Transcriptional activation of *Xenopus* class III genes in chromatin isolated from sperm and somatic nuclei

Alan P. Wolffe

Laboratory of Molecular Biology, Building 6, Rm 131, NIDDK, NIH, Bethesda, MD 20892, USA

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ABSTRACT

Xenopus sperm chromatin lacks class III transcription complexes and somatic histone H1. Inactive class III genes in sperm chromatin are easily programmed with transcription complexes *de novo* and transcribed in *Xenopus* oocyte nuclear extract. In contrast, repressed class III genes in somatic chromatin are not transcribed in the oocyte nuclear extract. Class III genes that are initially inactive or repressed in both types of chromatin can be efficiently transcribed in a cell free preparation of *Xenopus* eggs. Chromatin mediated repression of class III genes in somatic nuclei is reversible in *Xenopus* egg extract, but not in the oocyte nuclear extract. Any inhibition of transcription attributed to chromatin assembly onto a gene, will therefore depend on the extract in which transcription is assayed.

INTRODUCTION

An important element of transcriptional control in eukaryotes is the accessibility of promoter elements in chromatin (1-3). Prior assembly of nucleosomes *in vitro* onto eukaryotic genes has the potential to prevent subsequent binding of transcription factors and RNA polymerase (4-10). *In vitro* chromatin assembly systems (11, 12) lead to the binding of many proteins to DNA other than histones (13), or generate close packed nucleosomes (6). This chromatin may differ significantly from chromatin present in a normal somatic nucleus.

Chromatin isolated from a normal somatic nucleus retains the same genes in an active or repressed state as found *in vivo* (14, 15). *Xenopus* oocyte 5S RNA genes are maintained in a repressed state by a chromatin structure dependent on histone H1. Removal of histone H1 by ion exchange chromatography allowed oocyte 5S DNA to be transcribed in a complete transcription extract (15, 16). The readdition of histone H1, to somatic chromatin depleted of histone H1, prevented oocyte 5S RNA gene transcription. Treatment of somatic nuclei with salt concentrations that begin to dissociate histone H1 (17) allowed the activation of repressed oocyte 5S RNA genes after transplantation of these nuclei into *Xenopus* oocytes (18). These studies suggest that histone H1 can exert an inhibitory effect on transcription in addition to any possible inhibitory effect from the presence of nucleosomes alone.

This study extends these observations using a novel template : chromatin derived from Xenopus sperm nuclei. Sperm chromatin does not contain normal somatic histone H1 (19, 20), although it is packaged into a regular 'nucleosomal' repeat. Xenopus laevis sperm nuclei contain normal amounts of histones H3 and H4, but reduced levels of H2A and H2B, sperm specific histone variants are substituted (19, 20). Sperm chromatin does not contain class III transcription factors and is therefore an ideal template to examine the de novo programming of class III genes with transcription complexes using a chromatin template. In experiments examining the replication of class III genes in vitro using sperm and somatic nuclei in activated Xenopus egg extracts (21), I observed the active transcription of normally repressed class III genes. This work describes differences between the transcriptional activation of sperm nuclei or chromatin, and that of somatic nuclei or chromatin in Xenopus transcription extracts. Surprisingly, at high transcription factor concentrations in Xenopus activated egg extract, even genes assembled in chromatin containing histone H1 can be transcribed.

MATERIALS and METHODS

Plasmid DNAs.

The Xenopus laevis satellite I DNA clone called E190 was the gift of D. Carroll (22); the major oocyte 5S RNA gene was pX1o316 (23).

Transcription extract

Xenopus laevis oocyte nuclear extract was prepared by a modification of the procedure of Birkenmeier (16). Ovaries were removed from adult frogs, fragments of ovary were swollen for 3 hours in 5mM Tris (pH 7.8) and 10mM MgCl₂. Individual fragments of ovary were transferred to ice cold 'J' buffer (70mM NH₄Cl, 7mM MgCl₂, 0.1mM EDTA, 2.5mM DDT, 10% (v/v) glycerol, 10mM HEPES (pH 7.4) and 2% polyvinyl pyrrolidone). The oocytes were broken using two pairs of forceps. Nuclei were taken up into a pipette tip. Five hundred nuclei were collected into a microfuge tube in a total volume of 1ml. The nuclei were disrupted by brief vortexing. The lysate was centrifuged at 10,000 xg for 1 min at 4°C to pellet nuclear debris.

Activated egg extract was prepared as previously described (21). Unfertilized Xenopus laevis eggs were dejellied in 2% cysteine (pH 8.0) in egg laying buffer (110mM NaCl, 2mM MgCl₂, 1mM MgSO₄, 0.5mM NaH₂PO₄, 2mM NaHCO₃, 15mM Tris HCl (pH 7.4). The eggs were washed 5 times in 20% MBS (18mM NaCl, 0.2mM KCl, 0.15mM NaHCO₃, 2mM HEPES, (pH 7.5), 0.15mM MgSO₄, 0.05mM Ca(NO₃)₂, 0.1mM CaCl₂). They were then activated by pulsing with 200V for 0.5s. Eggs that formed a visible white spot in the animal pole were washed with 20% MBS and then with ice cold extraction buffer (50mM HEPES (pH 7.4), 50mM KCl, 5.0mM MgCl₂,

2mM β -mercaptoethanol, 3 μ g/ μ l leupeptin (Sigma). Cooled 4ml SW50 tubes were filled with eggs, excess buffer was removed, and spun in a Beckman SW 50 Ti rotor at 9,000 xg at 4°C for 15 min. The brown cytoplasmic fraction was removed with an ice cold Pasteur pipette and recentrifuged at 9,000 xg at 4°C for 30 min. The activated egg extract was then made 40% with glycerol and frozen in liquid nitrogen. Transcription reactions using these extracts or purified RNA polymerase III (24) were as described (25, 26).

Preparation of sperm nuclei, somatic nuclei and chromatin

Xenopus laevis sperm nuclei were prepared as previously described (21). Testes were homogenized in 2ml SuNaSp (250mM sucrose, 75mM NaCl, 0.5mM spermidine, and 0.15mM spermine). Debris was removed by centrifugation at 1000 xg for 5 min and resuspended in 0.5ml SuNaSp. Demembration was performed by the addition of 20 μ l 1mg/ml lysolecithin. After 10 min, the reaction was stopped by the addition of 1ml SuNaSp containing 3% bovine serum albumin at 0°C. The sperm nuclei (> 95% pure) were washed three times in SuNaSp and finally resuspended in SuNaSp plus 30% glycerol.

Nuclei were also prepared from *Xenopus* liver and erythrocytes by established methods (21, 27). Liver tissue was dounced and erythrocytes were resuspended in 50% glycerol, 50mM Tris-HCl (pH 7.5), 100mM KCl, 5mM MgCl₂, 2mM DTT, 0.5mM PMSF, and 0.1% NP40. The lysate was spun down at 4,000 xg for 10 min. The pelleted materials was resuspended in nuclei buffer (50mM Tris (pH 7.5), 70mM KCl, 7mM MgCl₂, 0.1mM EDTA, 2mM DTT, 0.4mM PMSF, 25% glycerol).

The method of McGhee (28) was used to convert nuclei to chromatin. Nuclei at a concentration of 1-2 x 10⁸/ml were incubated in nuclei buffer containing 1mM CaCl₂ with 2U/ml of micrococcal nuclease (Wormington) at 37°C for 15 min. EGTA was then added to 2.5mM and the nuclei pelleted at 850 xg for 10 min. The lightly digested nuclei were resuspended in 0.25 mM EDTA (pH 7.0), 1mM DTT, and 0.4mM PMSF and dialyzed for 24 hours at 4°C. The digests were then dialyzed for 3hr against chromatin storage buffer (10mM Tris (pH 7.5), 0.1mM EDTA, 1mM DTT, 0.4mM PMSF and 10% glycerol). Insoluble material was removed by centrifugation at 1000 xg for 5 min.

Preparation of transcription factors

The 5S RNA gene specific transcription factor TFIIA was isolated as described (29). Transcription fraction TFIIC with the characteristics described (23) was fractionated by published methods (30).

Preparation of histone H1 and antibodies to histone H1

Histone H1 was prepared from *Xenopus laevis* liver (31). The protein was extracted from nuclei with 5% (v/v) perchloric acid and was purified on Biogel P-60

using 20mM-HCl and 50mM NaCl as elution buffer. The purified protein was dialyzed against water. Polyclonal antiserum to histone H1 was prepared in rabbits, immunoblotting was as described (32, 33).

RESULTS.

Xenopus sperm chromatin is not pre-programmed with class III gene transcription complexes.

Xenopus sperm chromatin is not programmed with transcription complexes on any class III gene (Figure 1A). The addition of RNA precursors and RNA polymerase III to soluble sperm chromatin prepared by micrococcal digestion of sperm nuclei results in a general nonspecific accumulation of RNA (lanes 3 and 4). In contrast, the addition of a complete transcription extract prepared from oocyte nuclei (16) allows vigorous transcription of specific class III genes (lane 2). Transcripts were initially identified by using cloned class III gene transcription products as markers, and subsequently by hybridisation. The activation of sperm chromatin in the oocyte nuclear extract is comparable to the transcription of naked genomic DNA (lane 1). This lack of repression in sperm chromatin correlates with the absence of somatic histone H1 (20). Immunological straining of squashed Xenopus testes confirms the absence of histone H1 from mature sperm (Figure 2).

Xenopus sperm chromatin retains features of normal somatic chromatin as revealed by its sensitivity to nucleases. Sperm nuclei are relatively resistant to micrococcal nuclease digestion, in comparison to erythrocyte nuclei (Figure 1B). The bulk of the DNA digests to a limit size (lane 1) similar to that of a normal nucleosome repeat (lane 3). Class III genes are assembled into 'nucleosomal arrays' (Figure 3). This packaging of Xenopus sperm chromatin is similar to that seen with sea urchin sperm chromatin, which also has H1 and H2B sperm specific variants (34).

These experiments indicate that the inactive class III genes packaged into sperm chromatin are accessible to both transcription factors and RNA polymerase III. In contrast, transcription factors and RNA polymerase III cannot bind productively to repressed class III genes (e.g. oocyte 5S RNA genes) in somatic chromatin (15, Figure 1A, lane 5).

Xenopus sperm nuclei require decondensation for active transcription.

The transcription of soluble Xenopus sperm chromatin indicated that de novo programming of inactive class III genes was readily accomplished with this template. However, high levels of transcription from highly condensed sperm nuclei were not seen in the oocyte nuclear extract (data not shown). The oocyte nuclear extract does not decondense sperm nuclei. This suggested that chromatin decondensation, either by

Figure 1. Xenopus sperm chromatin is not preprogrammed with class III transcription complexes.

A. Lane 1, Xenopus genomic DNA (200ng) transcribed using 10 μ l oocyte nuclear extract; lane 2, Xenopus sperm chromatin (200ng) transcribed using 10 μ l oocyte nuclear extract. Lane 3 and 4, sperm chromatin transcribed using 30 and 60 units of RNA polymerase III. Lane 5, erythrocyte chromatin transcribed using 10 μ l oocyte nuclear extract.

Lanes 3, 4 and 5 are exposed for twice the time of lanes 1 and 2.

B. Sperm chromatin contains a regular nucleosomal ladder. Lane 1, sperm nuclei were digested with micrococcal nuclease (45u) for 5 min, DNA extracted and resolved on a 1.5% agarose gel. Lane 2, no digestion. Lanes 3 and 4 as above using erythrocyte nuclei. ϕ x174 RF DNA cut with Hae III was used as a size standard.

micrococcal nuclease digestion or by incubation in Xenopus egg extract (21) might be a prerequisite for transcriptional activation. In fact, the decondensation of sperm nuclei in the egg extract (Figure 4A) correlates with transcriptional activation (B).

The predominant transcript from decondensed sperm nuclei in activated egg extract is from satellite I DNA (Figure 4B, lanes 2 and 4). Satellite I DNA shares general transcription factors (TFIIIC and TFIIIB) and similar promoter elements with other class III genes (22, 35, 36). The 5S RNA genes are not actively transcribed in sperm chromatin incubated in the activated egg extract (26). Addition of the purified transcription factor specific for 5S RNA genes (TFIIIA) to the activated egg extract greatly stimulates 5S RNA gene transcription (lanes 3 and 5). The transcriptional activation is greatest from the completely decondensed nuclei.

Figure 2. Mature sperm nuclei do not contain somatic histone H1.

Whole *Xenopus* testes were squashed and fixed, they were then reacted with preimmune serum or with a polyclonal serum against *Xenopus* liver histone H1, followed by rhodamine conjugated second antibody. Alternatively the same preparation was stained with DAPI. The bar in the top right corner of the figures represents 25 μ m.

1. Shows DAPI staining and inactivity with the pre-immune antiserum as shown by immunofluorescence.
2. Shows DAPI staining and reactivity with serum raised against somatic histone H1 as shown by immunofluorescence.

Transcription factor dependent activation of 5S RNA gene transcription from sperm nuclei.

The active transcription of class III genes using as templates, either sperm chromatin in the oocyte nuclear extract (Figure 1) or decondensed sperm nuclei in the activated egg extract (Figure 4) is in marked contrast to the low levels of transcription obtained using somatic chromatin or nuclei in the oocyte nuclear extract (5, 14, Figure 1A). As the 5S RNA gene specific transcription factor (TFIIIA) is limiting in activated egg extract (26), it is possible to directly test whether de novo transcriptional activation from sperm chromatin is equivalent to that from genomic DNA. The addition of TFIIIA to decondensed sperm nuclei gives identical 5S RNA gene transcription to that seen using an equal mass of genomic DNA as template (Figure 5A, compare lanes 4 and 6, results were quantitated by cutting out gel slices and counting Cherenkov radiation). This result demonstrates that 5S RNA genes, that

Figure 3. Class III genes are assembled into a 'nucleosomal' repeat in sperm nuclei

Southern blots of DNA resolved on a 1.5% agarose gel are shown. Lane 1. *Xenopus* genomic DNA (10 μ g) digested with micrococcal nuclease (5u) for 5 min. Lane 2, *Xenopus* sperm chromatin (10 μ g DNA) was digested with 50 units of micrococcal nuclease for 5 min, Lane 3 as in 2, except digestion was with 200 units of enzyme. Hybridization was to a 5S DNA specific probe (radiolabelled 5S RNA). Lanes 4 - 6, as above, except hybridization was to a satellite I DNA specific probe. ϕ x174 RF DNA cut with Hae III was used as a size standard.

do not have transcription complexes in the sperm nucleus, bind transcription factors and assemble transcription complexes efficiently. This transcriptional activation occurs even though oocyte 5S DNA is assembled into nucleosomes (Figure 3). It should be noted that the sperm chromatin nucleosomal array does become more distinct during incubation in the egg extract, this increase in resolution is probably due in part to an increase in efficiency of digestion as the nucleus decondenses (data not shown). However, this may also reflect the remodelling of chromatin structure due to changes in histone composition (See Discussion).

We have previously proposed that a second transcription fraction required for 5S RNA gene transcription may be limiting in *Xenopus* activated egg extract (26). This transcription fraction (TFIIIC) can selectively activate oocyte 5S RNA gene transcription from cloned 5S RNA genes in the activated egg extract (Figure 5B). This occurs specifically on 5S DNA, probably because TFIIIC increases the stability with which the 5S specific transcription factor (TFIIIA) binds to a 5S RNA gene (23). When both TFIIIC and TFIIIA are added to the activated egg extract, oocyte 5S RNA gene transcription is enhanced from both cloned DNAs and sperm nuclei (Figure 5B and C, hybridisation analysis not shown). These results show that both TFIIIA and TFIIIC are deficient in the activated egg extract and the sperm nucleus. As these two transcription factors are the first components of a transcription complex to bind to a 5S RNA gene, this provides strong evidence for *de novo* formation of

Figure 4. Decondensation and transcriptional activation of *Xenopus laevis* sperm nuclei in *Xenopus* activated egg extract.

A. *Xenopus* sperm nuclei ($5 \times 10^5/\mu\text{l}$ extract) were incubated in activated egg extract, before they were photographed under Nomarski optics and DAPI UV fluorescence at zero time (1) after 30 min (2), and after 120 min. (3).

B. The transcriptional activity of sperm nuclei after varying periods of incubation in *Xenopus* activated egg extract was assayed by carrying out a transcription reaction for 30 min at the time indicated. A radioautograph of the radiolabeled RNAs is shown, the positions of 5S RNA and satellite I DNA transcripts are indicated.

Lane 1, Sperm nuclei (5×10^4) with RNA precursors added for 30 min; Lanes 2 and 3, transcription in activated egg extract 30 min after addition, 300ng/ μl of TFIIA supplemented the reaction mixture in lane 3; Lanes 4 and 5, transcription in activated egg extract 120 min after addition, 300ng/ μl of TFIIA supplemented the reaction shown in lane 5.

transcription complexes in the sperm nucleus (37, 38, 39). Selective activation of transcription of 5S RNA genes by a TFIIC fraction using somatic chromatin in the activated egg extract also occurs (data not shown, 40).

The transcriptional activation of 5S RNA genes in the *Xenopus* egg extract occurs efficiently in the presence of concentrations of aphidicolin (20 $\mu\text{g/ml}$) that completely block DNA replication (data not shown). This result is in agreement with the observation that activation of *Xenopus* 5S RNA gene transcription in eggs and embryos *in vivo* does not require replication. (33, 41).

Activation of repressed somatic cell nuclei in *Xenopus* activated egg extract.

Satellite I DNA and oocyte 5S RNA genes do not have transcription complexes in somatic cells (15). These genes are assembled into a repressive chromatin structure containing histone H1. *Xenopus* activated egg extract is deficient in somatic histone H1 (42, 43). The changes in transcriptional activity in somatic nuclei transplanted

Figure 5. Transcriptional activation of sperm chromatin compared to naked DNA in egg extract.

A. Sperm chromatin compared to genomic DNA as a transcription template. Transcription reactions were for 90 min in the activated egg extract. Lane 1 and 2, transcription of a commixture of 500ng cloned major oocyte 5S RNA gene and 10ng cloned satellite I DNA in the absence or presence of 100ng/ μ l TFIIIA. Lanes 3 and 4 decondensed sperm nuclei ($5 \times 10^5/\mu$ l extract). Lanes 5 and 6 as above using genomic DNA (20ng/ μ l extract). In lane 6 the TFIIIA was prebound to the genomic DNA, before addition of the egg extract. The genomic DNA was incubated in the activated egg extract for the same period of time (120 min) required for complete decondensation of the sperm nuclei.

B. Activation of cloned genes in egg extract.

In all these transcription reactions a commixture of 500ng cloned satellite I DNA and 500ng of cloned major oocyte 5S RNA gene were incubated for 90 min in 20 μ l activated egg extract plus RNA precursors. To these reactions were added the transcription factors indicated. Lane 1, no addition; lane 2, plus 1 μ l fraction TFIIC; lane 3, plus 4 μ l fraction TFIIC; lane 4, plus 10 μ l fraction TFIIC; lane 5 has no addition; lane 6, plus 100ng/ μ l TFIIIA; lane 7, plus 100ng/ μ l TFIIIA and 4 μ l TFIIC, lane 8, plus 4 μ l TFIIC. The three bands from the oocyte 5S DNA come from inefficient termination.

C. Activation of sperm nuclei in egg extract.

Xenopus sperm nuclei ($5 \times 10^5/\mu$ l extract) were allowed to decondense for 120 min before the indicated transcription factors were added, plus RNA precursors, for 90 min.

Lane 1, no addition; lane 2, plus 100ng/ μ l TFIIIA; lane 3, plus 100ng/ μ l TFIIIA and 2 μ l TFIIC; lane 4 plus 2 μ l TFIIC.

into Xenopus eggs (44) led to an investigation of the transcriptional activation of somatic nuclei in Xenopus egg extract.

Figure 6A is a control for the relative efficiencies of satellite I DNA and 5S RNA gene transcription from sperm nuclei with or without supplementation with high concentrations of TFIIIA. When sperm nuclei are incubated in the activated egg extract with out addition of TFIIIA, satellite I DNA is much more transcriptionally

Figure 6. Transactivation of repressed oocyte 5S RNA genes in activated egg extract.

A. *Xenopus* sperm nuclei ($5 \times 10^3/\mu\text{l}$ extract) were incubated in activated egg extract for 120 min. At the end of this period RNA precursors were added and the reaction mixture incubated for a further 90 min. Transcription products are shown in the absence (lane 1) or presence of TFIIIA ($500\text{ng}/\mu\text{l}$) (lane 2).

B. As in A, using *Xenopus* liver, lanes 1 and 3; or *Xenopus* erythrocyte nuclei, lanes 2 and 4. Lanes 3 and 4 are the transcription products in the presence of TFIIIA ($500\text{ng}/\mu\text{l}$).

C. Parallel reactions to those shown in A and B were carried out. The RNAs were deproteinized and hybridized to single stranded complementary DNA before RNase analysis (15). Lane 1 shows the distribution of 5S transcripts from somatic chromatin, between the somatic and oocyte gene families in the absence of TFIIIA; lane 2 shows the distribution in the presence of $500\text{ng}/\mu\text{l}$ TFIIIA (26); lane 3, shows the distribution when sperm chromatin is transcribed in the presence of TFIIIA. Both oocyte (O) and somatic (S) 5S RNA gene transcription is activated by the addition of TFIIIA.

active than 5S DNA (Figure 6A, lane 1). When TFIIIA is added to the reaction mixture, strong transcription of both oocyte and somatic 5S RNA genes occurs (Figure 6A, lane 2; and Figure 6C, lane 3).

Both liver and erythrocyte nuclei have very low levels of transcriptional activity in activated egg extract in comparison to naked DNA or sperm nuclei (Figure 6B, lanes 1 and 2). Only low levels of satellite I DNA transcription can be seen in Figure 6B and longer exposures are required to see 5S RNA and tRNA gene transcription. The low activity of satellite I DNA is dependent on the presence of histone H1 in somatic nuclei. Removal of histone H1 from somatic nuclei facilitates the activation of satellite I DNA transcription to the levels seen using a sperm chromatin template

Figure 7. Histone H1 is stably associated with erythrocyte nuclei

An radioautogram is shown of *Xenopus* sperm nuclei or *Xenopus* erythrocyte nuclei incubated in activated egg extract for varying periods of time as indicated, followed by repurification of nuclei, resolution on an SDS polyacrylamide gel and immunoblotting against histone H1.

Lane 1, 20ng histone H1 control, lanes 2 -5, 5×10^4 sperm nuclei incubated in $10\mu\text{l}$ activated egg extract for 0, 1, 4 and 24 hours. Lane 6 - 9, 2.5×10^4 erythrocyte nuclei were incubated in $10\mu\text{l}$ activated egg extract for 0, 1, 4, and 24 hours.

(data not shown, see 42). Addition of a large excess of TFI_{II}A to the activated egg extract leads to strong 5S RNA gene transcription (lanes 3 and 4). The 5S RNA transcription comes from the activation of both somatic and oocyte 5S RNA genes (Figure 4C, lane 2). This agrees with previous data showing activation of both somatic and oocyte 5S RNA gene transcription from cloned DNAs following the addition of TFI_{II}A to activated egg extract (26). The activation of 5S RNA gene transcription was found to be equivalent in both sperm and somatic nuclei (quantitated by Cherenkov counting of the radiolabelled 5S RNA synthesized). This result is important because it indicates that somatic cell chromatin templates can behave in a similar fashion to cloned DNAs *in vitro* (26, 45).

It has been proposed that 'activating oocytes' (18), in which oocyte 5S RNA gene transcription is turned on in a somatic nucleus transplanted into an oocyte, was the result of alterations in chromatin structure caused by proteolysis of histone H1 (15). Although the removal of histone H1 from somatic chromatin facilitates oocyte 5S RNA gene transcription (data not shown, see 42), immunoblotting indicates that histone H1 is stable in the activated egg extract (Figure 7). Histone H1 remains associated with somatic nuclei in spite of the activation of the oocyte 5S RNA genes. It therefore seems likely that the activated egg extract is reproducing the phenomena described for 'activating oocytes' or eggs (18, 42), without requiring the degradation of histone H1. The experiments described here suggest that either an excess of TFI_{II}A and/or TFI_{II}C in 'activating oocytes' could explain the earlier results of Korn and Gurdon (18).

DISCUSSION

The major conclusion from these experiments is that inactive class III genes in sperm chromatin or repressed oocyte 5S RNA genes in somatic chromatin can be activated in Xenopus transcription extracts. Activation of 5S RNA genes can be achieved solely by the addition of excess TFI_{IIA} or TFI_{IIIC} to nuclei in Xenopus activated egg extract (Figure 4, 5 and 6), it does not require replication or complete removal of histone H1 from a nucleus. Activation is more efficient if histone H1 is not present (as in sperm, Figure 2 and 7) or is removed completely from somatic nuclei (see also 42). Two examples of this are given here : oocyte nuclear extract can activate oocyte 5S RNA genes in sperm chromatin, but cannot activate repressed oocyte 5S RNA genes in somatic chromatin (Figure 1). Secondly, egg extract activates satellite I DNA weakly in somatic chromatin when compared to sperm chromatin (Figure 4), however satellite I DNA transcription increases when histone H1 is removed. Restoration of histone H1 to histone H1-deficient chromatin can repress oocyte 5S and satellite I DNA (42). In spite of the presence of histone H1 in the somatic nucleus, at high transcription factor TFI_{IIA} concentrations, oocyte 5S RNA genes can be transcribed as efficiently in both sperm and somatic nuclei in the activated egg extract (Figure 6). Repressed class III genes in somatic nuclei must become accessible to RNA polymerase and transcription factors under these conditions.

These results help to reconcile the conflict between the concept of stable states of gene activity and inactivity (1, 46) and the observations of nuclear totipotence from transplantation (47-49) and heterokaryon experiments (50-52). Although chromatin is inhibitory to transcription, especially if purified transcription factors are added to a chromatin template (4-10, Figure 1), cytoplasmic activities present in the Xenopus egg extract permit gene reprogramming in vitro (Figure 6). Any inhibition of transcription attributed to chromatin assembly onto a gene (4-10) will depend on the extract in which transcription is assayed. Presumably in the examples of gene activation from chromatin templates given here, the repressed chromatin is being remodelled. Other examples of the restructuring of nucleoprotein complexes in the Xenopus egg extract, are the decondensation of sperm nuclei (Figure 4; 53), and the removal of pre-assembled transcription complexes (26). Perhaps cytoplasmic activities in the egg extract allow the exchange of core histones and histone H1 from chromatin (54) as they allow the exchange of transcription factors from genes (26). It is important to note that the activation process is reversible, because increasing histone H1 concentration can have a dominant repressive effect (42). The potential importance of the reversibility of chromatin assembly in crude Xenopus extracts has already been recognized (55).

Sperm chromatin : an unprogrammed state

Xenopus sperm nuclei differ from somatic nuclei in that they do not have transcription factors associated with their class III genes in chromatin, nor do they possess the somatic form of histone H1 (Figures 2 and 7, 19, 20). This is presumably a consequence of the chromatin condensation processes of spermiogenesis. Chromatin remodeling is a well documented phenomenon during gametogenesis (56), although relatively little is known about the binding of transcription factors (57). Moreover during spermiogenesis transcription is known to be progressively inhibited (58). The displacement of transcription complexes and normal somatic histone H1 from Xenopus sperm chromatin demonstrates the potential importance of histone variants and chromosome condensation in regulating gene activity.

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