Association of the nucleolar transcription factor UBF with the transcriptionally inactive rRNA genes of pronuclei and early Xenopus embryos

Peter Bell1,*; Christine Mais1; Brian McStay2 and Ulrich Scheer1

1Department of Cell and Developmental Biology, Theodor-Boveri-Institute, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany
2Biomedical Research Center, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland, UK

*Author for correspondence (e-mail: bell@biozentrum.uni-wuerzburg.de)

SUMMARY

When nuclei (pronuclei) were assembled from sperm chromatin in Xenopus egg extract and examined by immunofluorescence microscopy, UBF was concentrated at a single intranuclear dot-like or more extended necklace-like structure. These UBF-foci contained rDNA as demonstrated by in situ hybridization and hence represent the chromosomal nucleolus organizing regions (NORs). Besides UBF, other components of the transcription machinery such as the TATA-box binding protein (TBP) and RNA polymerase I (pol I) as well as several nucleolar proteins could not be detected at the NORs. Immunodepletion experiments indicated that UBF is maternally provided and taken up by the pronuclei. Essentially the same results were obtained when we examined the NORs of early Xenopus embryos up to the midblastula stage. After this stage, when transcription of the rRNA genes has begun, nucleoli developed and the NORs acquired TBP and pol I. Our results support the hypothesis that UBF is an architectural element which converts the rDNA chromatin into a transcriptionally competent form.

INTRODUCTION

The accurate and efficient transcription of the ribosomal RNA (rRNA) genes requires RNA polymerase I (pol I) and at least two protein factors which bind to the promoter region of these genes to form a stable pre-initiation complex. One transcription factor is the upstream binding factor (UBF; Learned et al., 1986; Bell et al., 1988; McStay et al., 1991a; Schnapp and Grummt, 1991; for a recent review see Reeder et al., 1995). The other is a multiprotein complex consisting of the TATA-box binding protein (TBP) and several TBP-associated factors (TAFIs) and has been variously termed SL1, TIF-IB or Factor D in mammalian species (Mishima et al., 1982; Learned et al., 1985; Tower et al., 1986; Bell et al., 1988; Schnapp et al., 1990; Smith et al., 1990) and Rib1 in Xenopus (McStay et al., 1991a; Bodeker et al., 1996). Since Rib1 has been identified as the Xenopus equivalent of mammalian SL1 (Bodeker et al., 1996) we will collectively refer to this transcription factor as SL1. While SL1 exclusively functions as a pol I-specific transcription factor, its TBP subunit is also a component of trans-acting factors involved in pol II- and pol III-mediated transcription (reviewed by Hernandez, 1993; Goodrich and Tjian, 1994). Except for mouse and rat SL1, which have been shown to be capable of binding to the rDNA promoter and to induce basic levels of transcription in the absence of UBF (Schnapp et al., 1991; Kuhn and Grummt, 1992; Smith et al., 1993), human and Xenopus SL1 require UBF for formation of initiaton complexes (Bell et al., 1988, 1990; McStay et al., 1991a). Using a Xenopus in vitro system it has recently been shown that UBF, by binding to the enhancer elements upstream of the rRNA gene promoter, may act by recruiting SL1 to the promoter (McStay et al., 1997). The role of UBF as an assembly factor for pre-initiation complexes might also explain its capability of neutralizing the in vitro repression of pol I transcription by histone H1 and other DNA-binding proteins (Kuhn and Grummt, 1992; Kuhn et al., 1993).

A number of recent studies have allowed a detailed functional characterization of various UBF domains in DNA binding and bending, interaction with SL1 and pol I, and nuclear and nucleolar targeting (reviewed by Reeder et al., 1995). The DNA binding property of UBF is mediated by multiple sequence motifs termed HMG boxes which are related to the DNA binding motifs of the chromatin-associated high mobility group proteins HMG1 and 2 (Jantzen et al., 1990; Landsman and Bustin, 1993). UBF is believed to organize the promoter region of ribosomal DNA in such a way that SL1 can bind and form a stable pre-initiation complex (Bell et al., 1988; McStay et al., 1991a). A prerequisite for the tight binding of SL1 could be the bending of the promoter DNA into a complete circle which is mediated by UBF (Bazett-Jones et al., 1994; Putnam et al., 1994).

In keeping with its role as a pol I-specific transcription factor, UBF is found localized during interphase to the nucleolus where transcription of the rRNA genes and assembly of the ribosomes takes place (e.g. Jantzen et al., 1990; Chan et al., 1991; Rodrigo et al., 1992; Roussel et al., 1993; Zatsepina...
et al., 1993). When cells enter mitosis, rRNA synthesis ceases and nucleoli disappear (for references see Hadjiolov, 1985; Weisenberger and Scheer, 1995; Roussel et al., 1996). Thus, the rRNA genes of cycling cells alternate between transcriptionally active and inactive states and it is an interesting question whether this modulation of transcriptional activity is correlated with changes of the pol I-dependent transcription machinery. However, it turned out that in spite of the mitotic repression of the rRNA genes essential components of the transcription machinery such as UBF, SL1 and pol I remain bound to the chromosomal NORs (Weisenberger and Scheer, 1995; Jordan et al., 1996; Roussel et al., 1996). They are probably never disengaged from the rDNA template indicating that rRNA gene expression is regulated during mitosis at the level of transcription elongation by a hitherto unknown mechanism (Weisenberger and Scheer, 1995). Hence these studies did not allow an analysis of the sequence of events involved in the assembly of stable pol I transcription complexes in live cells.

During early embryogenesis of *Xenopus laevis* the rRNA genes are repressed until the midblastula stage when the embryonic genome is being activated (Brown and Littna, 1964; Shiokawa et al., 1977; Crampton and Woodland, 1979; De Capoa et al., 1983; Shiokawa, 1984; see also Miller, 1972, for the frog, *Rana pipiens*). The preblastula stage therefore represents an easily amenable model system to analyze the protein composition of rRNA genes prior to transcriptional activation. Furthermore, the postfertilization events leading to the formation of male pronuclei can be reproduced in vitro by adding sperm chromatin to *Xenopus* egg extract. The nuclei that will form spontaneously under such conditions can be regarded as the equivalents of male pronuclei (Lohka and Masui, 1983; Newport, 1987; reviewed by Almouzni and Wolffe, 1993). Our results demonstrate that maternally provided UBF binds to rDNA very early in embryogenesis and suggest that it plays a structural role in rendering the rRNA genes permissive for subsequent transcriptional activation.

**MATERIALS AND METHODS**

**Biological materials**

*Xenopus laevis* were purchased from the South African Snake Farm (Fish Hoek, Cape Province, South Africa). The 1-nu mutants were kindly provided by M. F. Trendelenburg (German Cancer Research Center, Heidelberg). *Xenopus* XTC-2 cells were grown as described (Smith and Tata, 1991).

**Antibodies**

Antibodies directed against the following antigens were used: xUBF (rabbit antiserum raised against recombinant full length xUBF; Cairns and McStav, 1995); xTBP (rabbit antiserum raised against three *Xenopus* TBP peptides; Bodeker et al., 1996); RNA polymerase I (human autoimmune serum S18, Reimer et al., 1987a); fibrillarin (mAb 72B9, Reimer et al., 1987b); xNopp180 (mAb No-114, Schmidt-Zachmann et al., 1984); *Xenopus* nucleolin (mAb b6-6E7-M12, Messmer and Dreyer, 1993) and *Xenopus* B23/NO38 (mAb No-185, Schmidt-Zachmann et al., 1987). The reactivity of these antibodies with *Xenopus* nuclear material has been documented by Bell et al. (1992) and Bell and Scheer (1997).

**Nuclear reconstitution**

In vitro assembly of nuclei from *Xenopus* sperm chromatin or λ-DNA was carried out as described previously (Bell et al., 1992). In brief, either demembranated *Xenopus* sperm nuclei prepared by the method of Blow and Laskey (1986) or λ-DNA (Gibco-BRL, Egggenstein, Germany) were added together with an ATP-regenerating system to extract made from activated *Xenopus* eggs (Newport, 1987). Extracts containing the reconstituted nuclei were processed for immunofluorescence microscopy or in situ hybridization.

**Squash preparations of *Xenopus* embryos**

Eggs of *Xenopus laevis* were raised after in vitro fertilization as described (Wolf and Hedrik, 1971). After removal of the embryonic jelly coat by incubation in 2% cysteine, pH 7.8, the embryos were squashed under a coverslip and the glass slide was dipped into liquid nitrogen. The coverslip was then flipped off and specimens were fixed in cold (–20°C) acetone for 10 minutes, air dried and processed for immunofluorescence microscopy.

**Immunofluorescence microscopy**

In vitro assembled nuclei were fixed by adding 4 volumes of PBS containing 1% formaldehyde (freshly prepared from paraformaldehyde) to the extract. Nuclei were adsorbed to a coverslip by centrifugation for 10 minutes at 1,000 rpm in a cytocentrifuge (Cytospin 2, Shandon, Frankfurt/Main, Germany), fixed in acetone at –20°C for 10 minutes and air dried. Specimens were then incubated for 30 minutes each with the primary antibodies used at a 1:100 dilution (sera) or undiluted (hybridoma supernatants) followed by the appropriate secondary antibodies conjugated to Texas Red or FITC (Dianova, Hamburg, Germany; diluted 1:50). In the case of double-label experiments the preparations were additionally incubated with another set of primary and secondary antibodies. DNA was visualized by staining with Hoechst 33258 (5 μg/ml in the final PBS wash).

Cultured *Xenopus* cells grown on coverslips were fixed for 10 minutes with PBS containing 1% formaldehyde followed by immersion in cold (–20°C) acetone for further 10 minutes and processed for immunofluorescence as described above. Photographs were taken with a Zeiss Axiospho equipped with epifluorescence optics (Carl Zeiss, Jena, Germany).

**In situ hybridization**

In vitro assembled nuclei were processed for immunofluorescence as described above and photographed. After removing the coverslip preparations were equilibrated in pK-buffer (20 mM Tris–HCl, pH 8.0, 2 mM CaCl2) and digested with proteinase K (10 μg/ml in pK-buffer for 10 minutes at room temperature). After a brief wash step in PBS the preparations were fixed with 1% formaldehyde in PBS for 10 minutes. Denaturation of DNA was induced by incubation in 70% formamide in 2x SSC at 70°C for 1 minute followed by immersion in cold (–20°C) 70% ethanol. After dehydration through a graded ethanol series specimens were air dried and incubated overnight at 37°C in the hybridization mixture (10 μg/ml digoxigenin labelled riboprobe in 50% formamide, 7.5% dextran sulfate in 2x SSC). The digoxigenin labelled sense probe was synthesized by in vitro transcription of a 505 bp EcoRI-HindIII fragment of the 28S region of *Xenopus* rDNA derived from the full length clone pXlr101A (Bakken et al., 1982) and subcloned into pBluescript using an RNA labelling kit (Boehringer Mannheim). After the hybridization reaction, specimens were washed in 50% formamide in 2x SSC and 2x SSC alone (both at 35°C) followed by several washes in PBS. Hybridized riboprobes were visualized by immunofluorescence microscopy with digoxigenin-antibodies (Boehringer Mannheim) as described above. Controls were performed by using digoxigenin labelled antisense neo RNA as a hybridization probe (Boehringer Mannheim).

**Depletion of egg extract**

Rabbit IgG directed against xUBF was coupled to paramagnetic beads (Dynabeads M-280 sheep anti-rabbit IgG, Dynal, Hamburg, Germany) according to the manufacturer’s instructions. For immunodepletion, a 100 μl sample of egg extract was incubated with approx-
imately $10^8$ beads for 30 minutes at room temperature. After incubation, the beads were removed with the help of a magnet and the depleted extract was used for in vitro assembly of nuclei as described above. Samples of depleted extract and the paramagnetic beads were analysed by SDS-PAGE. Beads with preimmune IgG served as controls.

**Gel electrophoresis of proteins and immunoblotting**

After retrieving the beads from the extract, both fractions (10 µl of extract, $10^7$ beads) were analyzed by SDS-PAGE. Beads were washed several times in PBS + 0.1% BSA, resuspended in sample buffer, heated for 5 minutes to 95°C and removed with a magnet. Supernatants were loaded on a SDS-10% polyacrylamide gel (Laemmli, 1970) and separated by electrophoresis. Samples of the complete extract were heated in sample buffer and analyzed in parallel. After transferring the proteins to nitrocellulose (Towbin et al., 1979), the filters were blocked in 5% nonfat dry milk in PBS for 1 hour, washed in PBS + 0.05% Tween and incubated with the anti-UBF serum for 1 hour followed by secondary peroxidase-conjugated antibodies (30 minutes; Medac, Hamburg, Germany). Dilution of the anti-UBF serum was 1:1,000 in blocking buffer. Bound antibodies were visualized by the ECL-system (Amersham Buchler, Braunschweig, Germany) according to the manufacturer's instructions.

**RESULTS**

**Characterization of the antibodies directed against UBF, TBP and RNA polymerase I by immunofluorescence microscopy**

Since the antibodies raised against full-length xUBF (Cairns and McStay, 1995) and xTBP peptides (Bodeker et al., 1996) have been characterized only biochemically it was necessary to demonstrate their applicability to immunofluorescence microscopy. Both antibodies stained the nucleoli of cultured Xenopus cells, as might be expected of components of the pol I transcription apparatus (Fig. 1A',B'). Besides the nucleoli, the nucleoplasm was also positive with the TBP-antibodies illustrating the well-known fact that TBP is likewise involved in pol II- and pol III-mediated transcription (Fig. 1B'; for references see Introduction). A nucleolar labelling was also obtained with the human autoimmune serum S18 directed against pol I (Fig. 1C'; for a characterization of S18 see Reimer et al., 1987a).

**Localization of UBF, TBP and RNA polymerase I in nuclei assembled from sperm chromatin**

Nuclei were assembled from sperm chromatin in Xenopus egg extract and the distribution of UBF was analysed by immunofluorescence microscopy using rabbit antibodies directed against xUBF. On top of a moderate nuclear fluorescence indicating the existence of a diffusible and obviously non-localized intranuclear UBF-pool, each nucleus contained a conspicuous UBF-positive focus, usually in the form of a strongly fluorescent dot-like or slightly elongated structure (Fig. 2A'). Sperm chromatin that failed to decondense was negative. When viewed with phase contrast optics, a structure underlying the fluorescent dot could not be identified. Not infrequently these UBF-foci displayed a more relaxed and partially unravelled organization and were seen to consist of smaller subunits. Occasionally they were arranged in the form of several (~25) tandemly arrayed fluorescent granules (Fig. 2B'). From these

---

**Fig. 1.** Immunofluorescence microscopy of Xenopus XTC-2 cells with antibodies directed against xUBF (A'), xTBP (B') and pol I (C'). All three antibodies stain the nucleoli. An additional labelling of the nucleoplasm at various intensities can be observed with the antibodies to xTBP (B'). The corresponding phase contrast images are shown in A-C. Bar, 20 µm.
observations we conclude that the UBF-antibodies decorate linear arrays of fluorescent entities which, depending on their specific packing density, occur either in the form of extended necklace-like beaded strands or compact aggregates.

Fig. 2. Immunofluorescence microscopy of nuclei assembled from sperm chromatin in *Xenopus* egg extract with antibodies directed against xUBF. Besides being diffusely distributed throughout the nuclear interior, UBF is highly concentrated at one specific site per nucleus. These UBF-foci appear either as brightly fluorescent dots (A′) or necklace-like structures consisting of tandemly arrayed fluorescent granules (B′). The corresponding DNA-staining with Hoechst dye 33258 is shown in A and B. Bar, 10 μm.

Fig. 3. The TATA-box binding protein (TBP) is not concentrated at specific sites but rather dispersed throughout the interior of the in vitro assembled nuclei (A′′). The corresponding phase contrast image (A) and Hoechst fluorescence (A′) are shown. Bar, 10 μm.
We next wanted to find out whether the UBF-positive structure contained other components of the pol I transcription machinery. Antibodies directed against xTBP produced a weak uniform labelling of the synthetic nuclei indicating that TBP was more or less uniformly distributed throughout the nuclei without any obvious local accumulation sites (Fig. 3A'').

Antibodies directed against pol I (human autoimmune serum S18) revealed an intranuclear speckled pattern with several bright foci dispersed throughout the interior of the synthetic nuclei (Fig. 4A' and 4B'). However, none of these pol I foci colocalized with the UBF positive aggregate as shown by double label immunofluorescence microscopy (Fig. 4A' and 4A''). Since the pol I-positive foci resembled to some extent the nuclear bodies which have been previously shown to be present in high numbers in synthetic nuclei and to contain a specific set of nucleolar proteins as well as p80 coilin (hence termed variously prenucleolar bodies, Bell et al., 1992, or coiled bodies, Bauer et al., 1994), we have also carried out double label experiments with antibodies to the nucleolar protein fibrillarin. A comparison of the distribution pattern of fibrillarin and pol I reveals that the nuclear bodies do not colocalize with the pol I-foci (Fig. 4B' and 4B''). This result corroborates our earlier finding that the nuclear bodies are devoid of pol I (Bell et al., 1992). Taken together we conclude that UBF is specifically and selectively enriched at a single locus in the in vitro assembled nuclei.

Nucleolar proteins do not colocalize with the UBF-positive sites

To find out whether the UBF-positive site might contain nucleolar proteins other than the pol I transcription machinery we performed immunofluorescence microscopy with antibodies directed against nucleolin, xNopp180, B23/NO38 and fibrillarin. All the antibodies labelled the same population of nuclear bodies (prenucleolar bodies, Fig. 4B'' and Bell et al., 1992). Detailed double label analyses with antibodies directed against UBF and fibrillarin ruled out a structural association of the UBF-positive sites with nuclear bodies (data not shown). From this we conclude that the UBF-focus is not a nucleolus.

UBF of the in vitro formed nuclei is maternally derived

Is the UBF present in in vitro assembled nuclei derived paternally from the sperm chromatin or maternally by nuclear

Fig. 4. UBF, pol I, and fibrillarin localize at three different sites within nuclei assembled in Xenopus egg extract. (A) Double label immunofluorescence microscopy with antibodies directed to pol I (A') and UBF (A'') reveals that pol I is concentrated at several sites scattered throughout the nucleoplasm (A') but not at the UBF-positive spot (A''). (B) Double label experiment with antibodies against pol I (B') and fibrillarin (fib; B''). The pol I foci (B') are not identical with the nuclear bodies which contain a specific set of nucleolar proteins such as fibrillarin (B''). The corresponding Hoechst fluorescence is shown in A and B. Bar, 10 μm.
uptake from the egg extract? By immunofluorescence and immunoblotting analyses we were unable to detect UBF in Xenopus sperm nuclei (data not shown). Similarly, sperm chromatin which failed to decondense and form a nuclear envelope upon incubation in egg extract did not fluoresce with UBF-antibodies. However, since such negative results cannot prove the absence of UBF (the antigenic determinants within the tightly packed sperm chromatin may be inaccessible to the antibodies and sperm UBF may be insoluble in the electrophoretic sample buffer), we have studied the role of the soluble maternal UBF pool for the generation of the UBF-foci during the nuclear assembly process. To this end we have removed UBF from the egg extract by an immunodepletion assay. The absence of UBF was monitored by immunoblotting experiments in combination with the sensitive ECL detection system (Fig. 5A). On western blots of control egg extract the UBF-antibodies reacted strongly with a relatively broad polypeptide band of approximately 85 kDa which often appeared to consist of 2-3 closely spaced bands (Fig. 5A, lane 1, arrow). Indeed, three bands with apparent molecular masses ranging from 82 to 87 kDa have been described after electrophoresis of UBF purified from Xenopus kidney cells (McStay et al., 1991a,b). The rabbit serum used in the present study further reacted on immunoblots with two unidentified proteins of approximately 130 and 200 kDa (Fig. 5A, lane 1). After incubation of the extract with paramagnetic beads coated with the UBF-antibodies, the 85 kDa UBF band was no longer detectable (Fig. 5A, lane 3). In contrast, the unidentified 130 and 200 kDa proteins were still present in the UBF-depleted extract indicating that their native forms were not recognized...
by the antibodies. Preimmune IgGs coupled to the paramagnetic beads did not remove the UBF-band (Fig. 5A, lane 1). As a further control we analyzed the proteins bound to the paramagnetic beads after incubation in the egg extract. UBF was recovered from the beads coated with the UBF-antibodies (Fig. 5A, lane 4, arrow) contrary to beads containing preimmune IgGs (Fig. 5A, lane 2).

When nuclei were assembled in control egg extract treated with preimmune IgGs, they contained UBF-positive foci essentially as described above for untreated extract (Fig. 5B'). In striking contrast such foci were absent when the nuclei were assembled in extract depleted of UBF (Fig. 5C'). We conclude that the generation of the UBF-foci requires maternal UBF which is imported into the newly formed nuclei, most likely by a nuclear pore complex mediated transport process (for characterization of nuclear localization sequences of xUBF see Dimitrov et al., 1993).

The UBF-foci contain rDNA

Since UBF is a pol I transcription factor it was tempting to speculate that rDNA may provide the framework to which UBF binds thus generating the conspicuous fluorescent dots or necklace-like patterns after staining with the UBF-antibodies. If this hypothesis was correct, then the UBF-foci should be absent from nuclei assembled from DNA lacking rRNA genes. In fact, nuclei formed around bacteriophage λ-DNA fluoresced homogeneously indicating that they had imported and accumulated UBF but lacked UBF-foci (Fig. 6A').

In another set of experiments we assembled nuclei from sperm chromatin derived from 1-nu Xenopus mutants. The diploid genome of these nucleolar mutants contains one set of normal rDNA and one set of rDNA comprising extremely deleted rRNA genes unable to direct the synthesis of rRNA (Brown and Gurdon, 1964; Tashiro et al., 1986). The homozygous 0-nu mutants are defective in ribosome biogenesis, do not develop nucleoli and die as young tadpoles when the materi-

![Fig. 6. Nuclei reconstituted from λ-DNA in egg extract accumulate UBF as shown by immunofluorescence with UBF-antibodies (A'). However, they lack the UBF-focus. DNA staining with Hoechst is shown in A. Bar, 20 μm.](image)

![Fig. 7. Nuclei assembled from sperm chromatin of wild type (A, A') and 1-nu Xenopus mutants (B, B') analyzed by immunofluorescence with UBF-antibodies. Almost all nuclei derived from sperm chromatin of wild-type (2-nu) animals reveal the characteristic UBF-focus (A'). In contrast, about half of the nuclei assembled from sperm chromatin of 1-nu mutants lack the UBF-positive dot (B'). The corresponding Hoechst fluorescence is shown in A and B. Bars, 10 μm.](image)
was evaluated). This result is consistent with the view that intact rDNA serves as a nucleation site for the assembly of the UBF-foci.

To finally prove the existence of rDNA within the UBF-foci we localized UBF by immunofluorescence and then, on the same specimen, rDNA by in situ hybridization using a digoxigenin labelled sense riboprobe complementary to the 28S region of Xenopus rDNA. Both the UBF-antibodies and the rDNA-specific probe labelled the same site within the sperm nuclei (Fig. 8). To rule out nonspecific binding of riboprobes to the UBF-foci we carried out control in situ hybridizations with digoxigenin-labelled neo RNA. Under such conditions no hybridization signals were detectable within the nuclei (data not shown).

**UBF binds to the chromosomal NORs of preblastula Xenopus embryos**

Our results described above suggested that after fertilization maternal UBF is rapidly taken up by the pronuclei and binds to the rDNA. During the subsequent cleavage stages up to the midblastula transition the embryonic nuclei remain transcriptionally inactive and lack nucleoli (Wallace, 1963; Brown and Littna, 1964; Hay and Gurdon, 1967; De Capoa et al., 1983). It was therefore of interest to find out whether UBF, in spite of the absence of transcriptional events, remained associated throughout these early cleavage events with the rDNA. In squash preparations of preblastula embryos UBF was clearly detectable in the form of brightly fluorescent dots (blastomeres from a 64 cell stage and an early blastula are shown in Fig. 9A' and B', respectively). From a careful inspection of a large number of squash preparations it became clear that two UBF-positive dots were associated with each chromosome set. Since Xenopus laevis cells have only one NOR-bearing chromosome pair (no. 12; Funaki et al., 1975) it is quite likely that the two UBF-positive dots correspond to the chromosomal NORs, i.e. the sites of the rDNA.

In striking contrast to the results obtained with the UBF-antibodies, antibodies directed against pol I and xTBP failed to recognize any discrete structure on squash preparations of early Xenopus embryos (data not shown). Thus, unlike UBF, both TBP and pol I are apparently absent from the NORs of preblastula embryos. Around the midblastula stage transcription of the rDNA genes commences and the embryonic nuclei eventually develop nucleoli which react with antibodies to UBF, TBP and pol I (for UBF see Fig. 9C'). Thus, UBF seems to be targeted and bound to the rDNA from the pronuclear stage onwards throughout embryogenesis, independent of their transcriptional status. In contrast, the association of TBP and pol I with the rDNA marks the onset of transcriptional activation of the rRNA genes.

**DISCUSSION**

In the present study we show that Xenopus eggs contain a storage pool of the rDNA transcription factor UBF. When sperm chromatin was incubated in Xenopus egg extract, UBF was taken up by the newly formed nuclei and concentrated at a single intranuclear spot as revealed by immunofluorescence microscopy. Since this UBF-focus was dependent on the presence of rDNA and colocalized with rDNA as shown by a combined immunofluorescence and in situ hybridization protocol we conclude that it represents the chromosomal NOR with the tandemly repeated rRNA genes. In fact, the haploid sperm nuclei of Xenopus laevis cells have only one NOR-bearing chromosome (no. 12; Funaki et al., 1975). When we removed, by immunodepletion experiments, UBF from the egg extract and added sperm chromatin, nuclei formed normally but lacked the characteristic UBF-positive foci. This finding rules out the possibility that UBF is transmitted paternally by the sperm nuclei. Furthermore we were unable to detect UBF in sperm nuclei by biochemical and immunolocalization experiments. Since the in vitro reconstituted nuclei are equivalent to male pronuclei it is quite likely that in vivo, after egg fertilization, UBF is recruited from the maternal storage pool and rapidly translocated into the pronuclei where it binds to the rDNA.

Interestingly, the UBF-foci were occasionally seen to consist of linear arrays of smaller fluorescent entities forming more or less extended necklace-like patterns. Similar structures have also been observed by immunofluorescence microscopy with antibodies directed to UBF, pol I, and TBP in nucleoli of normal cells and especially after exposure to the adenosine analog DRB (5,6-dichloro-β-D-ribofuranosylbenzimidazole)
which induces the reversible unfolding of nucleoli (Scheer et al., 1984; Haaf et al., 1991; Roussel et al., 1993; Zatsepina et al., 1993; Matera et al., 1994; Jordan et al., 1996). It is generally believed that each bead of a necklace represents a single transcriptionally active rRNA gene (see Weisenberger and Scheer, 1995). In contrast, the beaded elements of the in vitro nuclei apparently reflect the binding of UBF to transcriptionally inactive rDNA since they apparently lack TBP and pol I. Moreover, we have been unable to detect biochemically newly synthesized pre-rRNAs in egg extract supplemented with sperm chromatin (data unpublished). The precise structural relationships between the ~25 UBF-positive beads and the ~500 tandemly arranged rRNA genes present in a pronucleus remains to be determined.

To find out whether UBF is a constitutive component of the chromosomal NORs throughout the subsequent cleavage stages we analyzed squash preparations of early *Xenopus* embryos by immunofluorescence microscopy. NORs of pre-blastula stages were clearly positive with antibodies to UBF but failed to react with antibodies to TBP and pol I. Only after
the midblastula transition, i.e. concomitant with the transcriptional activation of the rRNA genes, UBF, TBP and pol I were detectable at the NORs comparable to the situation encountered in actively growing cells (e.g. Jordan et al., 1996; Roussel et al., 1996). Our results support the view that UBF is an architectural element which maintains an open structure of the rDNA containing chromatin independent of transcriptional events (Reeder et al., 1995).

The presence of UBF at the NORs of early *Xenopus* embryos in the absence of TBP and pol I is remarkable since the NORs of these developmental stages lack silver-staining proteins in contrast to later stages characterized by actively transcribing rRNA genes (De Capoa et al., 1983). The major proteins which are believed to be responsible for the strong silver affinity of NORs of normal somatic cells are the large subunit of pol I, protein pp135, and UBF (Williams et al., 1982; Roussel and Hernandez-Verdun, 1994). Our present results rule out UBF as an Ag-NOR protein and suggest that pol I and/or pp135 give rise to the strong silver affinity of NORs.

The finding that UBF binds to rDNA in the absence of TBP and pol I is in good accordance with in vitro studies suggesting that UBF is the first factor of the pol I transcription machinery that associates with the rRNA genes (reviewed by Reeder et al., 1995). However, whereas in vitro bound UBF recruits SL1 to the promoter (Learned et al., 1986; Bell et al., 1988; Jantzen et al., 1992; McStay et al., 1997), in early *Xenopus* embryos SL1 is somehow prevented from joining the UBF-rDNA complex which in turn may explain the inability of pol I to associate with the rRNA genes and to form stable initiation complexes. The capability of the egg extract to support transcription of class II and class III genes from appropriate templates demonstrates that functionally active TBP is present in the extract (Toyoda and Wolff, 1992; Barton and Emerson, 1994; Gottesfeld et al., 1994; Ullman and Forbes, 1995; Leresche et al., 1996). Likewise, repression of pol II transcription in preblastula embryos is not due to a deficiency in TBP (Prieleau et al., 1994). It will be interesting to find out whether the active SL1-complex (i.e. TBP plus the three TAFIs) is present during early embryogenesis and what mechanisms prevent its interaction with the UBF-rDNA-promoter.

We thank the following individuals for their kind gifts: C. Dreyer (mAb b6-6E7-M12), R. Reeder (clone pXlr101A), G. Reimer (mAb 72B9, serum S18), M. Schmidt-Zachmann (mAbs No-114, No-185) and M. F. Trendelenburg (*Xenopus* I-nu mutants). We also thank Corinna Zünkler for help with cell culture and Silke Hofbauer for preparation of the figures. This work received financial support from the Deutsche Forschungsgemeinschaft (Sche 157/10-1).

REFERENCES


(Received 21 May 1997 - Accepted 25 June 1997)