multiple cellular factors in addition to RNA polymerase III are necessary to transcribe class III genes (14, 15). Two factors (designated IIIB and IIIC) are necessary for transcription of the tRNA and the adenovirus VA RNA genes in vitro, whereas the 5S genes require these same factors plus a third gene-specific factor (IIIA) (14, 15). Formation of a stable transcription complex in vitro, containing one or more of these factors, is a general feature of these genes (2, 9, 13). The finding that isolated cellular chromatin is associated with all the factors necessary to promote transcription of the endogenous 5S and tRNA genes by purified RNA polymerase III suggests that such complexes may exist in vivo (11, 17). To establish whether these chromatin-associated factors are specifically bound in a stable complex on class III genes, we have studied their interaction with a model chromatin template—the simian virus 40 (SV40) minichromosome.

Numerous class III genes cloned into SV40 have been found to be transcriptionally active in the infected cell (8, 18, 19). During infection, SV40 DNA is packaged by the host cell histones into a chromatin structure termed the minichromosome (16). In light of the stable transcription complexes observed on class III genes in vitro (2, 9, 13) and the association of factor with cellular chromatin (11, 17), we decided to investigate whether the transcription factors that interact with these genes remain bound to the template in a stable complex during the isolation of recombinant minichromosomes containing such genes. Studies were conducted with a *Xenopus laevis* tRNA^{Met} gene and a *Xenopus borealis* 5S RNA gene cloned into the late region of SV40. Previous work has shown that formation of a stable complex in vitro requires factors B and C for the tRNA^{Met} gene and factors A and C for the 5S gene (9). Here we show that the tRNA^{Met} gene in the minichromosome can be isolated in a stable complex with factors B and C and is an active in vitro template for purified RNA polymerase III. In contrast, the 5S gene in the minichromosome is not isolated in a stable complex with transcription factors and remains refractory to

transcription by purified RNA polymerase III in either the absence or the presence of other factors.

MATERIALS AND METHODS

Virus construction. The tRNA SV40 fragments, orientations 1 and 2, were constructed as follows. An *EcoRI*-linked, 3,055-base-pair fragment of wild-type SV40, extending from the *Bam*HI site to the *Hpa*II site, was cloned into the *EcoRI* site of pBR322 (this plasmid, designated OY, is described in reference 7). OY was partially digested with *EcoRI*; linear molecules were isolated by agarose gel electrophoresis and ligated to an *EcoRI*-linked, 777-base-pair fragment containing the *Xenopus laevis* tRNA^{Met}1 gene derived from pXltmet1 (3) by *EcoRI* digestion. tRNA OY clones containing the tRNA insert located in either of two orientations adjacent to the former *Hpa*II site of SV40 were isolated and characterized. Two such recombinants, containing the tRNA gene in either orientation 1 or 2 (Fig. 1A), were partially digested with *EcoRI*, and the 3,835-base-pair fragment containing the tRNA gene and the SV40 insert was isolated by agarose gel electrophoresis. These tRNA SV40 linear fragments (orientations 1 and 2) were circularized by treatment with DNA ligase and then used to transfect African green monkey kidney cells together with tsA239 SV40 DNA as the temperature-sensitive conditional lethal helper (7). The 5S SV40 fragments, orientations 1 and 2, were constructed in a fashion similar to that described above; however, a 853-base-pair fragment containing the *Xenopus borealis* somatic 5S gene was used in place of the tRNA gene insert. This fragment was isolated by *Hind*III digestion of pXBS1 (12) and was subsequently blunt ended and ligated to *EcoRI* linkers. The 5S tRNA SV40 was constructed as follows: The 5S OY recombinant containing the 5S gene (see legend to Fig. 1) in orientation 2 (with respect to the SV40 insert) was partially digested with *EcoRI*. The 8,269-base-pair linear fragment was isolated by agarose gel electrophoresis and ligated to an *EcoRI* linked, 777-base-pair fragment containing the tRNA^{Met}1 gene. A clone containing the tRNA insert, located in orientation 1, adjacent to the former *Bam*HI site of SV40 was isolated. This molecule was digested with *Hae*II, and the 4,438-base-pair fragment (containing the 5S gene, SV40 fragment, and tRNA gene) was isolated by agarose gel electrophoresis. The linear fragment was used to transfect Afri-

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can green monkey kidney cells together with *tsA239* DNA as described above. The linear fragment was circularized in the monkey cells to regenerate an intact *Hae*II site. All of the resulting viral stocks contained approximately 70% recombinant virus and 30% helper. The structures of all plasmids, restriction fragments, and viruses used in this work were confirmed by at least three restriction endonucleases.

Minichromosome preparation. BSC1 cells (approximately 2×10^7 cells per flask) were infected with a recombinant virus stock at a multiplicity of infection of about 10 and incubated at 40°C. Cells were labeled with [*methyl*-³H]thymidine (2.5 μ Ci/ml of medium) from 20 to 42 h postinfection. The cell monolayer was rinsed three times with phosphate-buffered saline, followed by the addition of cold lysis buffer {0.25% Triton X-100, 10 mM EDTA, 10 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] [pH 6.8], 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 2 μ g of Leupeptin per ml and 100 U of Aprotinin per ml}. The cells were left in lysis buffer for 10 min at 4°C and were subsequently dislodged from the plate by agitation. After the addition of NaCl to 0.12 M, the lysate was dispersed by siphoning through a 10-ml pipette and centrifuged at $2,000 \times g$ for 5 min. The pellet was washed once with lysis buffer containing 0.12 M NaCl and centrifuged at $2,000 \times g$ for 5 min. The pellet from 2×10^7 cells was suspended in 0.15 ml of extraction buffer (0.25% Triton X-100, 10 mM EDTA, 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] [pH 7.9], 120 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 2 μ g of Leupeptin per ml and 100 U of Aprotinin per ml) and stirred with a magnetic flea for 4 h on ice. (In Fig. 1 and 4 the minichromosome preparation was extracted overnight on ice.) The suspension was centrifuged at $10,000 \times g$ for 10 min, and the resulting supernatant was centrifuged through a linear 5 to 20% sucrose gradient containing 10 mM PIPES (pH 6.8), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM

dithiothreitol, 2 μ g of Leupeptin per ml, 100 U of Aprotinin per ml, and either 0.1 M KCl or 0.6 M KCl (NaCl in Fig. 4), in an SW60 rotor at 54,000 rpm for 35 min at 4°C. The peak of ³H-labeled viral minichromosomes were pooled, dialyzed against 10 mM HEPES (pH 7.9), 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 2 μ g of Leupeptin per ml, and concentrated in the dialysis membrane against Sephadex G-200. The minichromosome preparation was stored on ice and used within 2 weeks of preparation.

Transcription factors. The unfractionated cell-free transcription system (KBS100) was derived from human tissue culture cells as described in reference 14. Human transcription factors and RNA polymerase III were purified by the procedure of Segall et al. (14). However, factor B was sequentially purified on the following chromatographic columns: phosphocellulose (P-11), carboxymethyl-cellulose (C-25), DEAE-Sephadex (A-25), and Bio-Rex-70. Factor C was sequentially purified on phosphocellulose (P-11), DEAE-cellulose (DE-52) and a Bio-Gel A 1.5 M column. Factor B was present in 1.0 μ g of a fraction from the Bio-Rex-70 column; factor C was present in 0.4 μ g of the excluded fraction from the Bio-Gel A 1.5 M column. The *Xenopus* gastrula extract was made as described previously (15); *Xenopus* factor A was purified as described previously (3). Transcription reactions contained 0.1 to 0.2 μ g of minichromosome template, as determined by 4',6-diamidino-2-phenylindole dihydrochloride fluorescence. A 0.2- μ g sample of pBR322 was included in reactions containing the unfractionated KBS100 to saturate nonspecific DNA-binding proteins in this extract. The reaction volume was 50 μ l and contained 20 mM HEPES (pH 7.9), 70 mM KCl, 7 mM MgCl₂, 12% glycerol, 5 mM dithiothreitol, 600 μ M each ATP, UTP, and CTP, 25 μ M [α -³²P]GTP (54 Ci/mmol), and 10 U of placental RNase inhibitor (Bolton Biologicals). The transcription reaction was incubated for 1 h at 30°C; after transcription was terminated the RNAs were purified (15) and fractionated by electrophoresis in 10% polyacrylamide gels containing 7 M urea.

RESULTS

The *Xenopus* tRNA^{Met1} and the somatic 5S genes were cloned, in both orientations, into the late region of SV40 (Fig. 1A). Each recombinant molecule, containing an intact early region, was transfected into monkey kidney cells in the presence of viral DNA from a temperature-sensitive mutant containing a thermolabile T antigen. After incubation at the nonpermissive temperature a primary virus stock was obtained consisting of over 70% recombinant virions.

SV40 minichromosomes containing the various genes were isolated from infected cells by a Triton X-100-EDTA extraction procedure (16), yielding 75S particles; no nucleosome-free DNA that sediments at 21S was observed in these preparations. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that numerous proteins in addition to cellular histones cosediment with the minichromosomes (data not shown). Sedimentation of the minichromosomes through 0.6 M KCl yield 55S particles which lack histone H1, but which still cosediment with an array of nonhistone proteins (data not shown). The tRNA gene in the recombinant minichromosome is accurately transcribed when added to a cell-free transcription system (KBS100) derived from human tissue culture cells (Fig. 1B). Minichromosomes with associated H1 (lanes 1 and 4) are transcribed at about one-fourth the rate of deproteinized DNA (lanes 3 and 6), whereas those sedimented through 0.6 M KCl and therefore

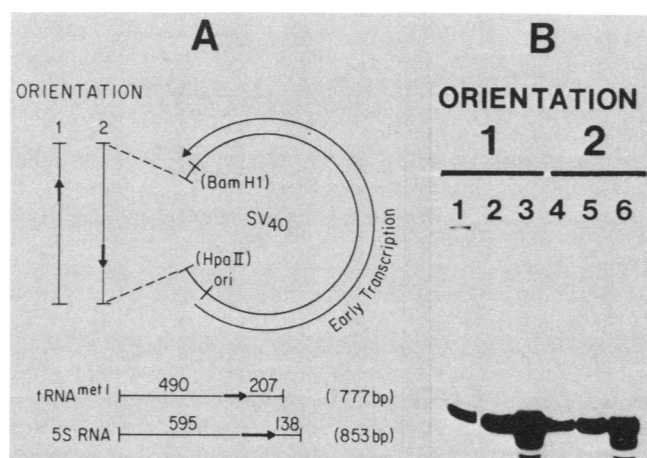


FIG. 1. (A) Map of a recombinant SV40 containing either a *Xenopus* tRNA^{Met1} gene or a 5S gene. (B) Transcription of minichromosomes containing a tRNA gene in an unfractionated human extract. An autoradiograph of transcription products separated by gel electrophoresis is shown. Transcription reactions contained 20 μ g of KBS100, 0.2 μ g of pBR322 (included to saturate nonspecific DNA binding proteins in the unfractionated transcription system) and 0.1 μ g of the following templates: tRNA SV40 minichromosomes sedimented through 0.1 M KCl (lanes 1 and 4), tRNA SV40 minichromosomes sedimented through 0.6 M KCl (lanes 2 and 5), or tRNA SV40 DNA (lanes 3 and 6). The tRNA gene was present in orientation 1 (lanes 1 through 3) or orientation 2 (lanes 4 through 6).

lacking histone H1 (lanes 2 and 5) are transcribed at about one-half the rate of DNA. Both orientations of the tRNA gene in the late region of the minichromosome are transcribed equivalently (lanes 1 through 3 and 4 through 6). After incubation in a reconstituted transcription system (see below), the sedimentation rate of the minichromosomes is unaltered, and the density distribution (after formaldehyde cross-linking) of the entire minichromosome population remains distinct from that of purified DNA after a similar incubation (Lassar, Ph.D. thesis, Washington University, St. Louis, Mo., 1983). These analyses suggest that the recombinant templates remain in a minichromosome configuration during the transcription reaction. In agreement with these results, micrococcal nuclease digestion indicates that a regular nucleosome repeat is maintained on the minichromosomes after a transcription reaction (data not shown). We emphasize, however, that physical characterization of the bulk of the minichromosomes may not reflect the chromatin conformation of the transcribed genes if they constitute only a small fraction of the templates.

Earlier work has established that, in addition to RNA polymerase III, two factors (designated IIB and IIC) are necessary for transcription of the tRNA and adenovirus VA RNA genes *in vitro*, whereas the 5S genes require these same factors plus a third gene-specific factor (IIIA) (14, 15). (For convenience we refer to the partially purified fractions as factors; although further purification has failed to reveal additional multiplicity, this possibility cannot be excluded.) To determine whether the recombinant minichromosome was purified in association with one or more such transcription factors, the minichromosome was incubated with various combinations of exogenous factors (Fig. 2A). Transcription of the tRNA^{Met1} gene in the recombinant minichromosome is dependent only upon the addition of exogenous RNA polymerase III (lanes 1 and 2). The addition of exogenous factors B and C did not further augment transcription of the gene (lanes 3 through 5). Thus, we presume that the recombinant minichromosome was purified in association with factors B and C, but not RNA polymerase III, although this has not been shown by direct physical characterization. The addition of RNA polymerase III alone to the template (lane 2) results in more transcription than does the addition of RNA polymerase III plus factors B and C (lane 5), suggesting that the template was purified in association with saturating amounts of factors B and C. However, because it is feasible that an excess of unbound factor (or contaminating inhibitor in the factor preparation) may depress transcription (lanes 4 and 5), we feel that relative factor stoichiometries cannot be determined by this analysis. The presence of factors B and C and the absence of RNA polymerase III were observed on minichromosomes purified in 0.1 or 0.6 M KCl (Fig. 2A and 3C). The maintenance of these factors on the minichromosome in 0.6 M KCl attests to their tight association with the template.

The interaction of the factors with the wild-type minichromosome was studied to distinguish whether these factors are specifically associated with tRNA gene-containing minichromosomes, or whether they fortuitously copurify with minichromosomes. When incubated with wild-type minichromosomes, the purified tRNA^{Met1} gene in a plasmid is transcribed in the presence of RNA polymerase III and factors B and C (Fig. 2C, lane 4). However, the tRNA gene is not transcribed in the presence of nonrecombinant SV40 minichromosomes if one or both of the factors is absent (lanes 1 through 3). This analysis indicates that the transcription factors do not copurify with wild-type minichromosomes

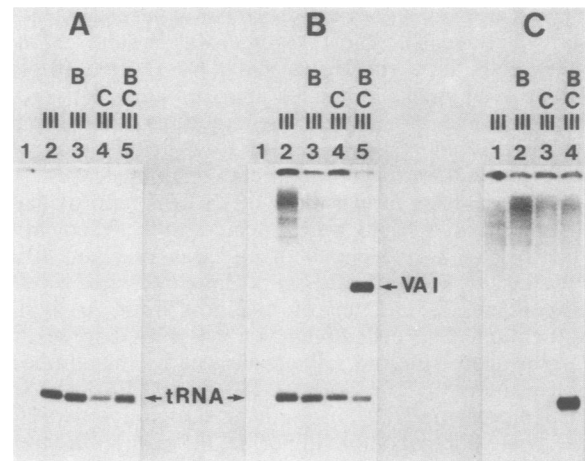


FIG. 2. Transcription factors are stably associated with the tRNA gene in the minichromosome. An autoradiograph of transcription products separated by polyacrylamide gel electrophoresis is shown. (A) The tRNA SV40 minichromosome (0.12 μ g) was incubated with the factors indicated above each lane. (B) The tRNA SV40 minichromosome and the purified adenovirus VAI gene (4) (pVA; 0.05 μ g) were together incubated with the factors indicated above each lane. (C) The wild-type SV40 minichromosome and the purified tRNA^{Met1} gene (3) (pXltmet1; 0.05 μ g) were together incubated with the factors indicated above each lane. The above experiments were performed with minichromosomes that had been stripped of histone H1 by sedimentation through 0.06 M KCl; the same results were obtained with minichromosomes sedimented through 0.1 M KCl. The tRNA gene was present in orientation 1.

in a dissociable form and suggests that the transcription factors are specifically associated with the recombinant minichromosome population. This experiment does not rule out the possibility that transcription factors are bound in a non-dissociable form to minichromosomes that do not contain a tRNA gene; however, we consider this possibility unlikely (see Discussion). Direct characterization of the transcription factors on the minichromosome will be necessary to distinguish what components are present or absent from the wild-type, tRNA, and 5S RNA (see below) recombinant minichromosomes.

Both factors B and C are necessary to form a stable transcription complex on a purified tRNA^{Met1} gene (9). If these factors are bound in a similar complex on the tRNA gene in the recombinant minichromosome, neither factor should exchange to a competing template during *in vitro* transcription of the minichromosome. Because transcription of the adenovirus VAI gene also requires factors B and C, this gene was employed as the competing template. When the recombinant minichromosome and a plasmid containing the adenovirus VAI gene are incubated with RNA polymerase III alone (Fig. 2B, lane 2) or RNA polymerase III plus either factor B (lane 3) or factor C (lane 4), transcription occurs only on the minichromosome-encoded tRNA gene. Transcription of the VAI gene is dependent upon the inclusion of both transcription factors B and C (lane 5). The absence of VAI gene transcription by an incomplete mixture of exogenous factors (lanes 2 through 4) indicates that both factors B and C are bound in a non-dissociating complex on the recombinant minichromosome.

Previous studies have revealed that transcription factors remain associated with the developmentally regulated *Xenopus* oocyte and somatic 5S genes in oocyte and somatic chromatin, respectively (11, 17). We were interested in

determining whether a similar transcription complex would be maintained on a 5S gene in an SV40 minichromosome. The presence of endogenous transcription factors was assayed on a recombinant minichromosome containing both the *Xenopus* tRNA^{Met}1 and the somatic 5S genes (Fig. 3A). The addition of RNA polymerase III to this minichromosome preparation supports only tRNA synthesis; the 5S gene is not transcribed (Fig. 3C, lane 2). In the absence of exogenous RNA polymerase III, neither gene in the minichromosome is transcribed (Fig. 3C, lane 1). Sedimentation of the minichromosome through 0.6 M KCl stimulates subsequent transcription of the tRNA gene by exogenous RNA polymerase III, but does not activate transcription of the 5S gene (Fig. 3C, lane 4). Incubation of the purified recombinant viral DNA with an unfractionated *Xenopus* gastrula extract results in the transcription of both genes, indicating that the 5S gene in viral DNA can interact with transcription

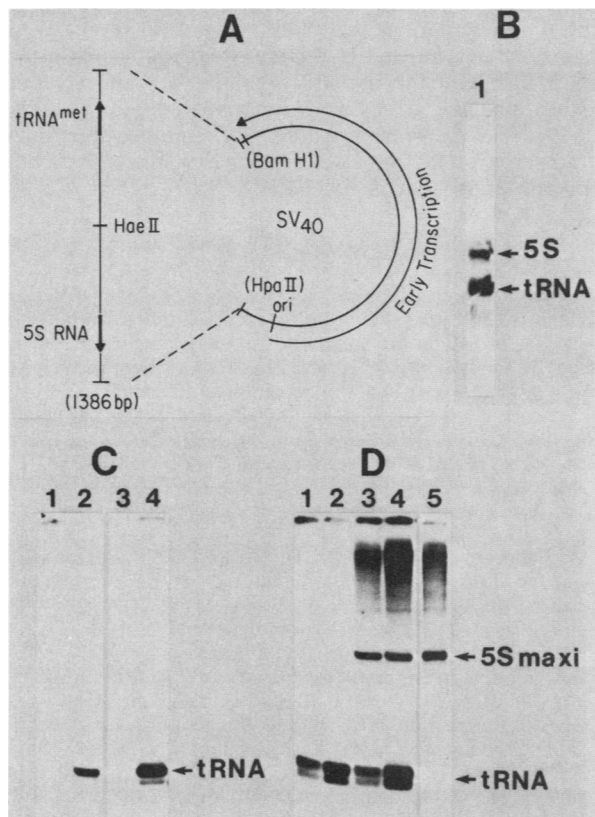


FIG. 3. Transcription of a minichromosome containing both 5S and tRNA genes. An autoradiograph of transcription products separated by gel electrophoresis is shown. (A) A map of the recombinant 5S/tRNA-SV40 containing both the *Xenopus* 5S RNA and tRNA^{Met} genes. (B) DNA purified from the 5S tRNA SV40 virus was transcribed in a *Xenopus* gastrula extract (lane 1). (C) 5S/tRNA SV40 minichromosomes (0.2 μg) sedimented through 0.1 M KCl (lanes 1 and 2) or 0.6 M KCl (lanes 3 and 4) were incubated under transcription conditions with nucleoside triphosphates plus the following components: none (lane 1), RNA polymerase III (lane 2), none (lane 3), and RNA polymerase III (lane 4). (D) Purified *Xenopus* factor A (0.18 μg), human factors B and C, and RNA polymerase III were incubated with the following templates: 0.2 μg of the 5S/tRNA SV40 minichromosome sedimented through either 0.1 M KCl (lane 1) or 0.6 M KCl (lane 2); 0.1 μg of the purified 5S maxigene (p115-77; reference 1) plus 0.2 μg of the 5S/tRNA SV40 minichromosome sedimented through either 0.1 M KCl (lane 3) or 0.6 M KCl (lane 4); 0.1 μg of the purified 5S maxigene (lane 5).

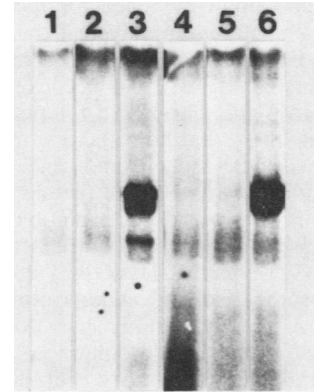


FIG. 4. Transcription of minichromosomes containing a 5S gene in an unfractionated human extract. An autoradiograph of transcription products separated by gel electrophoresis is shown. Transcription reactions contained 20 μg of KBS100, 0.2 μg of pBR322, and 0.2 μg of the following templates: 5S SV40 minichromosomes sedimented through either 0.1 M NaCl (lanes 1 and 4) or 0.6 M NaCl (lanes 2 and 5) and 5S SV40 DNA (lanes 3 and 6). The 5S gene was present in orientation 1 (lanes 1 through 3) or orientation 2 (lanes 4 through 6).

factors (Fig. 3B, lane 1). Thus a transcription factor complex, lacking only RNA polymerase III, is tightly associated (i.e., resistant to 0.6 M KCl) with the tRNA gene, but not the 5S gene, in the minichromosome.

To examine whether the 5S gene in the minichromosome could interact with exogenous transcription factors, the minichromosome was incubated with RNA polymerase III, factors B and C, and a vast excess of the 5S gene-specific factor A (Fig. 3D). The 5S gene in the minichromosome, sedimented through 0.1 or 0.6 M KCl, is not transcribed by these exogenous factors, whereas the tRNA gene in the same minichromosome is actively transcribed (lanes 1 and 2). Incubation of the factors with both the minichromosome and a plasmid containing a 5S maxigene (an insertion mutant that contains a fully functional promoter) results in the transcription of the tRNA gene and the 5S maxigene, whereas the minichromosome-encoded wild-type 5S gene is not transcribed (lanes 3 and 4). Thus, the absence of wild-type 5S RNA synthesis is not due to a *trans*-acting inhibitor of 5S gene transcription, but rather to a *cis*-acting effect of the minichromosome structure.

We have previously shown that the 5S and tRNA genes can each stably bind to at least one common transcription factor (9). To establish whether competition by the tRNA gene for a common factor was precluding transcription of the 5S gene in the minichromosome, studies were performed with recombinant minichromosomes containing only a 5S gene (Fig. 1A). When incubated in an unfractionated human extract, neither orientation of the 5S gene in the minichromosome supports 5S RNA synthesis (Fig. 4, lanes 1 and 4), whereas DNA purified from the minichromosome is transcribed (lanes 3 and 6). Removal of histone H1 by sedimentation of the minichromosome through 0.6 M NaCl does not activate subsequent 5S gene transcription (lanes 2 and 5). Thus the 5S gene remains transcriptionally quiescent in both minichromosomes containing or lacking a tRNA gene.

DISCUSSION

In this study we have demonstrated that the same factors (IIIB and IIIC) required to form a stable preinitiation complex on a purified tRNA^{Met} gene are apparently associated with a minichromosome containing this gene. We empha-

size, however, that direct evidence for factor association with the tRNA gene will require physical characterization of the factors. Because both factors B and C remain bound to viral chromatin in 0.6 M KCl and because neither factor apparently cycles off this chromatin template during transcription, it seems likely that both factors are stably bound to the tRNA gene in the minichromosome. However, this study does not indicate whether the factors were bound to the gene before minichromosome isolation or associated during the isolation procedure. In contrast to factors B and C, detectable RNA polymerase III activity does not copurify with minichromosomes isolated under isotonic or elevated salt conditions. A previous study has documented that isolated *Xenopus* erythrocyte chromatin is associated with class III gene transcription factors, but lacks detectable RNA polymerase III activity (17). These observations indicate that a stable transcription complex can be maintained on the gene in the absence of associated RNA polymerase III and suggest that the polymerase may be the only component to release from the template after transcription.

The maintenance of a stable noncycling transcription factor complex on class III genes may affect the chromatin structure of the gene and thus provide an epigenetic basis for control of the transcription of these templates (2). In accord with this hypothesis, Brown and colleagues noted that in *Xenopus* somatic cells the active somatic 5S genes are stably associated with factor A, whereas the quiescent oocyte 5S genes lack this factor (17). These workers have proposed that a repressive chromatin structure of the oocyte 5S genes precludes interaction with transcription factors. In the present study we document that the 5S gene in the isolated SV40 minichromosome remains refractory to transcription by exogenous factors because of a *cis*-acting effect of the minichromosome structure. The inhibition of 5S gene transcription was not relieved by sedimentation of the minichromosome through 0.6 M KCl (or NaCl). In contrast to this result, Brown and colleagues have observed that inactive 5S genes in cellular chromatin can interact with exogenous factors after exposure to 0.6 M NaCl (2, 17). This may reflect either a difference between cellular and viral chromatin or differences in the transcription systems used in this study and the oocyte nuclear extract employed by Brown and co-workers.

Transcriptional inactivation of the 5S gene has been observed after *in vitro* chromatin reconstitution on the gene in the absence of factor A, suggesting that factor A cannot interact with a nucleosome assembled 5S gene (2, 6). However, it is unclear whether nucleosomes directly interfere with factor A interaction, the assembly of a stable complex of factors A and C, the subsequent interaction of factor B and RNA polymerase III, or all of these steps that are necessary for transcription of the 5S gene (9). If the number of virus-coded 5S genes (about 500,000 copies per cell) exceeds the amount of cellular factor A, the majority of 5S genes may be assembled into minichromosomes in the absence of this factor and thereby rendered transcriptionally inert. Paradoxically, the 5S gene in the recombinant SV40 virus is transcribed during infection (George Pavlakis and Dean Hamer, personal communication); thus a portion of these templates must have associated transcription factors. Previous studies have found that about 1% of wild-type SV40 minichromosomes isolated by the Triton X-100-EDTA extraction procedure contain elongating RNA polymerase II molecules (5, 10); moreover, at late times of infection the amount of 5S RNA transcribed from the 5S recombinant minichromosome is approximately one-third the amount

transcribed from the endogenous 5S genes, which likely number only a few hundred (George Pavlakis and Dean Hamer, personal communication). Therefore the transcriptionally active 5S genes may constitute a small portion of the isolated recombinant minichromosome population and thus remain undetected *in vitro*.

The differential expression of the 5S and tRNA genes in the minichromosome may reflect either (i) different stabilities of transcription complexes on these two genes or (ii) a 5S gene-specific inhibition of transcription factor interaction. Whereas there is some suggestion that factor A cannot bind to a 5S gene after *in vitro* chromatin assembly (2, 6), it is unclear whether transcription factors B and C are able to interact with a chromatin packaged tRNA gene. Transcription factors B and C may associate with minichromosome packaged tRNA genes (either in the infected cell or during the minichromosome isolation), but factors A, B, and C (or a subset thereof) may be excluded from association with 5S genes in the same minichromosome. Future studies will address (i) whether transcription factors that interact with a nucleosome-assembled tRNA gene alter the chromatin structure of this template and (ii) what factors and structural modifications are necessary to activate 5S genes. These studies also suggest the feasibility of isolating or enriching for transcription factors for other genes via their association with appropriate minichromosomes.

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