The EMBO Journal vol.9 no.2 pp.573 - 582, 1990

Competition between transcription complex assembly and chromatin assembly on replicating DNA

Genevieve Almouzni[1,2], Marcel Méchali[2] and Alan P.Wolffe[1]

[1]Laboratory of Molecular Biology, Bldg 6, Rm 131, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA and [2]Institut Jacques Monod, CNRS - Tour 43, 2 place Jussieu, 75251 Paris Cédex 05, France

Communicated by M.Yaniv

We have used a *Xenopus* egg extract to show that a competition exists between the assembly of transcription complexes and nucleosomes on replicating 5S DNA. This competition results in the establishment of a transcriptionally repressed state for 5S DNA that is dependent on core histones but not on the precise positioning of the cores. The repression is selective, since satellite I DNA is not significantly repressed under these conditions. We demonstrate that the efficiency of chromatin assembly compared with transcription complex assembly is an important variable in determining gene activity.

Key words: chromatin/replication/5S RNA gene/TFIIIA/*Xenopus*

Introduction

In vivo the process of DNA replication is coordinated with the assembly of chromatin (Senshu *et al.*, 1978; Worcel *et al.*, 1978). Both nucleosomes and the complexes of transcription factors responsible for specific gene activity are assembled on newly replicated DNA (Brown, 1984; Wolffe and Brown, 1986). An important question is whether these two events interfere with one another. We have approached this problem using a *Xenopus* egg extract that supports DNA replication (Méchali and Harland, 1982; Blow and Laskey, 1986; Hutchison *et al.*, 1987), chromatin assembly (Almouzni and Méchali, 1988a) and transcription (Wolffe and Brown, 1987).

With a circular single stranded DNA molecule as template, complementary DNA strand synthesis occurs in the *Xenopus* egg extract with an efficiency exceeding 90%. The enzymatic processes resemble those occurring on the lagging strand of the chromosomal replication fork (Méchali and Harland, 1982). Chromatin assembly progresses coincidentally with DNA synthesis at a rate comparable with chromosomal replication in early *Xenopus* embryos (Almouzni and Méchali, 1988a).

We have examined the assembly of transcription complexes on *Xenopus* class III genes using single stranded templates that are duplicated and assembled into chromatin in the extract. Both the transcriptional activity and the chromatin structure of the newly assembled complexes have been analyzed. Duplex DNA does not replicate in these extracts and is assembled into chromatin relatively slowly in comparison with replicating single stranded DNA (Almouzni and Méchali, 1988a). We have therefore compared the transcriptional activity of duplex DNA with that of replicating single stranded DNA.

We find that histones can compete with transcription factors for binding to the promoter sequences of genes. This competition occurs only when nucleosome assembly is very efficient, as seen using replicating single stranded DNA templates. No repression is seen using duplex DNA as a template. The repression attributed to nucleosome assembly on replicating DNA is gene specific. Repression of 5S RNA genes occurs under conditions in which satellite I DNA is still actively transcribed.

Results

Satellite I DNA and somatic 5S DNA are differentially transcribed on replicating DNA

Two class III genes, the somatic 5S RNA gene of *Xenopus borealis* (Peterson *et al.*, 1980), and the satellite I DNA of *Xenopus laevis* (Ackerman, 1983; Lam and Carroll, 1983), were used in this study. Both the 5S RNA gene and satellite I DNA require transcription factors TFIIIB and TFIIIC to be transcribed by RNA polymerase III. In addition, the 5S RNA gene requires a specific transcription factor TFIIIA (Engelke *et al.*, 1980; Segall *et al.*, 1980; Shastry *et al.*, 1982). *Xenopus* egg extracts require exogenous TFIIIA for efficient 5S RNA gene transcription (Engelke *et al.*, 1980; Gottesfeld and Bloomer, 1982; Wolffe and Brown, 1987). In all our experiments TFIIIA was present in excess over gene sequence, and the protein was usually mixed with the DNA before addition of the egg extract.

Incubation of single stranded circular satellite I DNA or 5S DNA in the *Xenopus* egg extract leads to their almost complete conversion to a supercoiled double stranded form within 60 min (Figure 1A) (Méchali and Harland, 1982; Almouzni and Méchali, 1988a). The supercoiling of the replicated DNA (Figure 1A) is a consequence of the assembly of regularly spaced nucleosomes (approximately every 190 bp) (Figure 1B). A detailed comparison of the supercoiling of replicating single stranded DNA with that of duplex DNA has shown that chromatin assembly is both faster and more efficient on a replicating template than on duplex DNA under these reaction conditions (Almouzni and Méchali, 1988a,b).

Preliminary experiments using inhibitors of replication, e.g. aphidicolin (Huberman, 1981), demonstrated that complementary strand synthesis was required for transcription from single stranded DNA added to the extract (see also Cortese *et al.*, 1980). The requirement for DNA replication before a single stranded DNA template could be transcribed led us to use a pulse labeling protocol to examine the relative transcription efficiency of replicating single stranded and duplex DNA. Both satellite I DNA and somatic 5S RNA genes are efficiently transcribed when duplex DNA is added to the egg extract in the presence of excess TFIIIA (Figure

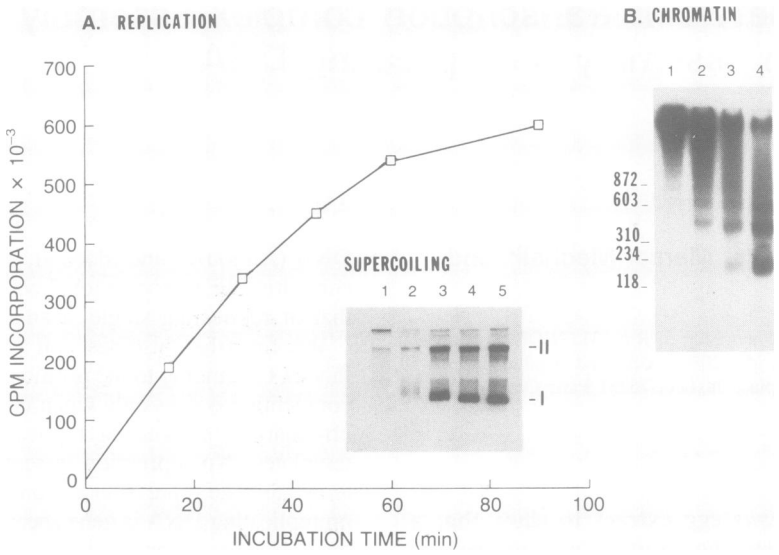

Fig. 1. Replication kinetics and chromatin assembly in the egg extract. Single stranded DNA was incubated in the reaction mixture at a concentration of 100 ng DNA/10 μl extract. The reaction mixture was supplemented with 10–20 μCi [α-^{32}P]dATP, 3 mM ATP and 5 mM $MgCl_2$ as described in Materials and methods. **Panel A** shows a plot of TCA precipitable counts against time (REPLICATION) and an autoradiograph of DNA taken at the indicated times and resolved on an agarose gel (SUPERCOILING): **lane 1**, 15 min; **lane 2**, 30 min; **lane 3**, 45 min; **lane 4**, 60 min, **lane 5**, 90 min. At the end of the reaction, 100% of the material was replicated, calculating from the number of counts incorporated into acid precipitable material (Materials and methods; Almouzni and Méchali, 1988a). Forms I and II of replicated M13 DNA are indicated. **Panel B** shows the micrococcal nuclease digestion pattern (CHROMATIN) obtained from single stranded DNA replicated as described in panel A during a 3 h incubation. Aliquots were taken during digestion afer 1, 2, 4 and 8 min respectively, resolved by 1.5% agarose gel electrophoresis and radioautographed as described in Materials and methods. A *Hae*III digest of ϕx 174 DNA was used as mol. wt markers. The average size of the repeat in the nucleosome array observed is ~190 bp (for example see Noll and Kornberg, 1977).

2A). In contrast, when single stranded DNA is replicated and transcribed under these conditions, satellite I DNA is transcribed much more efficiently than somatic 5S DNA. Even after 90 min, somatic 5S DNA transcription is barely detectable.

We considered the possibility that the preincubation of TFIIIA with the templates might give the non-replicated duplex DNA a non-physiological advantage for the formation of transcription complexes compared with replicating DNA. This is because a transcription factor would be specifically bound to the 5S RNA gene before nucleosome assembly began on duplex DNA. The experiment was therefore repeated with TFIIIA being mixed into the egg extract before addition of the templates. The results of this experiment, shown in Figure 2B, are almost identical to those of Figure 2A. Both satellite I and 5S DNA are transcribed using the non-replicating duplex templates, whereas with the replicating template only satellite I DNA is active. The preaddition of TFIIIA to the non-replicated duplex DNA does not appear to give this template any selective transcriptional advantage. A close inspection of 5S DNA transcription using the replicating templates suggests that preincubation of TFIIIA with single stranded 5S DNA might give more transcription after replication. However, the major point is that duplex satellite I DNA and somatic 5S DNA that have been synthesized in the egg extract from single stranded templates are differentially transcribed. What is responsible for this differential transcription?

We have excluded the unlikely possibility that differential precipitation or differences in the association of non-specific proteins with the two templates could have accounted for these results. This was carried out by cloning the satellite I DNA repeat next to somatic 5S DNA and repeating the transcription assays. The same selective inactivation of somatic 5S DNA occurred on replicating single stranded, but not duplex DNA (Figure 3, lanes 1 and 2).

Control experiments examining the stability of transcripts in the egg extract, using α-amanitin or purified RNAs, showed that the preferential accumulation of satellite I DNA transcripts relative to 5S RNA, when replicating DNA was used as a template, was not due to the selective degradation of 5S RNA (not shown). Additional controls revealed that there are no differences in the kinetics of replication or of DNA supercoiling between templates containing satellite I DNA, 5S DNA or no class III gene at all. We also purified the replicated duplex satellite I and 5S DNA. When added back to the egg extract, both templates were actively transcribed (not shown).

Next, we considered the possibility that the single stranded nature of the templates might selectively inhibit 5S DNA transcription. Non-specific single stranded DNA is a potent inhibitor of transcription in many *in vitro* systems because of its capacity to sequester RNA polymerase (Roeder, 1974) as well as transcription factors (Hanas *et al.*, 1984, 1985; Stillman *et al.*, 1985; Hayes *et al.*, 1989). Duplex or single stranded M13 DNA and a 5S RNA gene were mixed at concentrations typically used in the transcription experiments. No inhibition by single stranded DNA was observed when a double stranded 5S RNA maxi gene was used as the transcription template (Figure 3, lanes 4–6). These experiments were extended to mixtures of double stranded 5S RNA maxi gene with satellite I DNA and alternatively of a wild-type 5S RNA gene with satellite I DNA, both sets of templates being mixed with single stranded 5S DNA (Figure 3, lanes 7 and 8). These results exclude any specific inhibitory effect on transcription complex formation mediated by the single stranded form of the 5S RNA gene during replication.

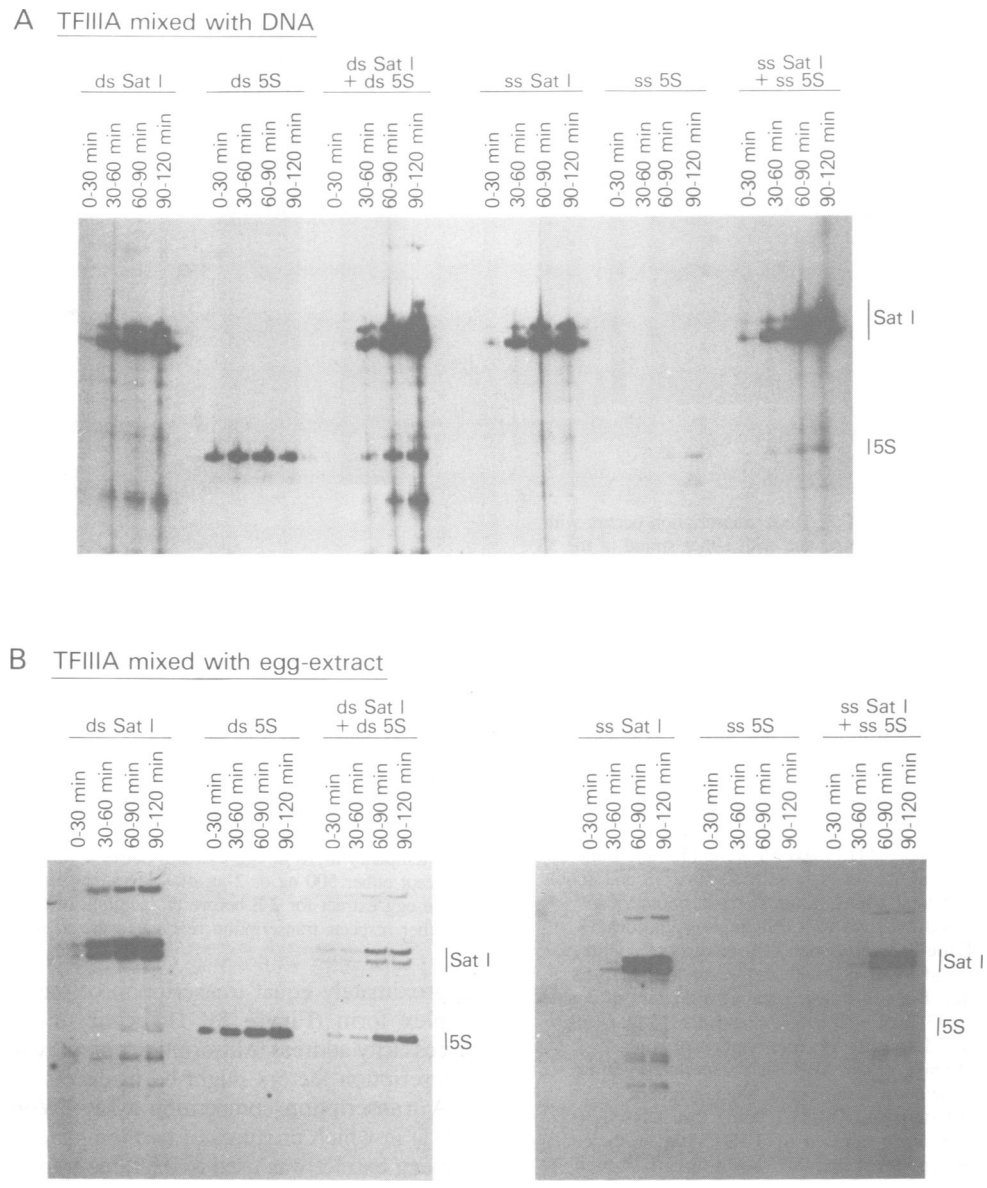

Fig. 2. Time course of transcription on single stranded or double stranded templates with satellite I DNA or the 5S RNA somatic gene. All reactions were performed under standard conditions (Materials and methods). These reactions were supplemented with 500 ng of TFIIIA. In each set of four lanes, the labeling precursor [α-^{32}P]UTP (10 μCi) was added at various times after starting the incubation for a 30 min pulse at 0, 30, 60 and 90 min as indicated. Templates were M13-Sat I as double stranded DNA; M13-5S as double stranded DNA; a mixture of each template; M13-Sat I as single stranded DNA; M13-5S as single stranded DNA; and a mixture of both single stranded forms as indicated. In (**A**) TFIIIA was mixed with the DNA before addition of the extract. In (**B**) TFIIIA was mixed with the extract before addition of the DNA.

We next asked whether a particular secondary structure adopted by single stranded 5S DNA might be important for nucleating or preventing transcription complex assembly (Andersen, 1987). It is important to note that 5S RNA gene transcription is not completely abolished when replicated single stranded 5S DNA is the template. We can therefore investigate variables that may elevate transcription or depress if further. The major secondary structures proposed to exist in 5S RNA, and by inference in the non-coding strand of 5S DNA (Pieler *et al.*, 1986) are likely to be disrupted in a 5S RNA maxi gene (pXbs 115/77; Bogenhagen and Brown, 1981). This maxi gene contains an insertion of 38 bp at the 3′ side of the binding site of TFIIIA. We compared the transcription of both the maxi gene and a wild-type gene as single stranded DNA, and as duplex DNA. Both genes were transcribed equivalently in each case, although transcription from the replicated single stranded DNA was much less than that from duplex DNA added to the extract (Figure 3, lanes 3 and 12).

Finally, each strand of the somatic 5S RNA gene formed an effective template for complementary strand synthesis and low levels of 5S RNA gene transcription (Figure 3, lanes 13 and 14). These results suggest that non-specific single stranded DNA or particular structures adopted by single stranded 5S DNA are not responsible for the low efficiency of transcription of replicated 5S DNA.

We conclude that the differential transcription of satellite I DNA and somatic 5S DNA is not due to experimental artifact. The differential transcription of these two genes is similar to the observations of Lassar *et al.* (1985) in

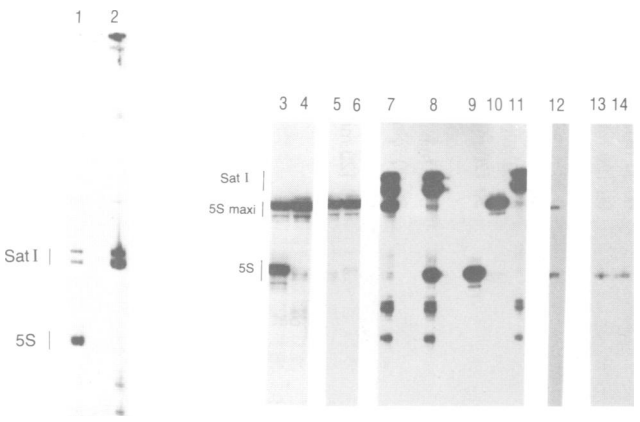

Fig. 3. The selective inactivation of 5S DNA transcription occurs with the 5S DNA in *cis* to satellite I DNA, and with either strand of the 5S RNA gene as template. All reactions were under standard conditions using [α-^{32}P]GTP. Reaction mixtures were incubated for 2 h at 22°C. DNA was preincubated with 500 ng TFIIIA before addition of 10 μl of the egg extract. **Lane 1**; transcription of 100 ng of satellite I and 5S DNA cloned in *cis* (M13-SatI/5S), when added to the reaction mixture as duplex DNA. Labeling was for a pulse of 30 min after 2 h preincubation. **Lane 2**; transcription of 100 ng of satellite I and 5S DNA cloned in *cis*, when added to the reaction mixture as single stranded DNA. Labeling was for a pulse of 30 min after 2 h preincubation (note that this is twice the normal concentration of DNA). **Lane 3**; transcription of a mixture of double stranded M13-maxi 5S and M13-5S (100 ng of total DNA). **Lane 4**; transcription of double stranded M13-maxi 5S alone (50 ng). **Lane 5**; transcription of a mixture of double stranded M13-maxi 5S and double stranded M13 (100 ng of total DNA). **Lane 6**; transcription of a mixture of double stranded M13-maxi 5S and single stranded M13 (100 ng total DNA). **Lane 7**; transcription of a mixture of double stranded M13-maxi 5S and M13-Sat I with single stranded M13-5S (50 ng of each template). **Lane 8**; transcription of a mixture of double stranded M13-5S and M13-Sat I with single stranded M13-maxi 5S (50 ng of each template). **Lanes 9–11**; transcription of double stranded M13-5S, M13-maxi 5S and M13-Sat I respectively (50 ng each). **Lane 12**; transcription of a mixture of single stranded M13-maxi 5S and single stranded M13-5S (100 ng total DNA). **Lane 13**; transcription of single stranded M13-5S (50 ng) in the coding strand orientation. This experiment and that shown in lane 14 used [α-^{32}P]UTP as radioactive precursor. **Lane 14**; transcription of single stranded M13-5S (50 ng) in the non-coding strand orientation. The radiographs shown in **lanes 12–14** were from longer exposures than those in **lanes 1–11**.

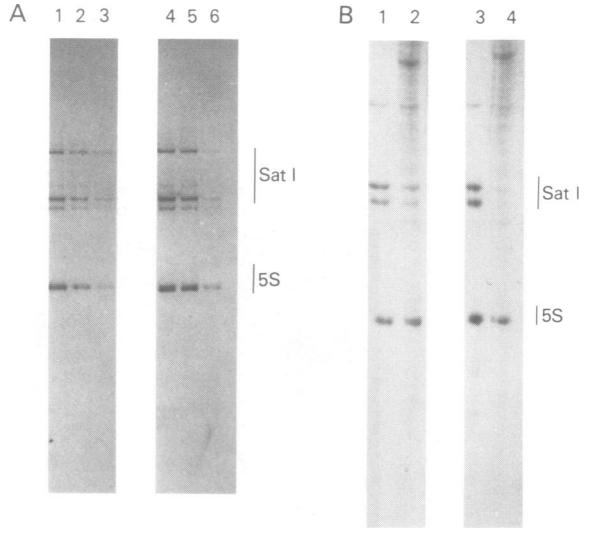

Fig. 4. Satellite I DNA and the 5S RNA somatic gene compete equivalently for transcription factors. (**A**) A mixture of double stranded M13-Sat I and M13-5S was incubated at various ratios in 10 μl egg extract supplemented with 10 μCi [α-^{32}P]GTP. The DNA was preincubated with 200 ng/μl TFIIIA. In **lanes 1** and **4**, 500 ng satellite I and 500 ng 5S RNA somatic gene are mixed (molar ratio of genes 1:1), **lane 2** has 1 μg satellite I DNA and 500 ng 5S RNA somatic gene (2:1), **lane 3** has 2 μg satellite I DNA and 500 ng of 5S RNA somatic gene (4:1), **lane 5** has 500 ng of satellite I DNA and 1 μg of 5S RNA somatic gene (1:2), **lane 6** has 500 ng of satellite I DNA and 2 μg of 5S RNA somatic gene (1:4). (**B**) **Lanes 1** and **3**, transcription of a mixture of double stranded M13-Sat I and M13-5S (100 ng of each template) in 10 μl egg extract. **Lanes 2** and **4**, as in lanes 1 and 3 except either 500 ng or 2 μg of λ DNA respectively was incubated in the egg extract for 2 h before the addition of the class III genes, in all other respects transcription reactions were as in (A).

that 5S DNA (requiring TFIIIA, TFIIIB and TFIIIC) is selectively repressed in chromatin. A gene requiring a different transcription complex (containing only TFIIIB and TFIIIC) for transcription (a tRNA gene) remained active. Satellite I DNA and a tRNA gene share similar promoter elements distinct from 5S DNA. These authors proposed two possible explanations for differential expression of 5S RNA and tRNA genes in the SV40 minichromosome: either the two transcription complexes have different stabilities, or there was a 5S RNA gene specific inhibition of transcription factor interactions. We next examine both of these possibilities.

A 5S RNA gene transcription complex is not assembled efficiently on replicating DNA

The possibility that there was a difference in the stability of the transcription complexes assembled on satellite I DNA and on somatic 5S DNA appeared unlikely because of the approximately equal transcription of the two templates in duplex form (Figure 2). However, this result does not necessarily address transcription complex stability, because transcription factors might be in excess.

A transcription competition assay (Wormington *et al.*, 1981) in which mixtures of two templates are incubated in the egg extract was used to examine transcription complex stability in more detail. In these experiments, a fixed volume of extract is used to transcribe mixtures of genes in which the mass of one template is kept constant, while the mass of the second template is progressively increased. If the two templates bind transcription factors with the same efficiency, then the amount of transcription from each template will be proportional to the mass of each template. If one template binds transcription factors weakly, transcription from this template will be proportionately weaker. The high concentration of DNA used in order to see effective competition inhibits chromatin assembly (Wolffe and Brown, 1987; Almouzni and Méchali, 1988a). With TFIIIA in excess, satellite I DNA and somatic 5S DNA have approximately equivalent competitive strengths in this egg extract (Figure 4A). This suggests that transcription factors are sequestered onto naked duplex satellite I DNA or somatic 5S DNA with equivalent efficiency. Experiments in which high concentrations of each template were added sequentially to the egg extract (Bogenhagen *et al.*, 1982) further confirmed that transcription complexes assembled on satellite I or 5S DNA had equivalent stability in this extract (data not shown; Wolffe and Brown, 1987).

It is apparent from Figure 4A that high DNA concentrations lead to the non-specific inhibition of both satellite I DNA and 5S DNA transcription. In later experiments we make use of high concentrations of non-specific DNA to titrate out chromatin assembly. The least inhibitory DNA we discovered was that from bacteriophage λ. Preincubation of increasing concentrations of λ DNA in egg extract, before addition of a mixture of duplex satellite I and 5S DNA and radioactive RNA precursors, leads to a selective reduction in satellite I DNA transcription (Figure 4B). Although the exact cause of this selectivity has not been determined, we believe it could be due to a selective sequestration by λ DNA of transcription factors that bind the type of internal control region found in satellite I or tRNA genes. In any event both satellite I and 5S DNA remained active in the presence of adequate non-specific DNA to titrate out chromatin (see below; Wolffe and Brown, 1987). Therefore we next considered whether the formation of transcription complexes might be influenced by the assembly of chromatin onto the DNA.

Mixtures of single stranded satellite I DNA and somatic 5S DNA were incubated in the egg extract such that complete replication and chromatin assembly would proceed. Addition of radioactive ribonucleotides showed that satellite I DNA was transcribed more efficiently than somatic 5S DNA (Figure 5, lane 1). The assembled chromatin was purified through sucrose, either after the addition of isotonic buffer or following a 0.5 M salt wash at room temperature. This salt concentration is high enough to cause the disruption of regular nucleosomal arrays (Spadafora *et al.*, 1979; data not shown), whereas transcription complexes are stable to this salt concentration (Setzer and Brown, 1985). Disruption of chromatin structure with salt did not permit any additional transcription of somatic 5S DNA relative to satellite I DNA by purified RNA polymerase III (Figure 5, lane 3). In contrast, when the disrupted chromatin is incubated in an oocyte nuclear extract rich in class III gene transcription factors, somatic 5S DNA transcription is stimulated relative to that of satellite I DNA (Figure 5, lane 5). The chromatin that was not salt treated did not show any selective activation of somatic 5S RNA synthesis (Figure 5, lane 6). These results indicate that an intact chromatin structure can prevent transcription factors or RNA polymerase binding to 5S DNA. However, the disrupted chromatin is accessible to transcription factors and RNA polymerase. In the presence of RNA polymerase alone only satellite I transcripts accumulate, suggesting that the difference in transcription between satellite I DNA and 5S DNA can be explained by the failure to form competent transcription complexes on 5S DNA. Perhaps the assembly of nucleosomes including 5S DNA is responsible for preventing transcription complex formation.

Chromatin assembly is necessary for the repression of 5S DNA

The results presented to this point show that although satellite I DNA and 5S DNA are transcribed efficiently as duplex DNA, 5S DNA is inefficiently transcribed relative to satellite I DNA when replicating single stranded DNA is used as a template (Figures 2–4). The inactivity of 5S DNA may be due to the formation of nucleosomes. If this is true, then high nucleosome densities must be required since repression of 5S DNA is only seen on replicating templates. Titration

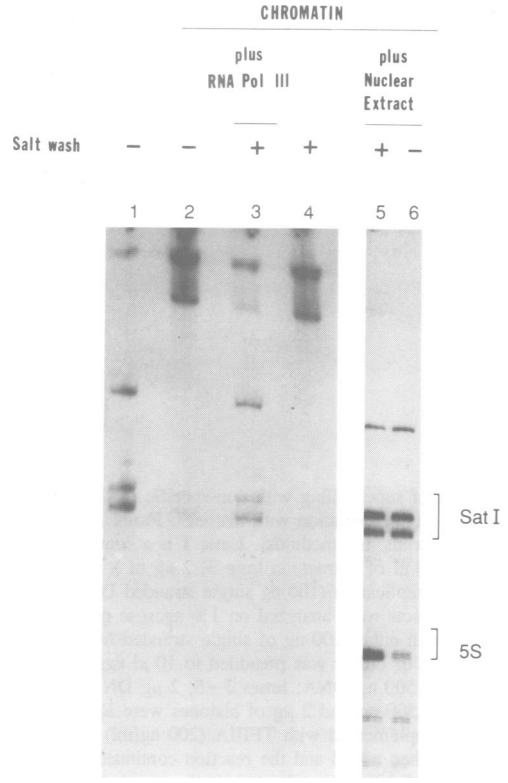

Fig. 5. Somatic 5S RNA genes do not form competent transcription complexes using single stranded 5S DNA as a template, instead, the gene is repressed. A mixture of single stranded M13-Sat I and M13-5S was incubated in 10 μl of the egg extract supplemented with [α-^{32}P]GTP for 90 min in the presence of 200 ng/μl TFIIIA. Radioactive nucleotide was used to monitor the position of the chromatin within the sucrose cushion. [α-^{32}P]GTP was converted to deoxy form and incorporated into DNA during replication. This radioactive DNA was recovered as chromatin, whereas radiolabeled RNA was lost in the cushion. The reaction mixture was then either mixed with buffer (**lanes 1, 2** and **6**) or with salt to make the reaction up to 0.5 M NaCl (**lanes 3–5**). The reaction mixtures (**lanes 2–6**) were then centrifuged through a 7% sucrose layer in J buffer (Materials and methods). The pellet was then used for transcription experiments under the conditions described (Materials and methods). Reactions were supplemented either with nothing (**lanes 2** and **4**), with pol III (60 U) (**lane 3**) or with oocyte nuclear extract (**lanes 5** and **6**) and incubated for 2 h. **Lane 1** is a control for transcription in the egg extract without fractionation, rNTPs and [α-^{32}P]GTP were added after a 90 min incubation and the transcription allowed to continue for 2 h. The gel lanes derived from templates transcribed in the oocyte nuclear extract were exposed for a shorter time.

of chromatin assembly with excess non-specific DNA should prevent the repression of 5S DNA.

We used non-specific λ DNA to titrate out chromatin assembly without preventing transcription of specific genes (Wolffe and Brown, 1987, Figure 4B). Concentrations of non-specific DNA as low as 50 μg/ml begin to titrate out the supercoiling of replicating single stranded DNA and 200 μg/ml of λ DNA titrates DNA supercoiling almost completely (Figure 6A, lane 3). The titratable component in this system appears to be core histones, as the readdition of purified core histones to the replication reaction that has been previously preincubated with competitor DNA restores supercoiling (not shown).

The presence of this excess of λ DNA has no major effects on the efficiency of replication of single stranded DNA. Satellite I DNA transcription is reduced ~3- to 5-fold in

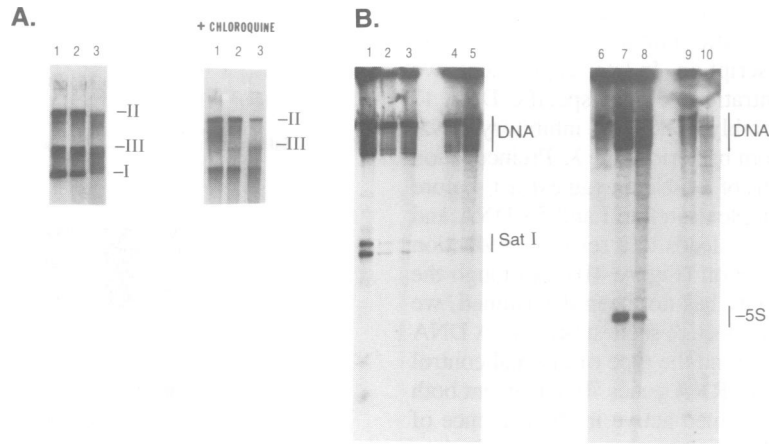

Fig. 6. Titration of supercoiling with non-specific DNA and the effect on transcription of replicating single stranded satellite I and 5S somatic DNA with or without supplementation with histones. **Panel A**. The egg extract was preincubated for 2 h with competitor DNA in the presence of Mg^{2+}/ATP (Materials and methods). **Lane 1** is a control to which buffer was added without competitor DNA. In **lane 2**, 500 ng of λ DNA was preincubated in 10 μl of extract, in **lane 3**, 2 μg of λ DNA was preincubated in the extract. After 2 h TFIIIA was added to the extract (200 ng/μl). The template for replication (100 ng single stranded DNA) and 10 μCi of [α-^{32}P]dATP were then added and replication allowed to proceed for 90 min. The products were analyzed on 1% agarose gels without chloroquine or with 30 μg/ml chloroquine (+CHLOROQUINE). **Panel B**. All reactions were with either 100 ng of single stranded M13-Sat I (**lanes 1–5**) or 100 ng of single stranded M13-5S (**lanes 6–10**). In **lanes 1–5**, non-specific DNA or buffer was preadded to 10 μl egg extract for 2 h as indicated in the presence of Mg^{2+}/ATP (Materials and methods): **lane 1**, no DNA; **lane 2**, 500 ng DNA; **lanes 3–5**, 2 μg DNA. In **lanes 1–3** no exogenous histones were added before addition of single stranded DNA, in **lanes 4** and **5**, 500 ng and 2 μg of histones were added to the reaction mixture for 30 min before addition of the single stranded DNA. All reactions were supplemented with TFIIIA (200 ng/ml) at this point as well. Replication was then allowed to proceed for 90 min. Radioactive [α-^{32}P]GTP was then added and the reaction continued for 1 h. **Lanes 6–10** are as in lanes 1–5 except single stranded M13-5S is used. Note that the incorporation of ^{32}P into replicated single stranded M13 DNA varies in the sample lanes (marked DNA). This incorporation depends on the reduction of the ribonucleotide to the deoxyribonucleotide and incorporation of the radiolabeled deoxyribonucleotide into the replicating template. Ideally this incorporation would be uniform. We have found that the efficiency of solubilization of high mol. wt DNA in the formamide sample buffer varies especially in the presence of quantities of λ DNA. Such a variation is not seen with low mol. wt RNA. We therefore attach no significance to the apparent variation in radioactive DNA recovered and visualized on the autoradiogram.

the presence of λ DNA (Figure 6B, lanes 1–3). Note that these concentrations of λ DNA have a general inhibitory effect on class III gene transcription, probably due to the non-specific sequestration of transcription factors or RNA polymerase (Figure 4; see Wolffe and Brown, 1987). There also appears to be a selective inhibition of satellite I DNA transcription relative to that of 5S DNA in the presence of λ DNA (see Figure 4B). The addition of core histone concentrations sufficient to restore the supercoiling of replicating DNA did not alter satellite I DNA transcription significantly (Figure 6B, lanes 4 and 5). In contrast, addition of λ DNA greatly stimulates transcription of a replicating somatic 5S DNA template (Figure 6B, lanes 6–8). The reduction in DNA supercoiling is quite small when 5S DNA transcription is maximally stimulated (Figure 6A, cf. lanes 1 and 2). This result emphasizes the requirement for efficient chromatin assembly in order to see repression of 5S DNA transcription. Supplementation of the reaction mixture with core histones dramatically reduces the efficiency of 5S RNA gene transcription (Figure 6B, lanes 9 and 10). We conclude that chromatin assembly is the major event preventing transcription of the somatic 5S RNA gene in this system.

Chromatin structure of 5S DNA

In vitro reconstitution of nucleosomes using short linear DNA fragments have shown that the *X.borealis* somatic 5S RNA gene positions a nucleosome over the first 70 bp of the gene including 30 bp of the internal control region, which is the binding site for TFIIIA (Sakonju and Brown, 1982; Rhodes, 1985). A similar nucleosome position is seen when closed circular DNAs are reconstituted with purified core histones, and is correlated with an inhibition of transcription complex formation (D.J.Clark and A.P.Wolffe, unpublished data). Experiments using *in vitro* chromatin assembly systems for *Xenopus* oocytes also report a nucleosome assembling at this position (Shimamura *et al.*, 1988). Transcription is also inhibited in the oocyte extracts, although the interpretation of these results is complicated by the use of non-physiological temperatures to assemble chromatin (37°C) (Shimamura *et al.*, 1988; Wolffe *et al.*, 1984). However, *in vivo* experiments have failed to show a precise positioning of a nucleosome on cloned 5S DNA assembled into chromatin following injection into oocyte nuclei (Gargiulo and Worcel, 1983). The position, if any, of a nucleosome on a somatic 5S RNA gene *in vivo* is unknown.

Primer extension footprinting (Gralla, 1985) of both duplex 5S DNA and of the replicated single stranded 5S DNA after 90 min incubation in the egg extract did not show either a nucleosome footprint or a TFIIIA footprint over the 5S RNA gene (Figure 7, lanes 2 and 4). Parallel reactions to which radioactive RNA precursors were added revealed the duplex template to be transcriptionally active and the replicated single stranded template to be repressed. DNase I digestion of single stranded DNA replicated in the presence of [α-^{32}P]dATP in a parallel reaction revealed a clear 10–11 bp modulation of cleavage indicating that the DNA was assembled into chromatin (Figure 7, lane 5). The lack of a TFIIIA footprint is not surprising because <5% of templates are active under these conditions (calculated from the measured transcription rate and the maximal rate observed *in vitro*; Wolffe *et al.*, 1986). The absence of a nucleosome footprint was unexpected; however, clear changes in DNase I cleavage from the digestion pattern of naked DNA are seen, perhaps indicating multiple preferred

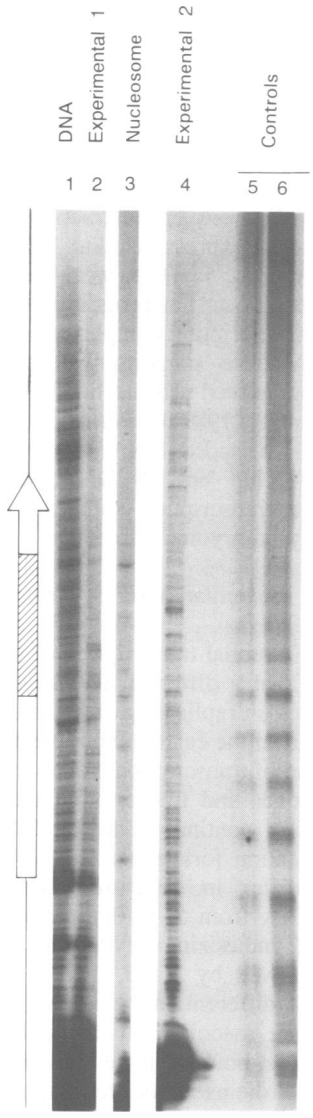

Fig. 7. DNase I footprinting of chromatin assembled on replicating single stranded DNA containing a somatic 5S RNA gene. Primer extension analysis was performed as described by Gralla (1985). The primer was homologous to the coding strand of 5S DNA from −39 to −19 relative to the start of the gene. **Lane 1** shows the digestion pattern of duplex 5S RNA gene (M13-5S) added to the egg extract and digested immediately with DNase I as described in Materials and methods. **Lane 2** ('Experimental 1') shows the digestion pattern of duplex 5S DNA added to the egg extract and digested after 90 min of incubation. **Lane 3** ('Nucleosome') shows the digestion pattern of the same DNA reconstituted into nucleosomes by salt−urea dialysis of purified core histones (Camerini-Otero *et al.*, 1976). The DNA was incubated for 90 min in the egg extract before DNase I digestion. **Lane 4** ('Experimental 2') shows the DNase I digestion pattern of M13-5S which was added to the egg extract in single stranded form in the presence of 200 ng/μl TFIIIA. The reaction mixture was incubated for 90 min before treatment with DNase I. **Lane 5** shows a control reaction ('Control') in which the same reaction mixture as described in Figure 3 was supplemented with 10 μCi [α-^{32}P]dATP. The reaction was incubated for 90 min before DNase I treatment. The radioactive products, following digestion and purification, were resolved directly on a 6% polyacrylamide−7 M urea gel and autoradiographed. The position of the 5S RNA gene (arrow) and the internal control region (ICR) are indicated (hatched box). **Lane 3** is from a separate experiment run with markers, the other lanes are from the same gel.

nucleosome positions (Figure 7, cf. lanes 1 and 4). Control experiments confirmed that the DNA in parallel experiments was supercoiled, and therefore assembled into chromatin as expected. As a positive control for the stability of a nucleosome to DNase I digestion in the egg extract, nucleosomes assembled onto closed circular duplex 5S DNA by salt/urea dialysis (Camerini-Otero *et al.*, 1976) were incubated in the egg extract followed by DNase I footprinting. A 10−11 bp modulation of DNase I cleavage is seen (Figure 7, lane 3), indicating that the nucleosome once formed was stable in the extract. We do not detect a similar modulation of DNase I cleavage in the chromatin assembled in the egg extract. As our analysis allows only the bulk population of molecules to be examined, we cannot exclude the possibility that a population of molecules do have a nucleosome positioned on the 5S RNA gene as previously described (Rhodes, 1985). However, this result suggests that precise nucleosome positioning is not required for repression of 5S RNA gene transcription.

Discussion

The major conclusion from this work is that competition exists between transcription factors and histones for binding to promoter elements on replicating DNA. Histones compete out transcription factors on somatic 5S RNA genes more effectively than on satellite I DNA (Figures 2, 3, 5 and 6). The efficient and rapid assembly of replicating templates into chromatin inhibits somatic 5S RNA gene transcription. The assembly of duplex DNA into chromatin appears to be too slow and inefficient under the conditions used to inhibit 5S RNA gene transcription (Almouzni and Méchali, 1988a). Small reductions in the efficiency of chromatin assembly on replicating DNA are sufficient to activate 5S DNA (Figure 6). The inhibition of 5S RNA gene transcription is due to a failure to form competent transcription complexes (Figure 5).

Transcription factor binding to naked DNA does not explain differential gene transcription

Transcription complexes form on class III genes with efficiencies that depend on the affinity of individual transcription factors for particular promoter elements (Pieler *et al.*, 1987; Wolffe and Brown, 1987, 1988; Wolffe, 1988). Transcription factors already bound to class III genes can also be dissociated to different extents, depending on the affinity of the transcription factors for each other and the promoter (Sakonju and Brown, 1982; Wolffe and Brown, 1987). In this *Xenopus* egg extract with excess TFIIIA and without chromatin assembly taking place, a somatic 5S RNA gene competes effectively for common transcription factors with satellite I DNA (Figure 4). Transcription complexes, once assembled, also appear to have comparable stabilities on satellite I and 5S DNA in this extract. Therefore, preferential binding of transcription factors to satellite I DNA rather than to 5S DNA, when naked in solution, is not the explanation for the selective reduction in 5S DNA transcription.

The contribution of chromatin structure to differential gene transcription

Chromatin structures are known to maintain genes in a repressed state (Schlissel and Brown, 1984; Lassar *et al.*, 1985). Changes in chromatin structure can selectively repress genes; e.g. the late addition of histone H1 to transcriptionally active chromatin can cause changes in differential class III gene activity (Wolffe, 1989a). The cell free systems used

to assemble chromatin derived from *Xenopus* eggs and oocytes do not appear to add endogenous histone H1, probably because the normal somatic form of histone H1 is not present in these extracts (Dilworth *et al.*, 1987; Shimamura *et al.*, 1988; see also Wolffe, 1989a,b). Titration of core histones by the addition of non-specific DNA to the replication reaction, as revealed by the reduction in DNA supercoiling (Germond *et al.*, 1975), leads to activation of 5S DNA transcription (Figure 6). Readdition of core histones restores DNA supercoiling and the repressed state. We conclude that repression is dependent on the association of core histones with the replicating DNA.

The repression of gene activity by core histones is in agreement with earlier conclusions in which chromatin was assembled onto promoter elements before transcription factors were added (Bogenhagen *et al.*, 1982; Gottesfeld and Bloomer, 1982; Weisbrod *et al.*, 1982; Knezetic and Luse, 1987; Workman and Roeder, 1987). In these experiments no attempt was made to ascertain whether precise nucleosome positions were responsible for the repressed state. When assayed, repression was established at low levels of chromatin assembly. In contrast, our experiments suggest that the efficiency of nucleosome assembly will have to be very high in order to cause 5S RNA gene repression (Figures 1 and 6; Almouzni and Méchali, 1988a; Morse, 1989). Evidence from *in vivo* experiments suggests that precisely positioned nucleosomes may be important in repressing gene expression (Almer and Horz, 1986; Almer *et al.*, 1986; Han and Grunstein, 1988; Han *et al.*, 1988). In our experiments, repression of 5S DNA appears independent of precise nucleosome positioning (Figure 7).

Biological significance

These *in vitro* experiments were designed to examine the formation of transcription complexes under conditions of chromatin assembly on replicating DNA. We believe that these conditions more closely resemble those that occur *in vivo* than the addition of non-replicating duplex DNA to transcription extracts, that may or may not assemble chromatin. Although much more experimentation will be required before we have a clear view of the relevance of these experiments to the *in vivo* expression of satellite I and 5S DNA, several interesting similarities appear.

Satellite I DNA is normally transcribed at high levels only in oocytes (Ackerman, 1983; Wakefield *et al.*, 1983). However, isolation of embryonic chromatin indicates that satellite I DNA is partially programmed with transcription complexes at the mid-blastula transition (MBT) (Wolffe, 1989a). In this respect the developmental regulation of satellite I DNA resembles that of the oocyte 5S RNA genes (Wormington and Brown, 1983). Satellite I DNA and oocyte 5S DNA are then repressed more extensively through gastrulation (Wolffe, 1989a). The mechanism responsible for repressing these genes involves the association of histone H1 with nucleosomal arrays (reviewed by Wolffe and Brown, 1988). In contrast, somatic 5S RNA genes are active throughout oogenesis, at the MBT and through gastrulation. However, the absolute activity of somatic 5S DNA at these different developmental stages has not been determined. There is some evidence that all somatic 5S RNA genes are not transcribed in somatic cells (Wolffe, 1989b). Supplementation of somatic chromatin with excess TFIIIA in an egg extract activates both oocyte and somatic 5S RNA gene transcription substantially. Somatic 5S DNA may therefore be partially repressed during embryogenesis presumably by chromatin mediated mechanisms.

The similarities between our results using replicated single stranded DNA and the developmental regulation of class III genes perhaps exist in the early period of embryogenesis, during the period of rapid replication cycles (Graham and Morgan, 1966). *Xenopus* egg extracts program *Xenopus* sperm chromatin to express satellite I DNA vigorously, but oocyte and somatic 5S DNA weakly (Wolffe, 1989b). Presumably this reflects the programming of embryonic chromatin with transcription complexes prior to the MBT. Experimental evidence suggests that isolated pre-MBT chromatin is programmed with satellite I DNA transcription complexes (Wolffe, 1989a). Perhaps our results with replicating single stranded DNA reflect the capacity of satellite I DNA, but not 5S DNA, to be efficiently programmed with transcription complexes on a replicating template in early embryonic chromatin.

Models for the molecular basis of differential gene transcription

The basis for differential transcription of satellite I and 5S DNA might be that a different mechanism of chromatin assembly operates on replicating DNA in comparison with duplex DNA. All of the earlier experiments examining the general inhibition of transcription, following the sequential addition of histones and transcription factors, made use of long regions of continuous duplex DNA. In fact the eukaryotic replication fork generates two daughter DNA duplexes that differ in structure. The leading strand (synthesizing DNA from 5′ to 3′) is continuous, but the lagging strand (synthesizing DNA from 3′ to 5′) is discontinuous (reviewed by Hand, 1978). Not only is the structure of DNA different on the lagging strand, but many other proteins are associated with the replicating DNA including those that bind to single stranded regions (Wold *et al.*, 1989). Proteins associated with the replication machinery may facilitate chromatin assembly (Stillman, 1986; Almouzni and Méchali, 1988a). It might also be possible that histones associate with single stranded DNA and accelerate nucleosome formation during replication (Palter *et al.*, 1979). Chromatin assembly at the replication fork might therefore be expected to differ from that on duplex DNA, as is observed here. It may differ not only kinetically, but also biochemically. These possibilities remain to be tested.

Differential transcription of satellite I DNA and 5S DNA could depend on the relative kinetics of transcription complex assembly in the face of efficient chromatin assembly on replicating DNA. The results of Roeder and colleagues (Workman *et al.*, 1988) suggested possible regulatory events at this level. Efficient transcription of the adenovirus major late promoter in the face of nucleosome assembly was proposed to be dependent on the rate of preinitiation complex formation. Genes requiring only transcription factors TFIIIC and TFIIIB, i.e. satellite I DNA or tRNA genes, have been shown to assemble transcription complexes more rapidly than 5S RNA genes, which also require TFIIIA (Jahn *et al.*, 1987; Wolffe, 1989a). This could be the reason why 5S DNA is repressed whereas satellite I DNA remains active. Although most of our experiments differ from earlier work in that we use a preaddition protocol in which TFIIIA is prebound to

the 5S DNA before addition to the extract, we also compared reactions in which TFIIIA was mixed with the extract before the template was added (Figure 2). We find no significant differences in our results using either of these protocols. In fact, the preaddition protocol appears to make transcription complex assembly on duplex DNA as rapid as on satellite I DNA (Figure 2A). We conclude that the kinetics of transcription complex formation would seem not to be important for determining differential gene activity in this particular case.

An alternative explanation for the differences in transcription between satellite I DNA and 5S RNA genes is that a satellite I DNA transcription complex has a structure that can be accommodated into a regulated nucleosomal array, whereas a complete 5S RNA gene transcription complex is unstable in ordered chromatin (Lassar *et al.*, 1985). Changes in the organization of chromatin might therefore cause the displacement of transcription factors from genes (Wolffe, 1989a). The equilibrium binding of transcription factors in the presence of chromatin, rather than the rate of association of transcription factors would determine the final transcriptional state.

Materials and methods

Preparation of the X.laevis egg extract

Unfertilized eggs were obtained from *X.laevis* frogs, and extracts were prepared as detailed in Almouzni and Méchali (1988a,b). Briefly, dejellied eggs were disrupted by direct centrifugation (12 000 *g* for 30 min) at 4°C in a modified extraction medium (20 mM HEPES pH 7.5, 70 mM potassium chloride, 1 mM dithiothreitol (DTT), 5% sucrose, 10 μg/ml leupeptin). The supernatant was recentrifuged at 150 000 *g* for 60 min. The final supernatant was stored in small aliquots at −80°C.

Preparation of DNA

DNA from bacteriophage M13mp18 was prepared from phage purified by CsCl buoyant density centrifugation. Double stranded form I M13 DNA was isolated and prepared as described in Maniatis *et al.* (1982). Radiolabeled double stranded form I M13 DNA was prepared by incubating single stranded M13 in the egg extract in the presence of [α-^{32}P]dATP (10−20 μCi) and purifying the replicated DNA by deproteinization and ethanol precipitation. Class III genes were cloned into M13 vectors as described by Maniatis *et al.* (1982). The satellite DNA gene used was contained in a *Hin*dIII fragment from E190 (Lam and Carroll, 1983) cloned into M13 and designated as M13-Sat I. The somatic 5S RNA gene was contained in a BamHI fragment from pXP-10 (Wolffe *et al.*, 1986), designated as M13-5S. The somatic 5S RNA maxigene was cloned as *Hin*dIII fragment from pXbs 115/77 (Sakonju *et al.*, 1980), designated M13-maxi 5S. Satellite I DNA and 5S DNA were cloned in *cis* by sequential insertion of the *Hin*dIII fragment of E190 and the *Bam*HI fragment of pXP-10 into the M13 polylinker. This construct was designated M13-SatI/5S.

The oligonucleotide probe used for the primer extension footprinting was 20 nucleotides long, corresponding to the sequence 5′ to the 5S somatic 5S RNA gene from −39 to −19 relative to the start of transcription.

DNA synthesis, chromatin assembly and transcription reactions

Unless otherwise specified, our standard reaction mixtures contained 10−20 μg DNA/ml egg extract supplemented with 3 mM ATP and 5 mM $MgCl_2$. Supplementation of the egg extract with Mg^{2+}/ATP was carried out before DNA was added to the extract. DNA synthesis was followed by the addition of 10−20 μCi of [α-^{32}P]dATP to the reaction. Aliquots were taken at various times during incubation at 22°C and either transferred to Whatman GF/C filters and processed for the counting of acid-insoluble material as previously described (Méchali and Harland, 1982), or processed for digestion by micrococcal nuclease. When subjected to electrophoresis, the samples were deproteinized by proteinase K (500 μg/ml) followed by phenol extraction and ethanol precipitation. To follow transcription, 10 μl of the reaction mixtures contained 500 μM of ATP, UTP and CTP, 100 μM of GTP, 10 μCi of [α-^{32}P]GTP (3000 Ci/mmol) and 5 U human placental RNase inhibitor (BRL). When [α-^{32}P]UTP was used instead of [α-^{32}P]GTP, the concentrations of both of these compounds was reversed. Incubations were continued for the times indicated. The samples were then processed for sequencing gel analysis as previously described (Wolffe and Brown, 1987). Unless stated to the contrary, in all transcription reactions using 5S DNA, an excess of TFIIIA was added (50 ng/μl extract). TFIIIA was usually preadded to the template before the extract in these experiments, but mixing the protein with the extract before adding the template gave comparable results (see Figure 2).

In order to assess the presence or absence of intact transcription complexes on assembled minichromosomes we made use of the differential stability of chromatin and transcription complexes to salt (Spadafora *et al.*, 1979; Setzer and Brown, 1985). Transcriptional complexes on class III genes resist 0.5 M NaCl, although RNA polymerase III itself dissociates. Nucleosomes become close packed following treatment with 0.5 M NaCl, as determined by micrococcal nuclease digestion. The reaction mixture was therefore made 0.5 M with NaCl, incubated for 10 min at room temperature, and then pelleted in a microfuge through 7% sucrose containing 1 × J buffer (70 mM NH_4Cl, 7 mM $MgCl_2$, 10 mM HEPES pH7.4, 0.1 mM EDTA and 2.5 mM DDT). Transcription was reconstituted either with oocyte nuclear extract (Birkenmeier *et al.*, 1979; Wolffe, 1989b) or with 60 U purified RNA polymerase III (Cozzarelli *et al.*, 1983).

Preparation of transcription factors and histones

The 5S RNA specific transcription factor TFIIIA was isolated as described (Smith *et al.*, 1984). Core histones were purified from chicken erythrocytes as histone pairs H2A + H2B and H3 + H4 as described by Simon and Felsenfeld (1979).

Digestion with DNase I and micrococcal nuclease

Conditions for DNase I footprinting of DNA fragments with TFIIIA have been described (Wolffe *et al.*, 1986).

Primer-extension footprinting of circular DNA molecules was carried out by a modification of the method of Gralla (1985). Single stranded M13-5S was replicated in the egg extract supplemented with 200 μg/ml TFIIIA. This DNA was then digested lightly with varying amounts of DNase I. Samples were then processed as described by Wolffe and Brown (1987).

Control nucleosomal templates were assembled using closed circular duplex M13-5S and purified chicken erythrocyte core histones by salt−urea dialysis (Camerini-Otero *et al.*, 1976).

Unless otherwise specified, chromatin assembly reactions were digested by micrococcal nuclease (150 U/μg assembled DNA) after addition of 3 mM $CaCl_2$. Aliquots were taken during digestion, made up to 30 mM EDTA, 0.5% SDS, and treated as described above, either for gel electrophoresis (Maniatis *et al.*, 1982) or for counting of acid-insoluble material.

Acknowledgements

We would like to thank our many colleagues in the Laboratory of Molecular Biology and the Laboratory of Cellular and Developmental Biology, NIDDK for their comments on this work. We are especially grateful to Drs D.D.Brown, D.J.Clark, R.Martin, R.H.Morse and R.T.Simpson for their comments on the manuscript and to Thuy for its preparation.

References

Ackerman,E.J. (1983) *EMBO J.*, **2**, 1417−1422.
Almer,A. and Horz,W. (1986) *EMBO J.*, **5**, 2681−2687.
Almer,A., Rudolph,H., Hinnen,A. and Horz,W. (1986) *EMBO J.*, **5**, 2689−2696.
Almouzni,G. and Méchali,M. (1988a) *EMBO J.*, **7**, 665−672.
Almouzni,G. and Méchali,M. (1988b) *EMBO J.*, **7**, 4355−4365.
Andersen,J. (1987) *FEBS Lett.*, **217**, 197−202.
Birkenmeier,E.H., Brown,D.D. and Jordan,E. (1978) *Cell*, **15**, 1077−1086.
Blow,J.J. and Laskey,R.A. (1986) *Cell*, **47**, 577−587.
Bogenhagen,D.F. and Brown,D.D. (1981) *Cell*, **24**, 261−270.
Bogenhagen,D.F., Wormington,W.M. and Brown,D.D. (1982) *Cell*, **28**, 413−421.
Brown,D.D. (1984) *Cell*, **37**, 359−365.
Camerini-Otero,R.D., Sollner-Webb,B. and Felsenfeld,G. (1976) *Cell*, **8**, 333−347.
Cortese,R., Harland,R. and Melton,D. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4147−4151.
Cozzarelli,N.R., Gerrard,S.P., Schlissel,M.S., Brown,D.D. and Bogenhagen,D.F. (1983) *Cell*, **34**, 829−835.
Dilworth,S.M., Black,S.J. and Laskey,R.A. (1987) *Cell*, **51**, 1009−1018.
Engelke,D.R., Ng,S.-Y., Shastry,B.S. and Roeder,R.G. (1980) *Cell*, **19**, 717−728.

Gargiulo,G. and Worcel,A. (1983) *J. Mol. Biol.*, **170**, 699–722.
Germond,J.E., Hirt,B., Oudet,P., Gross-Bellard,M. and Chambon,P. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 1843–1847.
Gottesfeld,J. and Bloomer,L.S. (1982) *Cell*, **28**, 781–791.
Graham,C.F. and Morgan,R.W. (1966) *Dev. Biol.*, **14**, 439–460.
Gralla,J.D. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 3078–3081.
Han,M. and Grunstein,M. (1988) *Cell*, **55**, 1137–1145.
Han,M., Kim,U.-J., Kayne,P. and Grunstein,M. (1988) *EMBO J.*, **7**, 2221–2228.
Hanas,J.S., Bogenhagen,J.S., Bogenhagen,D.F. and Wu,C.-W. (1984) *Nucleic Acids Res.*, **12**, 2745–2758.
Hanas,J.S., Hazuda,D.J. and Wu,C.-W. (1985) *J. Biol. Chem.*, **260**, 13316–13320.
Hand,R. (1978) *Cell*, **15**, 317–325.
Hayes,J., Tullius,T.D. and Wolffe,A.P. (1989) *J. Biol. Chem.*, **264**, 6009–6012.
Huberman,J.A. (1981) *Cell*, **23**, 647–649.
Hutchison,C.J., Cox,R., Drepaul,R.S., Gomperts,M. and Ford,C.C. (1987) *EMBO J.*, **6**, 2003–2010.
Jahn,D., Wingender,E. and Seifert,K.H. (1987) *J. Mol. Biol.*, **193**, 303–313.
Knezetic,J.A. and Luse,D.S. (1986) *Cell*, **45**, 95–104.
Lam,B.S. and Carroll,D. (1983) *J. Mol. Biol.*, **165**, 567–585.
Lassar,A., Hamer,D.M. and Roeder,R.G. (1985) *Mol. Cell. Biol*, **5**, 40–45.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Méchali,M. and Harland,R.M. (1982) *Cell*, **30**, 93–101.
Morse,R.H. (1989) *EMBO J.*, **8**, 2343–2351.
Noll,M. and Kornberg,R.D. (1977) *J. Mol. Biol.*, **109**, 393–404.
Palter,K.B., Foe,V.E. and Alberts,B.M. (1979) *Cell*, **18**, 451–467.
Peterson,R.C., Doering,J.L. and Brown,D.D. (1980) *Cell*, **20**, 131–141.
Pieler,T., Guddat,U., Oei,S.L. and Erdmann,V.A. (1986) *Nucleic Acids Res.*, **14**, 6313–6326.
Pieler,T., Hamm,J. and Roeder,R.G. (1987) *Cell*, **48**, 91–100.
Rhodes,D. (1985) *EMBO J.*, **4**, 3473–3482.
Roeder,R.G. (1974) *J. Biol. Chem.*, **249**, 241–248.
Sakonju,S. and Brown,D.D. (1982) *Cell*, **31**, 395–405.,
Sakonju,S., Bogenhagen,D.F. and Brown,D.D. (1980) *Cell*, **19**, 13–25.
Schlissel,M.S. and Brown,D.D. (1984) *Cell*, **37**, 903–913.
Segall,J., Matsui,T. and Roeder,R.G. (1980) *J. Biol. Chem.*, **255**, 11986–11991.
Senshu,T., Fukada,M. and Ohashi,M. (1978) *J. Biochem.*, **84**, 985–988.
Setzer,D.R. and Brown,D.D. (1985) *J. Biol. Chem.*, **260**, 2483–2492.
Shastry,B.C., Ng,S.-Y. and Roeder,R.G. (1982) *J. Biol. Chem.*, **257**, 12979–12986.
Shimamura,A., Tremethick,D. and Worcel,A. (1988) *Mol. Cell. Biol.*, **8**, 4257–4269.
Simon,R.H. and Felsenfeld,G. (1979) *Nucleic Acids Res.*, **6**, 689–696.
Smith,D.R, Jackson,I.J. and Brown,D.D. (1984) *Cell*, **37**, 645–652.
Spadafora,C., Oudet,P. and Chambon,P. (1979) *Eur. J. Biochem.*, **100**, 225–235.
Stillman,B.W. (1986) *Cell*, **45**, 555–565.
Stillman,D.J., Caspers,P. and Geiduschek,E.D. (1985) *Cell*, **40**, 311–317.
Wakefield,L., Ackerman,E. and Gurdon,J.B. (1983) *Dev. Biol.*, **95**, 468–475.
Weisbrod,S., Wickens,M.P., Whytock,S. and Gurdon,J.B. (1982) *Dev. Biol.*, **94**, 216–229.
Wold,M.S., Weinberg,D.H., Virshup,D.M., Li,J.J. and Kelly,J.J. (1989) *J. Biol. Chem.*, **264**, 2801–2810.
Wolffe,A.P. (1988) *EMBO J.*, **7**, 1071–1079.
Wolffe,A.P. (1989a) *EMBO J.*, **8**, 527–537.
Wolffe,A.P. (1989b) *Nucleic Acids Res.*, **17**, 767–780.
Wolffe,A.P. and Brown,D.D. (1986) *Cell*, **47**, 217–227.
Wolffe,A.P. and Brown,D.D. (1987) *Cell*, **51**, 733–740.
Wolffe,A.P. and Brown,D.D. (1988) *Science*, **241**, 1626–1632.
Wolffe,A.P., Perlman,A.J. and Tata,J.R. (1984) *EMBO J.*, **3**, 2763–2770.
Wolffe,A.P.., Jordan,E. and Brown,D.D. (1986) *Cell*, **44**, 381–389.
Worcel,A., Han,S. and Wong,M.L. (1978) *Cell*, **15**, 969–977.
Workman,J.L. and Roeder,R.G. (1987) *Cell*, **51**, 613–622.
Workman,J.L., Abmayr,S.M., Cromlich,W.A. and Roeder,R.G. (1988) *Cell*, **55**, 211–219.
Wormington,W.M. and Brown,D.D. (1983) *Dev. Biol.* **99**, 248–257.
Wormington,W.M., Bogenhagen,D.F., Jordan,E. and Brown,D.D. (1981) *Cell*, **24**, 809–817.

Received on August 3, 1989; revised on October 23, 1989

Note added in proof

Recent experiments have successfully reproduced the nucleosome density-dependent inhibition of 5S DNA transcription at 27°C in an oocyte S150 extract, eliminating our reservations concerning earlier experiments at elevated temperature (37°C). This inhibition of transcription does not absolutely require a nucleosome to be specifically positioned on 5S DNA. (D.Tremethick, personal communication.)