The characterization of the TFIIIA synthesized in somatic cells of *Xenopus laevis*

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In somatic cells of *Xenopus*, transcription of the TFIIIA gene initiates >200 bp upstream from the start site used in oocytes. The resultant mRNA encodes a protein, S-TFIIIA, that is 22 amino acids longer at its amino terminus than the abundant form of TFIIIA in oocytes (O-TFIIIA). S-TFIIIA binds the 5S RNA gene and 5S RNA, and both O- and S-TFIIIA promote the formation of stable transcription complexes on oocyte-type 5S RNA genes in an oocyte nuclear extract. We have not found any functional difference between the two forms of TFIIIA. Different transcription start sites suggest differential promoter usage—one in oocytes that permits high levels of gene activity and another that is used in somatic cells for low-level TFIIIA mRNA synthesis.

[Key Words: *Xenopus; TFIIIA; zinc finger protein; transcription*]

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The 38-kD zinc finger protein TFIIIA is synthesized at high rates and stored in large amounts in *Xenopus* oocytes (Engelke et al. 1980). There are two oocyte-specific functions carried out by this protein (O-TFIIIA). The first of these functions is the activation throughout oogenesis of a large multigene family encoding oocyte-specific 5S RNA genes. These 5S RNA genes are transcribed actively during oogenesis and then repressed in somatic cells where a much smaller multigene family, called somatic 5S RNA genes, encodes the 5S RNA synthesized for ribosomes (for review, see Wolfe and Brown 1988). The second function is the interaction of O-TFIIIA with 5S RNA forming a 7S RNP storage particle that accumulates in early oogenesis (Honda and Roeder 1988; Pelham and Brown 1980). Accumulation of O-TFIIIA protein is due in part to the high rate of transcription of the TFIIIA gene in oocytes, TFIIIA mRNA is very abundant in oocytes with ~10^6 copies per oocyte, as compared with about 1 copy per somatic cell (Ginsberg et al. 1984; Taylor et al. 1986).

Soon after the discovery of the somatic form of TFIIIA in oocytes, it was noted that the predominant form of TFIIIA in somatic cells (S-TFIIIA), as detected with an antibody raised against O-TFIIIA, had a slower electrophoretic migration than O-TFIIIA. A band that comigrated with O-TFIIIA, but that was less abundant than S-TFIIIA, was usually also detected in somatic tissues. The difference in apparent molecular mass between the two is ~2 kD (Pelham et al. 1981; Shastry et al. 1984), yet peptide maps of the two immunoreactive proteins were identical (Shastry et al. 1984).

Three possible explanations for the difference have been considered. First, multiple TFIIIA genes may exist. This is unlikely because Southern blots probed with O-TFIIIA demonstrated that there is only one genomic gene for TFIIIA (Taylor et al. 1986). Second, post-translational modification may occur. A number of attempts to demonstrate post-translational modification of O-TFIIIA have failed. O-TFIIIA incubated in somatic extracts did not give rise to S-TFIIIA (Pelham et al. 1981). TFIIIA synthesized in *Xenopus* embryos following microinjection of synthetic mRNA, derived from a cDNA clone of O-TFIIIA, was always of the oocyte type even during stages of development when endogenous TFIIIA synthesis is mainly of the somatic type (Andrews and Brown 1987). Most recently, O-linked glycosylation of TFIIIA was not detected by Jackson and Tjian (1988). The third possibility, transcriptional processing, is the topic of this paper.

When first discovered, S-TFIIIA was hypothesized to be involved in the developmental control of the two kinds of 5S RNA genes either as a specific repressor of oocyte 5S RNA genes or perhaps as a specific activator of somatic 5S RNA genes in somatic cells. Early experiments did not support either of these possibilities. Tissue culture extracts that contain predominantly S-TFIIIA transcribe a mixture of somatic and oocyte 5S RNA genes with about the same relative efficiency as oocyte nuclear extract that contains only O-TFIIIA (Pelham et al. 1981). This relative transcription efficiency expressed as a somatic to oocyte 5S RNA ratio (S/O) is ~5–10. Shastry et al. (1984) partially purified...
S-TFIIIA and found it inactive in 5S RNA gene transcription, leading them to conclude that the active form of TFIIIA in somatic cell extracts is the minority O-TFIIIA component. In contrast, Pelham et al. (1981) calculated that the amount of O-TFIIIA in somatic cell extracts is too small to account for the extract’s ability to support 5S RNA gene transcription and therefore concluded that both O- and S-TFIIIA must be active transcription factors.

The oocyte 5S RNA genes are completely repressed by late gastrula (Wakefield and Gurdon 1983; Wormington and Brown 1983) before an embryo has detectable S-TFIIIA protein (Pelham et al. 1981; Shastry et al. 1984; Andrews and Brown 1987). Thus, O-TFIIIA stored during oogenesis is presumed to be the active factor for the transcription of somatic 5S RNA genes in early embryogenesis. These experiments taken together, despite the disagreement on whether S-TFIIIA is a functional transcription factor for 5S gene transcription, infer that the differential transcription of somatic versus oocyte 5S RNA genes in somatic cells does not depend on the presence of S-TFIIIA.

In this paper we show that, in somatic cells of Xenopus, transcription of the TFIIIA gene initiates upstream of the start site previously identified in oocytes. Translation of the somatic mRNA yields a protein that has 22 additional amino acids at the amino terminus and is identical in mobility to in vivo-derived S-TFIIIA. In transcription assays, both O-TFIIIA and S-TFIIIA behave identically and have equivalent activity on both oocyte and somatic 5S RNA genes.

Results

The two forms of TFIIIA are encoded by a single gene

Mouse L cells were stably transformed with either a full-length genomic clone of TFIIIA containing ~2 kb of DNA upstream of the O-TFIIIA transcription start site or a cDNA clone of O-TFIIIA (Fig. 1). Soluble extracts of the cell lines were analyzed for TFIIIA by Western blot, using a polyclonal rabbit antiserum raised against O-TFIIIA protein A. Control mouse L-cell extracts (lane 6) do not cross-react with the antiserum. The cell lines transformed with the genomic clone synthesize both 40- and 38-kD TFIIIA proteins, whereas the cDNA clone encodes only the smaller O-TFIIIA protein. From this, we conclude that both proteins are encoded by a single gene even though a minority of the Xenopus TFIIIA synthesized in mouse cells migrates as S-TFIIIA. In contrast, the majority of TFIIIA synthesized in Xenopus tissue culture cells (lane 1) is S-TFIIIA.

S-TFIIIA mRNA is longer at its 5' end than O-TFIIIA mRNA

We noted that the published 5'-flanking sequence of genomic TFIIIA (Tso et al. 1986) has an in-frame ATG codon 66 nucleotides upstream from the amino-terminal ATG of O-TFIIIA and 15 nucleotides upstream from the cap site of O-TFIIIA mRNA (Fig. 2). A clone ranging from −325 to +7 relative to the O-TFIIIA cap site (pTF35) was used to probe a Northern blot of mouse L-cell extract; a clone containing only sequence upstream of the O-TFIIIA cap site (pTF35) was used to probe a Northern blot of mouse L-cell extracts transfected with pTFG1. This probe did not hybridize with mRNA from oocytes, but there is a distinct hybridization band with somatic-derived poly[A] + RNA. There are two closely approximated bands in somatic mRNA that hybridize with O-TFIIIA cDNA probe (Fig. 3A, lane 4). Careful analysis has shown that it is the upper slower moving band that hybridizes with the upstream probe pTF35. The lower band is indistinguishable from that found in oocytes.

The 5' region of the TFIIIA mRNA in somatic poly[A]+ was amplified by an “anchor” polymerase chain reaction (PCR) method (Frohman et al. 1988). The PCR reaction was primed by oligonucleotides that span introns in the genomic TFIIIA gene (Fig. 4). Because the PCR products do not contain intron sequences, amplification of contaminating genomic DNA is ruled out. The amplified DNA was electrophoresed and hybridized by Southern blot with either full-length cDNA encoding O-TFIIIA (Fig. 4A) or a clone containing only sequence upstream of the O-TFIIIA cap site (Fig. 4B). Only the PCR products from amplification of somatic mRNA hybridize with the upstream probe confirming that a fraction of the somatic mRNA contains additional sequences upstream from the oocyte mRNA start site not found in the oocyte mRNA. Multiple bands are seen in the amplification products. These are due, at least in part, to incomplete extension at the 5' end in the reverse transcriptase reaction.

The sequence of the 5' region of S-TFIIIA mRNA and the protein translated from it

The PCR product from somatic mRNA was cloned using

Figure 1. Western blot analysis of soluble extracts of mouse L cells transfected with the Xenopus TFIIIA genomic clone pTFG1 and the cDNA expression plasmid pLTRTF6. Antiserum directed against purified TFIIIA was used, followed by 125I–protein A. (Lane 1) Xenopus XLA cultured cell extract; (lanes 2 and 3) extracts of two independent mouse L-cell lines transfected with pTFG1; (lanes 4 and 5) two independent mouse L-cell extracts transfected with pLTRTF6; (lane 6) control mouse L-cell extract; (lane 7) Xenopus oocyte extract. S and O denote S-TFIIIA and O-TFIIIA, respectively.
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Figure 2. Nucleotide sequence of the somatic TFIIIA clone. The nucleotide sequence upstream from the known transcription start site of O-TFIIIA (three vertical arrows) cloned from the PCR product of the Xenopus tadpole poly[A]+ RNA at the top line in each row is compared with the corresponding region (bottom line) from a Xenopus genomic clone reported by Tso et al. (1986). The asterisks (*) denote those residues in the cDNA (upper) sequence that are identical to another reported genomic clone of this region (Scotto et al. 1989). The nucleotides are numbered with +1, denoting the first of the three nucleotides of the transcription start site of O-TFIIIA (three vertical arrows) cloned from the PCR product of the Xenopus tadpole poly[A] RNA at the top line in each row is compared with the corresponding region (bottom line) from a Xenopus genomic clone reported by Tso et al. (1986). The asterisks (*) denote those residues in the cDNA (upper) sequence that are identical to another reported genomic clone of this region (Scotto et al. 1989). The nucleotides are numbered with +1, denoting the first of the three nucleotides of the transcription start site of O-TFIIIA mRNA; residues preceding it are indicated by negative numbers. The deduced additional amino acid sequence in-frame with the O-TFIIIA translation initiation codon is shown. The ATG translation initiation codons for O-TFIIIA and for the somatic S-TFIIIA are enclosed in the open box and the shaded box, respectively. The restriction site NsiI used for cloning S-TFIIIA in a translatable form is shown.

Figure 3. Northern blot hybridization of RNAs from the different stages of Xenopus laevis. Total RNAs (5 μg) from Xenopus laevis mature ovari (lane 1), immature ovari (lane 2), unfertilized egg (lane 3) and 15 μg of poly[A]+ RNA from tadpoles (lane 4) were subjected to Northern blot hybridization. Duplicate blots were hybridized with either 32P-labeled insert from the plasmid pSPTF15 (A) or pTF35 (B).
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Figure 4. Southern blot analysis of the PCR-amplified products. Products of PCR were electrophoresed and blotted, and duplicate filters were probed with radioactive pSPTF15 (A) or pTF35 (B). [Lanes 1 and 5] PCR product of oocyte mRNA; [lanes 2 and 6] PCR product of oocyte mRNA digested with SalI and BglII; [lanes 3 and 7] PCR product of tadpole mRNA; [lanes 4 and 8] PCR product of tadpole mRNA digested with SalI and BglII. The PCR products in lanes 1 through 4 were primed with P7, whereas those in lanes 5–8 were primed with P8. The dots on the left represent the location of qbX174/HaeIII DNA molecular weight markers whose sizes are 1353, 1078, 872, 603, and 310 bp, respectively. The diagrams of O- and S-TFIIIA mRNAs [below] show the regions covered by the two primers [P7 and P8] and the two probes, A [pSPTF15] and B [pTF35]. Transcription start sites are denoted by solid arrows; the start site for S-TFIIIA mRNA is not known precisely. The inverted open arrowheads refer to known locations of introns in the genomic sequence [Ts0 et al. 1986]. The SalI site is within the primer used for PCR, and the BglII site is in the gene downstream from the location of the two primers (for details, see Methods).

Figure 5. Western blot analysis of Xenopus XLA cell extract mixed with in vitro-translated O- or S-TFIIIA. Xenopus XLA cell extract (lane 1) was mixed with 3 μl of wheat germ translation extract containing in vitro-synthesized S-TFIIIA (lane 2) or O-TFIIIA (lane 3).

Figure 6. Radioactive in vitro translation products of O-TFIIIA [O], S-TFIIIA [S], and S^M-TFIIIA [S^M] in wheat germ extract detected by fluorography of a dried polyacrylamide gel.
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Figure 7. The interaction of O- and S-TFIIIA synthesized in vitro with 5S RNA and 5S RNA genes. Radiolabeled protein was synthesized in wheat germ extract from synthetic O- or S-TFIIIA mRNA and incubated with unlabeled 5S RNA or with a DNA fragment (66 bp) that spans the internal control region of the 5S RNA gene. The protein–nucleic acid complexes were resolved by nondenaturing gel electrophoresis [(O) O-TFIIIA; (S) S-TFIIIA]. To emphasize the difference in mobility, a mixture of the two proteins is also shown (lane O + S). In the absence of specific nucleic acids, the protein does not migrate into the gel. Open arrows indicate the position of the complexes; solid arrowheads show the position of the unlabeled nucleic acids.

Figure 8. Support of oocyte and somatic 5S RNA gene transcription by in vitro-synthesized O-TFIIIA, S-TFIIIA, and SM-TFIIIA. Depleted oocyte nuclear extract was supplemented with three concentrations of the three kinds of in vitro-synthesized TFIIIA s. [Lanes 1, 4, and 7] 0.45 μl of the translation reaction product was mixed with 10 μl of depleted oocyte nuclear extract; [lanes 2, 5, and 8] 0.15 μl; [lanes 3, 6, and 9] 0.05 μl. [Lanes 1–3] S-TFIIIA; [lanes 4–6] SM-TFIIIA; [lanes 7–9] O-TFIIIA; [lane 10] 0.45 μl of control wheat germ extract; [lane 11] no wheat germ extract. S and O refer to locations of somatic and oocyte 5S RNA, respectively.

and 4). Translation in vitro of synthetic mRNA with the upstream sequence gives a protein that comigrates with S-TFIIIA [Fig. 5]. Somatic cells synthesize variable amounts of a protein that comigrates with O-TFIIIA, along with the more abundant S-TFIIIA [Fig. 1, lane 1 and Fig. 5, lane 1]. This minority component might be a premature termination product of S-TFIIIA, as is the case in vitro [Fig. 6], or a small amount of O-TFIIIA mRNA may be synthesized in somatic cells.

S-TFIIIA should not be confused with proteins other than TFIIIA that have been reported to bind 5S RNA genes. Barrett and Sommerville [1987] claimed that both proteins in the 42S RNP storage particle of oocytes bind and footprint 5S RNA genes. We have shown that this is attributable to TFIIIA contamination of 42S RNP preparations [Joho et al. 1990]. One of the two proteins in the 42S particle that we refer to as p43 binds specifically to 5S RNA but not to 5S DNA. It is different but related to TFIIIA and only found in oocytes [Joho et al. 1990]. Recently, Blanco et al. [1989] described a 42-kD protein present in oocytes and somatic cells that they believe functions as a specific transcription factor for somatic 5S RNA genes. This protein cannot be the same as S-TFIIIA because their 42-kD protein is abundant in mature oocytes, whereas S-TFIIIA cannot be detected in oocytes.

The function of S-TFIIIA versus O-TFIIIA

The sequence of O-TFIIIA begins with a short amino-terminal sequence before the first zinc finger begins. Six of these amino acids can be deleted without influencing TFIIIA function [Vrana et al. 1988]. From our knowledge of the structure of TFIIIA, we believe that the amino terminus is oriented toward the 3' end of the 5S RNA gene [Smith et al. 1984]. The DNA-binding region is delimited by the nine zinc fingers, and a domain essential for transcription is located at the carboxyl end of the protein [Smith et al. 1984; Vrana et al. 1988]. The other transcription factors, to the extent that their orientation is known, are presumed to bind near the 5' part of the internal control region and the start site of the gene [Pieler et al. 1987]. Taken together, these findings suggest that the addition of 22 amino acids at the amino terminus of TFIIIA will influence neither S-TFIIIA binding to DNA nor its ability to support transcription of a 5S RNA gene. Mobility-shift experiments show that both S- and O-TFIIIA bind to 5S RNA and 5S DNA [Fig. 7]. However, this is a qualitative assay that does not assess subtle binding constant differences that could have large biological effects.

Next, we showed that S-TFIIIA is indistinguishable from O-TFIIIA in its ability to support 5S RNA gene transcription. The assay uses either S-TFIIIA or O-TFIIIA to restore 5S RNA transcriptional activity to an oocyte nuclear extract depleted of endogenous TFIIIA. S-TFIIIA is clearly not a repressor of oocyte 5S RNA genes. In fact, it supports the same relative efficiency of somatic to oocyte 5S RNA gene transcription [Fig. 8]. We proposed that differential gene expression of somatic 5S RNA genes in somatic cells depends on the ability of somatic 5S RNA genes to form stable transcription complexes in cells where the same complexes with oocyte 5S RNA genes are destabilized. A sensitive in vitro test for the stability of a transcription complex is a template
exclusion assay (Bogenhagen et al. 1982). In vitro-synthesized O-TFIIIA and S-TFIIIA form stable transcription complexes with oocyte 5S RNA genes by this assay (Fig. 9). It can be argued that oocyte nuclear extracts would not show differential transcription of the two genes by the two proteins even if it existed because somatic cell-specific transcription factors may be absent. Somatic 5S RNA genes are consistently transcribed 5 to 10 times more efficiently than oocyte 5S RNA genes in oocyte nuclear extract (Wormington et al. 1981). Although this level of discrimination is only a fraction of the >1000-fold S/O differential transcription characteristic of somatic cells, assays in egg extracts have reproduced the 1000-fold S/O discrimination using O-TFIIIA (Wolffe and Brown 1987).

We conclude that the additional amino acids at the amino terminus of S-TFIIIA do not interfere with the protein's ability to form stable transcription complexes and promote transcription of 5S RNA genes. Differential transcription of 5S RNA genes must depend on mechanisms that are independent of the type of TFIIIA present in a cell.

Biological implications of two forms of TFIIIA

We believe that the biological importance of these two forms of TFIIIA is not attributable to a change in function of the protein but to the fact that different promoters are used for the same gene in oocytes and somatic cells. This mechanism for gene control is common and was described first for the amylase gene in mammals (Young et al. 1981). What interests us is the evolutionary solution for synthesizing a needed protein at two vastly different levels in oocyte and somatic cells of Xenopus. All of the components of ribosomes are synthesized at greatly exaggerated rates in oocytes. This is accomplished by different mechanisms. Genes for the 18S and 28S RNAs are amplified (Brown and Dawid 1968; Gall 1968). The 5S RNA is encoded by a large auxiliary gene family that functions only in oocytes (Brown 1982), where 5S RNA synthesis begins in advance of 18S and 28S RNA accumulation (Wegnez et al. 1972; Ford and Southern 1973). The 5S RNA produced at this stage is stored in RNP particles for weeks before it is incorporated into ribosomes (Denis and le Maire 1983). There are several reasons for the oocyte (but not somatic cells) to synthesize large amounts of TFIIIA. TFIIIA is an essential transcription factor for the large oocyte 5S RNA multigene family in oocytes and also stabilizes 5S RNA in the form of an RNP particle before ribosome assembly. TFIIIA also serves as a maternally inherited transcription factor that will function in the early part of embryogenesis before S-TFIIIA is synthesized.

Studies on the delimitation of cis-acting sequences that control the TFIIIA gene have been reported (Matsumoto and Korn 1988; Hall and Taylor 1989; Scotto et al. 1989). The multiple regions that have been identified are located between -306 and -101 from the transcription start site of O-TFIIIA. Therefore, key elements controlling O-TFIIIA transcription are within the DNA encoding the 5'-untranslated region of S-TFIIIA. Hall and Taylor (1989) compared the function of sequences in oocytes versus somatic cells and reported one cis-acting sequence that influences transcription in oocytes but not in somatic cells. It is likely that the high level of synthesis of O-TFIIIA is accomplished by an oocyte-specific promoter that is identified by an oocyte-specific transcription factor(s).

Methods

Plasmid constructions

A full-length Xenopus TFIIIA genomic clone (pTFG1) and an O-TFIIIA cDNA expression plasmid were gifts from Drs. Y. Matsumoto, J. Tso, and L. Korn. The genomic TFIIIA plasmid pTFG1 was constructed by ligation of two EcoRI fragments derived from two original λ phage genomic clones (Tso et al. 1986) into the EcoRI site of pEMBL8+ vector. This plasmid is missing a 46-bp EcoRI fragment within intron 4. It contains ~2 kb of DNA upstream from the O-TFIIIA transcription start site. The cDNA plasmid pLTRTF6 was constructed by J. Tso, who ligated the murine sarcoma virus long terminal repeat promoter region to the Xenopus oocyte TFIIIA cDNA, which was followed by the SV40 polyadenylation signal, in the plasmid pEMBL9+ vector. The plasmid pTF35, derived from pCATF35 (Hall and Taylor 1989), was a gift from William Taylor. It contains the upstream region of genomic TFIIIA DNA from -325 to +7 (counting +1 as the cap site of O-TFIIIA) cloned into the polylinker region of pGEM1. The Xenopus S-TFIIIA cDNA
clone [pSTF1] was generated by ligation of the 570-bp SalI–BglII fragment obtained by PCR into the SalI–BglII site of the oocyte TFIIIA cDNA pSPTF14 (Vrana et al. 1988). The resultant DNA was then ligated as a 1.9-kb SalI–EcoRI insert into the SalI and EcoRI sites of pSP64 vector. This clone has a d[A-T] sequence at the 5' end of the insert. To remove the 5' d[A-T] region, the clone pSTF2 was constructed by ligation of the 784-bp NsiI–XhoI fragment from pSTF1 with the 5'-end SalI–BglII XhoI–EcoRI DNA from the oocyte TFIIIA cDNA fragment. This S-TFIIIA cDNA with the full coding sequence was cloned into the NsiI and EcoRI sites of the plasmid pGEMEX-1.

DNA transfection and Western blot analysis for TFIIIA expression

Either pTFFG1 or pLTRTF6 was mixed with the herpes simplex virus thymidine kinase gene, pLS119/109 tk (McKnight and Kingsbury 1982) as a selection marker and transfected into X. laevis oocytes, using a step program (94° 40 sec; 55° 2 min; 72° 3 min), followed by a 15 min final extension at 72° in a DNA Thermal Cycler. For PCR amplification, an oligo(dT)-adapter primer, 5'-d-GTCG containing the XhoI sites, was annealed to the poly(dA)-tailed cDNA at 42° for 2 min and then extended at 72° for 40 min to synthesize the cDNA synthesis was primed by 25 pmols of an antisense primer. The reaction mixture was extracted once with an equal mixture of phenol and chloroform, followed by chloroform extraction, and then precipitated with ethanol in the presence of 2.5 M ammonium acetate.

Poly(A)^+ RNA preparation and Northern blot analysis were carried out as described by Ausubel et al. (1989). Five micrograms of total RNA from the X. laevis ova, unfertilized egg, and 15 µg of poly(A)^+ RNA from tadpoles were electrophoresed on 1.2% agarose gel containing 2.2 M formaldehyde, transferred to GeneScreen, and hybridized with 32P-labeled full-length O-TFIIIA cDNA insert or the insert from the plasmid pTF35.

In vitro translation and transcription of the somatic TFIIIA cDNA

The O-TFIIIA cDNA pSPTF15 (Vrana et al. 1988) was linearized at the HindIII site, the S-TFIIIA cDNA constructs, pSTF2 and pSM–TF2, were linearized at the EcoRI site, and the three DNAs were used as templates to produce capped RNAs according to the procedure described by Melton et al. (1984). The synthetic capped RNA was translated in either wheat germ extract or rabbit reticulocyte extract, using a complete amino acid mixture (Vrana et al. 1988). The added radioactive amino acids were either 3S-methionine, 35S-cysteine, or a mixture of 3H-labeled leucine, lysine, phenylalanine, proline, and tyrosine (1 µM, 70–190 Ci/mmol). The proteins were analyzed by electrophoresis on an 18% polyacrylamide gel with a 6% stacking gel. Following electrophoresis, the gel was fixed for 30 min in a mixture of H2O, methanol, and acetic acid (60:30:10), treated with ENHANCE (New England Nuclear) for 60 min, dried, and autoradiographed.

Immunological depletion of TFIIIA from X. laevis oocyte nuclear extract and its reconstitution for transcription with in vitro-translated TFIIIA were carried out as described by Vrana et al. [1988]. Transcription reactions were incubated at 30°C in
20 µl of reaction mixture containing 10 µl of immunologically depleted oocyte nuclear extract, 0.2 µg of pXlobs [an oocyte 5S RNA gene; Brown and Schlissel 1985], 0.05 µg of pXxls115/105 [a somatic 5S RNA gene, Bogenhagen and Brown 1981], 7 µCi of [α-32P]UTP [800 Ci/m mole], 250 µM ATP, CTP and GTP, 10 units of RNase in 1 buffer (Birkenmeier et al. 1978) and were supplemented with in vitro-translated TFIIIAs in wheat germ extract diluted as described with H2O just before use. In template exclusion experiments, pXlobs was preincubated with the reconstituted extract for 20 min, pXxls115/105 was added for another 20 min, followed by a mixture of the labeled nucleoside triphosphates for 30 min. Transcription products were analyzed by electrophoresis on a 10% polyacrylamide/7 M urea gel and autoradiographed.

Mobility-shift assays

Radiolabeled protein (10 ng) was incubated with nonradioactive X. laevis oocyte type 5S RNA [300 ng] 20 mM Tris, 70 mM KCl, 10 µM ZnCl2, 5 mM MgCl2, 0.1% NP-40, 2.5 mM DTT, and 5% glycerol [pH 7.5] at room temperature for 15 min. For DNA binding, the radiolabeled protein (10 ng) was mixed in the above buffer with a 66-bp DNA fragment [100 ng] that included the internal control region of the X. laevis somatic 5S RNA gene and with 50 µg/ml of poly[d(A-T)]. The mixture was incubated with 30 µg/ml of RNase A for 15 min before electrophoresis. The protein nucleic acid complexes were electrophoresed on a 4.5% polyacrylamide gel containing 0.2% prepolymerized acrylamide in 20 mM HEPES/NaOH (pH 8.2) and 5% glycerol. The upper reservoir contained 5 mM DTT. Electrophoresis was performed at 120 V [20 mA] for 90 min.

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