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Formation of stable preinitiation complexes is a prerequisite for ribosomal DNA transcription *in vitro*

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ABSTRACT

Cytoplasmic extracts from cultured mouse cells contain the factor(s) required for specific transcription initiation of rDNA by RNA polymerase I. Prior to transcription the essential proteins bind to the ribosomal gene and remain bound to the template for several rounds of transcription. The assembly of these preinitiation complexes in vitro has been demonstrated by kinetic analysis of the transcription reaction and by competition experiments. Complex formation involves an initial, rapid binding of transcription factor(s) to rDNA sequences followed by additional events which arrange the DNA-protein complex into a transcriptionally active state. Once the complexes have formed they persist for at least 2 hours in vitro and are resistant to elevated salt concentrations. The assembly of the complexes was inhibited when the template DNA was incubated with histones prior to the addition of S-100 extract. If, however, preinitiation complex formation was allowed to occur before the addition of histones, the interference of histones with specific transcription was much less pronounced.

INTRODUCTION

The transcription of eukaryotic genes requires, in addition to DNA-dependent RNA polymerase, multiple protein factors that act in a concerted manner in the specific readout of defined classes of genes. The nature of these essential factors and their mode of action are largely unknown. The genes most intensively studied so far are the 5S RNA genes from Xenopus. Here a positive transcription factor which interacts specifically with an intragenic control region in the 5S gene has been purified (1-3). In addition to this 5S-specific factor and RNA polymerase III, at least two other components are necessary for accurate transcription of 5S DNA (4). It has been shown that in a cell-free system 5S DNA interacts specifically with transcription factors present in crude cellular extracts. This leads to the formation of very stable, transcribable complexes in vitro. DNA templates that have been assembled into transcription complexes undergo many rounds of 5S RNA synthesis (5). It has been suggsted that the formation of stable active or inactive transcription complexes prior to the initiation reaction may play an important role in the

expression or repression of specific genes according to the differentiated state of eukaryotic cells.
In a recent publication Davison et al. (6) have shown that the same mechanism may also account for transcriptional regulation of genes which are transcribed by RNA polymerase II. The advances in understanding gene control at the transcriptional level depend on developing cell-free systems which accurately reflect the gene regulation of intact cells.
Ribosomal genes which are transcribed by RNA polymerase I, represent an attractive experimental system to study gene regulation in eukaryotes, since they are effectively regulated on the transcriptional level which is also reflected in an in vitro system (7). The elucidation of the molecular mechanisms involved in the regulation of gene expression requires the identification and functional analysis of both transcription factors and DNA sequences that are essential for the readout of the gene. Recently the nucleotide sequence requirements for specific initiation of transcription by RNA polymerase I were determined (8). The protein factors that are involved in transcription initiation are not yet characterized. In order to reveal more information on the molecular mechanism of the initiation process and the factors involved in the transcription of ribosomal genes we investigated whether rRNA genes can form stable complexes with essential proteins as a prelude to active transcription.

MATERIAL AND METHODS

Recombinant DNA

The recombinant plasmid pMr600 used in the present study contains a 600 bp PvuII fragment of mouse rDNA inserted into the Sma I site of pUC9 by blunt-end ligation. The cloned rDNA fragment extends from position -319 in the non-transcribed spacer to +292 in the transcribed region. The transcription orientation is the same as that of the lac Z gene. Thus linearization of the plasmid yields a 297 bases run-off RNA in the cell-free transcription system. When the recombinant DNA is cleaved within the rDNA insert by SmaI, a 155 bases run-off transcript is produced in vitro.

Cell-free transcription system

The S-100 extract was derived from cultured Ehrlich ascites tumor cells and was prepared according to Weil et al. (9). The final volume of the standard RNA synthesis reaction mixture was 50 µl, 30 µl being contributed by the S-100 extract. The concentration of components in the assay were: 12 mM Hepes (pH 7.9), 85 mM KCl, 0.12 mM EDTA, 0.5 mM DTT, 5 mM $MgCl_2$, 10 mM creatine

phosphate, 0.66 mM each of ATP, CTP and UTP, 12.5 µM GTP, 2 µCi α-(^{32}P)GTP (spec. act. 400 Ci/mmol), and 0.1-0.5 µg template DNA. Under standard reaction conditions the mixture was incubated at 30°C for 1 hour and processed for gel analysis as described before (7). If quantitation of the transcripts was required the autoradiographs were scanned with a Joyce-Loebl densitometer and the relative transcriptional efficiency was expressed in arbitrary units.

Transcription complex formation

The experiments in which the transcriptional activity of two sequentially added templates was compared were performed with ammonium sulfate precipitated extracts which are devoid of endogenous nucleoside triphosphates. For this, S-100 proteins were precipitated by dropwise addition of an equal volume of saturated ammonium sulfate solution at 4°C. After standing in ice for 30 min the proteins were pelleted by centrifugation at 10.000 rpm for 20 min in a Sorval centrifuge, disolved in BC buffer (20 mM Hepes, pH 7.9; 20 % glycerol, 0.2 mM EDTA, 1 mM DTT) and dialyzed for 5 hours against a 100 fold volume of this buffer containing 100 mM KCl (BC_{100}). By this procedure no reduction of transcriptional activity of the extracts was observed. Omission of one nucleoside triphosphate from ammonium sulfate-precipitated extracts reduced rRNA synthesis to less than 5 % of control levels.

For complex formation, the first template (usually pMr600/EcoRI) was preincubated for 10 min at 30°C with the nucleotide-free extract in the complete reaction mixture without GTP. After addition of the second template (pMr 600/Sma I) transcription was initiated by addition of cold and (^{32}P)-labelled GTP. Further incubation was for 1 hour, except as noted.

RESULTS

Optimal DNA Concentration for rRNA Transcription in vitro

Cloned mouse rRNA genes can be faithfully transcribed in crude cytoplasmic extracts derived from cultured mouse cells. Initiation in such a cell-free system is accurate as shown by run-off transcripts, S1 mapping and 5'end analysis (10). The level of rDNA transcription shows a marked dependence on both extract and template concentrations. For the competition experiments described below, the optimal template concentration required to saturate the cell-free system had to be determined. As shown in Fig. 1A there was a sharp optimum within 0.25-1 µg DNA per 50 µl reaction. This indicates that at a low DNA concentration, the template is the limiting component which determines the number of transcripts synthesized. At higher template concentrations the transcriptional activity dropped considerably and the background increased.

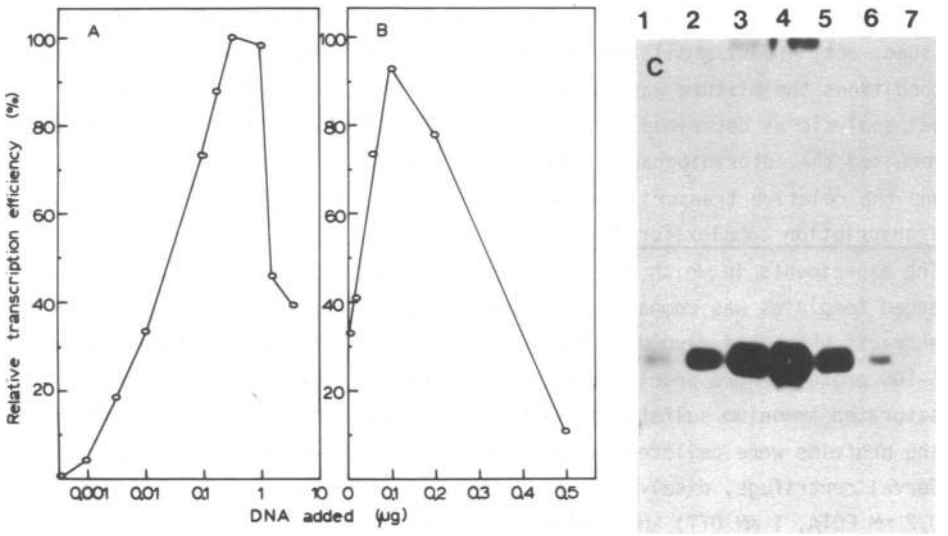

Fig. 1. Optimal DNA concentration for rRNA synthesis in vitro.
A) Different concentrations of pMr600/EcoRI DNA (0-5 µg/50 µl assay) were incubated with S-100 extracts for 60 min at 30°C under standard reaction conditions.
B) and C) Increasing amounts of pUC9 vector DNA were added to reaction mixtures containing a constant amount of pMr600/EcoRI DNA. After incubation the transcripts were processed as described in Materials and Methods. The autoradiographs (C) were scanned and the percentage of rRNA synthesized was plotted against the amount of DNA (B). A 50 µl assay contained 0.01 µg pMr 600/Eco RI (lane 1) plus 0.02 µg (lane 2), 0.07 µg (lane 3), 0.1 µg (lane 4), 0.2 µg (lane 5), 0.3 µg (lane 6) or 0.5 µg (lane 7) pUC9 vector DNA.

This finding suggests that there is more than one DNA binding protein involved in specific transcription initiation. In the presence of excess template these DNA binding proteins would interact with different DNA molecules thus preventing transcription complex formation and hence little correct transcription. Since the amount or activity of transcription factors varied between different extract preparations, the DNA optimum was determined for each individual extract and care was taken to keep the total amount of DNA per assay at or slightly below the optimal concentration.
Since the efficiency of rDNA transcription is dependent upon the DNA concentration of the assay we investigated whether unspecific vector DNA can partially substitute for template DNA. For this, 0.01 µg pMr600 DNA was assayed in the presence of increasing amounts of pUC9 vector DNA. As shown in Fig. 1 B and C, maximal rRNA synthesis could be achieved with as little as 0.01 µg pMr600 DNA when the total DNA concentration of the reaction was in-

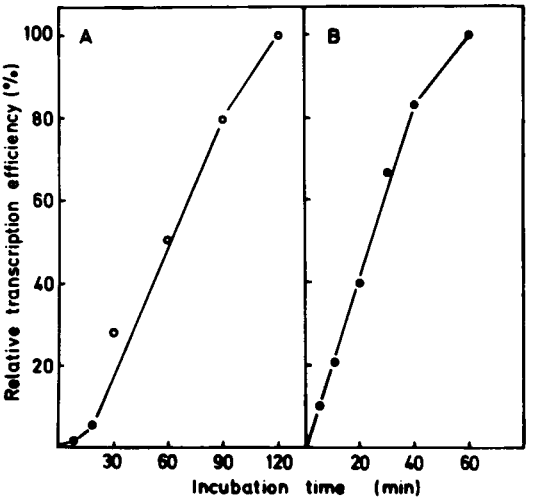

Fig. 2. Time course of rDNA transcription in vitro.
A: Kinetics without preincubation; B: kinetics after a preincubation period (without nucleoside triphosphates) for 10 min at 30°C.

creased by adding vector DNA. In this case maximal transcription was observed in the presence of 0.1 µg vector DNA. If the ratio of pUC9 to pMr600 DNA was higher than 10 to 1, a strong inhibition of transcription occured, though the total DNA concentration was still within the optimal range. The apparent inhibition of transcription by DNA which does not contain rDNA promoter sequences suggests that plasmid DNA competes for transcription factor(s) although with a much lower efficiency compared to rDNA. On the other hand an increase in the relative transcriptional efficiency of specific DNA in the presence of nonspecific competitor DNA has also been observed in RNA polymeraseIII transcription systems (11, 12) and has been interpreted as the selective capturing of inhibitory protein(s) in the extracts by plasmid DNA.

Kinetics of rDNA transcription

The time course of pMr600-directed rRNA synthesis is biphasic. Usually very little transcription takes place during the first 15 min. After this lag phase transcription proceeds linearly for 1 - 2 hours and later reaches a plateau (Fig. 2 A). To investigate the nature of this lag phase the template DNA was preincubated with a nucleotide-free extract for 15 min at 30°C before starting the reaction by the addition of nucleoside triphosphates. Under these conditions transcription occured at the maximal rate without the lag period (Fig. 2 B). This finding suggests that the interaction of essential proteins

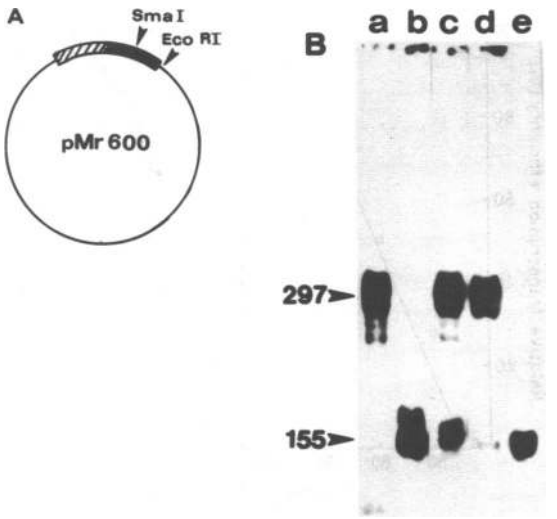

Fig. 3. Formation of transcription complexes
A) Schematic representation of the plasmid pMr600.
The thin line represents the vector pUC9. The hatched region of the ribosomal insert represents the non-transcribed spacer and the black region transcribed sequences of mouse rDNA.
B) The autoradiograph shows the transcription products of pMr600 DNA (0.2 µg/50 µl) which has been truncated with either EcoRI or SmaI.
Lane a: pMr600/EcoRI, lane b: pMr600/SmaI, lane c: equal amounts of pMr600/EcoRI and pMr600/SmaI added simultaneously to the reaction mixture, lane d: same as lane c except that the pMr600/EcoRI DNA was preincubated for 10 min before the pMr600/SmaI template; lane e: same as lane d except that the first template was pMr600/SmaI and the second template pMr600/EcoRI.

with defined rDNA regions, i.e. the formation of active transcription complexes or preinitiation complexes, takes place much more slowly than the actual transcription reaction. The amount of specific transcripts synthesized was calculated by running labelled DNA fragments with known numbers of counts for autoradiographic quantitation. Thus the band intensity could be translated into pmoles of RNA synthesized per pmole of template (not shown). These estimations revealed the transcription of 1 - 1.5 RNA molecules per input DNA template within an incubation period of 30 min, which indicates that the efficiency of the cell-free system is only about 1/1000 the RNA produced *in vivo*.

Assembly of Active Transcription Complexes

To study the formation of active transcripton complexes we used a quantitative assay that measures the ability of rRNA genes to compete for transcription factors present in the S100 extracts. This assay, referred to as the

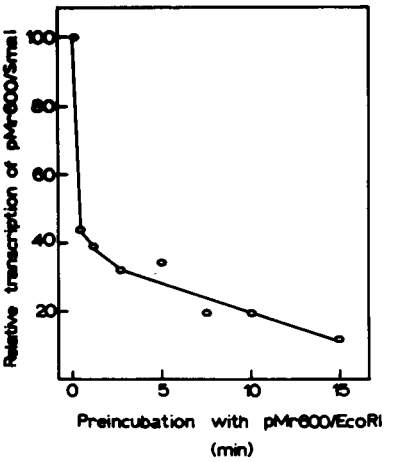

Fig. 4. Time course of transcription factor binding to rDNA
The time between the addition of the first template (pMr600/EcoRI) and the second template (pMr600/SmaII) to the nucleotide depleted S-100 extract was varied. After an additional 5 min incubation, transcription was initiated by addition of nucleoside triphosphates. The labelled RNA products were resolved by gel electrophoresis and quantified by scanning the autoradiograph. The amounts of the 297bp and 155 b RNA synthesized at time zero were added together and normalized to 100 %. The transcription efficiency of pMr600/SmaI relative to pMr600/EcoRI DNA was plotted against the time of preincubation with pMr600/Eco RI.

"prebinding" assay, is based on the assumption that, in conditions preventing transcription initiation (that is, in the absence of nucleoside triphosphates) initiation factor(s) bind more tightly to DNA sequences containing the rDNA promoter than to nonspecific DNA. Accurate transcription of a second, distinguishable, template added to the reaction mixture 15 min later, but before initiation of transcription by NTP addition, would then be strongly reduced or even eliminated, provided that the first template were present in excess and its interaction with the factor sufficiently stable. Our prebinding assay used the recombinant DNA pMr600 which has been truncated with either EcoRI or SmaI, as templates in the cell-free system. The run-off RNAs synthesized from each template can be distinguished by their different sizes. pMr600/Eco RI yields a 297 b transcript; pMr600/SmaI a 155 b RNA (Fig. 3 A, 3 B, lanes a and b).
Both templates added simultaneously in equimolar amounts to the extract were transcribed equally well (lane c). Any change in the molar ratios of both DNA templates resulted in a similar change of the ratio of 297 and 155 bases transcripts. However, a completely different transcription pattern was obtained

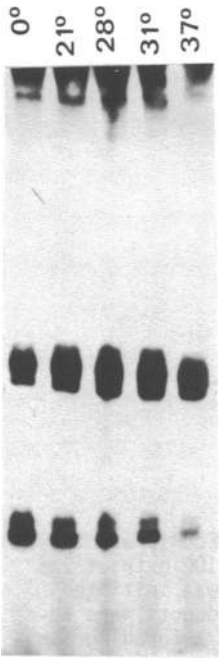

Fig. 5. Temperature-dependence of transcription complex formation
The preincubation temperature of the first template pMr600/EcoRI (0.1 µg/50 µl) with the nucleotide depleted extract was varied. The autoradiograph shows the efficiency of transcription complex formation after a preincubation period of 10 min at different temperatures. Transcription was started by addition of the second template (0.1 µg pMr600/SmaI) and the nucleoside triphosphates. The reactions were stopped after an additional incubation for 60 min at 30°C.

when the pMr600/EcoRI DNA was preincubated with the extract for 10 min at 30° before adding the second template pMr600/SmaI. At saturating concentration of the first template, transcription from the second template was decreased to less than 5 % of that seen with simultaneous DNA addition (lane d). The preferential transcription of the first template suggests that transcription factors present in the S100 extracts bind to the rDNA and remain stably associated with the DNA without dissociation and binding to a second template. The term stable is used here and below when referring to factor-DNA associations strong enough significantly to reduce accurate transcription from the second template added after the prebinding period.

Kinetics and Temperature Dependence of Complex Formation

The time dependence of the transcription complex formation was examined by varying the time of preincubation of the S-100 extract with the first template pMr600/EcoRI before adding the second template pMr600/SmaI. As shown in Fig.4 preferential transcription of the pMr600/EcoRI DNA was already observed after a preincubation period of only 30 seconds. Complex formation was essentially completed within 5-10 min. After 10 min there was only a slight decrease in the amount of the 155 b RNA - the transcript derived from the sequentially added pMr600/SmaI DNA. In this particular experiment transcription of the

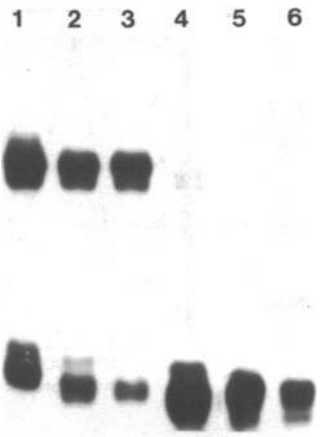

Fig. 6. Stability of preformed transcription complexes.
The reaction mixtures contained equal amounts (0.2 µg each) of pMr600 DNA truncated either with EcoRI or SmaI. In the first reaction (lanes 1-3) the two templates were added simultaneously. In the second reaction (lanes 4-6), pMr600/SmaI DNA was added 15 min before pMr600/EcoRI DNA. After further 15 min of incubation in the nucleotide-free extract, transcription was initiated by addition of nonradioactive nucleoside triphosphates. Aliquotes were removed after 0, 30 and 60 min and labelled with α-^{32}P-GTP for 1 hr.

second template was not completely inhibited since the amount of template DNAs was below the saturating DNA concentration.

The assembly of transcription complexes is temperature-dependent. As shown in Fig. 5 preincubation for 10 min of the first template (pMr600/EcoRI) with the extract at 0°C did not inhibit transcription of the second template (pMr600/SmaI). Increasing the temperature during the preincubation period up to 37°C resulted in a gradual decrease of transcription of the second template. Maximal complex formation occured at 37°C, though at this temperature a slight inhibition of total transcriptional activity as compared to 30°C was observed.

Specificity and Stability of Complex Formation

The competition assay allowed us to examine the stability of the preformed rDNA-protein complexes. Fig. 6 shows that preferential transcription of the first template continued for at least two hours. In this experiment pMr600/SmaI DNA was preincubated with extract in the standard reaction mixture without nucleoside triphosphates. After 15 min the second template (pMr 600/EcoRI) was added and transcription started by the adition of all four cold

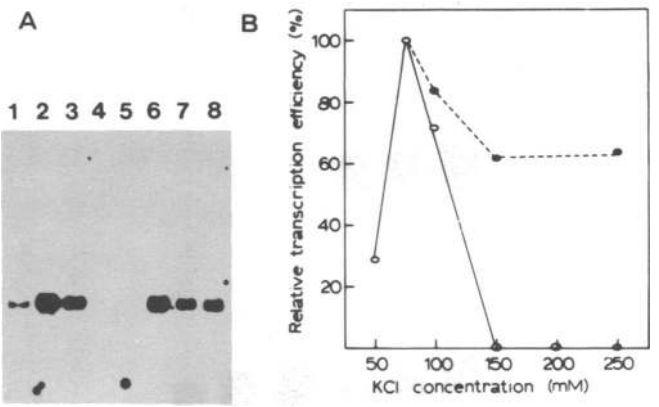

Fig. 7. Influence of increasing KCl concentrations on rRNA synthesis before or after transcription complex formation.
A) lanes 1-5: pMr600/EcoRI DNA (0.1 µg) was preincubated for 10 min in the standard reaction mixture in the presence of 50, 75, 100, 150 and 250mM KCl, respectively, before adding the nucleoside triphosphates and further incubation for 5 min. Lanes 6-8: pMr600/EcoRI DNA (0.1 µg) was allowed to form transcription complexes at 75 mM KCl by preincubation for 10 min at 30°C. Then the KCl concentration was increased to 100, 150 and 250 mM, and transcription was started by the addition of the nucleotides. The amount of labelled GTP was increased to 5 µCi per assay in this experiment, the transcription reaction was stopped after 5 min.

nucleoside triphosphates. At defined time points (0-30-60 min after the addition of the NTPs) rDNA transcription was monitored by the incorporation of ^{32}P-labelled GTP into the specific transcripts. Though in this experiment the transcriptional activity dropped slightly during prolonged incubation, the preferential transcription of the first template was found even after one hour of active rRNA synthesis (lanes 4-6). Evidently, the template-bound protein(s) that had the opportunity to redistribute to the subsequently added DNA during the incubation period, failed to do so. This indicates that, once the complexes had formed, they did not dissociate during the assay.
The stability of the preformed complexes could also be demonstrated by their relative resistance to increasing salt concentrations. The transcription system shows a very sharp optimum for monovalent cations. KCl-concentrations above 125 mM virtually abolished transcription initiation (Fig. 7, lanes 4 and 5). When, however, complex assembly was allowed to occur at 75 mM KCl before increasing the salt up to 250 mM KCl, rRNA synthesis was much less affected (lanes 7 and 8). There was a reduction of about 40 % in the amount of the specific run-off transcript observed at high salt compared to the optimal salt concentration. This could mean that either the complexes are not

fully stable against elevated salt or that this decrease in transcriptional activity reflects the ability of RNA polymerase I to elongate but not to re-initiate at higher salt concentration.
For RNA polymerase III transcription systems it has been shown that preformed transcription complexes are resistant to dilutions (5) as well as to competition by large amounts of vector DNA (12). Analogous experiments for the RNA polymerase I system (not shown here) indicate that the interaction of specific binding proteins with defined rDNA sequences seems to be much weaker than those with tRNA and 5S RNA genes. The rDNA complexes do not significantly resist dilution and can be dissociated by high concentrations (at a molar ratio of >10:1) of nonspecific vector DNA (see also Fig 1 B). Furthermore, preincubation of the extract with vector DNA significantly reduces rDNA transcriptional activity (not shown). These observations indicate that in the absence of rDNA, essential factor(s) bind presumably at nonspecific sites of the plasmid DNA and are not or are only partially displaced by sequential addition of rDNA.

Inhibition of Complex Formation by Histones

The results presented above suggested that a prerequisite for specific transcription *in vitro* is the interaction of essential proteins with defined regions of the rDNA. In the following experiments we studied whether proteins that bind nonspecifically to DNA would prevent this specific DNA-protein interaction. For this, increasing amounts of a mixture of all histones (H1, H2A, H2B, H3 and H4) were added to the reaction mixture prior to the addition of extract. Quantitation of rRNA synthesized revealed a progressive decrease of transcription as the histone-to-DNA ratio increased (Fig. 8). No inhibition of transcription was observed when up to 0.1 µg histones were added to 0.1 µg template DNA. Fifty percent inhibition of transcription occured in the presence of 0.25 µg histones, which corresponds to a histone-to-DNA ratio of 2.5:1 (w/w). In the presence of 1 µg histones transcription was completely suppressed.
When, however, the DNA and the S100 extract were preincubated for 5 min at 30°C before the additon of the histones the effect on transcription was much less pronounced. Approximately three fold higher histone concentrations were required to cause a similar degree of inhibition (Fig. 8). This suggests that the histones compete with some DNA binding factor(s) in the cytoplasmic extract that are required for complex formation. An effective competition occurs only at high histone-to-DNA ratios. The decrease in transcriptional activity in the presence of histones is certainly not due to the formation of nucleo-

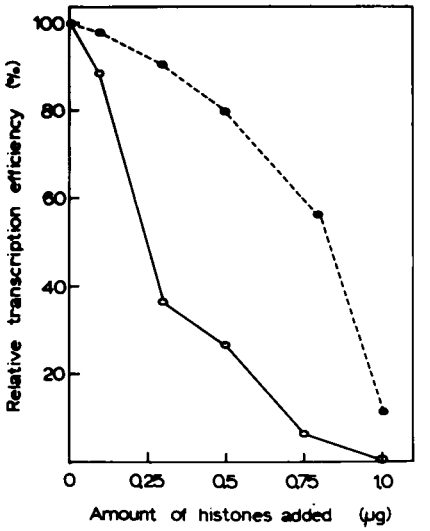

Fig. 8. Effect of exogenous histones on the transcriptional activity
0.15 µg pMr600/EcoRI DNA were incubated simultaneously with increasing amounts of histones and S-100 extract (o——o). The same concentrations of histones were added after the template has been incubated with the S-100 extract for 10 min at 30°C (o-----o). Transcription was initiated by the addition of nucleoside triphosphates and proceeded for 60 min at 30°C. The RNA was quantified by densitometric scanning of the autoradiograph. The amount of RNA synthesized in the absence of histones was normalized to 100 %.

somes because the same inhibition curves were also obtained with histones H1 or H3 alone (data not shown).

To demonstrate the interference of histones with the assembly of stable transcription complexes the effect of histones on the transcription of two sequentially added templates was investigated. Transcription of simultaneously added pMr600 DNA (0.15 µg each) truncated with either EcoRI or SmaI yielded two transcripts of comparable intensity (Fig. 9, lane a). In the presence of 1 µg histones transcription of both templates was completely inhibited (lane b). When, however, the first template was preincubated with the histones for 5 min at 30° C before addition of the second template and the S-100 extract, only RNA synthesis from the first DNA was suppressed whereas transcription of the second template was not impaired (lanes c and d). This demonstrates that histones - once bound to the DNA - do not interfere with either transcription factors or a sequentially added DNA.

Fig. 9. Competition of transcription factors and histones for rDNA
Lanes a+b: 0.15 µg each of pMr600/EcoRI and pMr600/SmaI DNA were assayed in the cell-free system in the absence (a) or presence of 1 µg total histones (b). Lane c: preincubation of pMr600/EcoRI DNA with 1 µg of histones for 5 min before addition of pMr600/SmaI, the S-100 extract and the nucleoside triphosphates. Lane d: same as lane c but the first template was pMr600/SmaI and the second template pMr600/EcoRI.

DISCUSSION

Cloned rDNA fragments which contain the region of transcription initiation by RNA polymerase I can be accurately transcribed in a cell-free system derived from cultured mouse cells. The data presented in this paper demonstrate that transription factors present in limiting concentrations in actively transcribing cell extracts specifically interact with defined sequences on the rDNA template and remain bound to the template during several rounds of transcription. An analogous specific binding of transcription factors to DNA fragments containing class III or II RNA polymerase promoter sequences has been demonstrated before and has been designated "transcription complex" or "preinitiation complex" formation (5, 6). In this paper we provide evidence that a similar mechanism also accounts for the transcription of ribosomal genes by RNA polymerase I *in vitro*. The binding of transcription factors to ribosomal gene promoter sequence is almost complete within the first minute of incubating template DNA with S-100 extracts as shown by the inhibition of transcription of a subsequentially added template. The initial DNA-protein interaction is followed by additional events which enable RNA polymerase I to recognize the specific deoxyribonucleoprotein complex. This is reflected by the lag period of 15-20 min before rDNA synthesis proceeds at maximal rates. Once the specific preinitiation complexes are formed they are strikingly more stable than those formed with non-promoter sequences
The following lines of evidence suggest that RNA polymerase I initiates transcription at DNA molecules that have bound essential factors and that repeated

transcription of the same gene occurs in vitro. Preferential transcription of the first template occured for at least 4-5 rounds of transcription over a period of 2 hours. The rounds of transcription have been calculated from the number of transcripts synthesized per rDNA template in the assay. They are certainly an underestimate of the real number since the dependence of the transcriptional activity on the DNA concentration indicates that not all templates are active in the extract. However, the fact that the preference of rRNA synthesis from the first template compared to that from a subsequently added DNA was maintained over incubation periods of more than one hour provides evidence that the template for transcription is not naked DNA but a deoxyribonucleoprotein complex and that transcription factors remain bound to the same DNA molecule without exchanging to the second template.
The fact that probably only a small percentage of the rDNA added to the extracts served as active templates in transcription may be due to either the sequestering of essential DNA binding proteins by the vector DNA or by the inhibition of specific transcription complex assembly by the interaction of nonspecific DNA binding proteins with the rDNA sequences required for complex formation. Such an interference with transcription complex formation could be demonstrated by adding histones to the reaction mixture. A strong inhibition of transcription was observed if the histones were added to the DNA prior to the addition of S-100 extract. The inhibitory effect was much less pronounced if the histones were added to the reaction after complex formation had been allowed to occur. This suggests that histones block the access of positive transcription factor(s) to the rDNA template. This inhibitory effect of exogenous histones has been observed both with a mixture of all five histones as well as with H1 or H3 alone. It is therefore unlikely that the formation of nucleosomes may account for the suppression of transcription.

The results obtained for the RNA polymerase I transcription system are very reminiscent of the data described for the transcription of RNA polymerase III genes (5, 12). It has been shown that in cell-free extracts 5S RNA and tRNA gene templates are assembled into stable, transcriptionally active complexes that persist for many rounds of transcription. It has been suggested that the formation of transcription complexes on 5S RNA genes involves an initial binding of the positive transcription factor TF IIIA followed by rearrangement to an actively transcribing state or binding of other, yet unidentified, factors. RNA polymerase III then recognizes this DNA complex to initiate transcription.
The same sequence of events seems to be involved in transcription of ribosomal

genes by RNA polymerase I which suggests that the proposed mechanism of initiation may be of general significance for the transcription of other classes of genes. In both gene systems complex formation is a rapid, temperature-dependent process which is followed by a delayed onset of RNA synthesis. In either case the RNA polymerase initiates transcription at the preformed complexes and reads the same template several times.

We do not know yet which factors are involved in the formation of transcription complexes and how the assembly takes place. However, the activity of different extract preparations to direct specific rDNA transcription appears to be due largely to differences in the concentration of positive transcription factors which are involved in complex formation. The further purification and analysis of the various factors should lead to an understanding of their respective roles in transcription initiation and their sites and mechanism of action.

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