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Differential Expression of Oocyte-Type Class III Genes with Fraction TFIIIC from Immature or Mature Oocytes

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The *Xenopus* OAX genes can be expressed in oocytes but are virtually inactive in somatic tissues. The $tRNA^{Met1}$ (tMET) genes also appear to be developmentally regulated. We have examined the reason for the differential expression of these class III genes. Analysis of the transcriptional activities of extracts derived from immature and mature oocytes revealed that the developmental regulation of these genes can be reproduced in vitro. We have partially purified the required transcription factors B and C from these extracts to ascertain the components responsible for this differential activity. The immature oocyte C fraction activates the tMET and OAX genes when reconstituted with either the immature or mature oocyte-derived B fraction. In contrast, the mature oocyte C fraction fails to activate these genes regardless of which B fraction is used. Both C fractions activated the somatic 5S gene. Purification of the oocyte C fractions by phosphocellulose or B box DNA affinity chromatography failed to separate additional activities responsible for the differential expression of OAX or tMET. By using template exclusion assays, the inability of the mature oocyte C fraction to activate transcription was correlated with an inability to form stable transcription complexes with the tMET or OAX gene.

A central question in development biology concerns the mechanisms which bring about stage-specific gene expression. The *Xenopus* class III system provides several examples of genes which are expressed specifically in oocytes. The developmental regulation of the oocyte 5S gene has been studied in detail (for reviews, see references 16 and 36). However, the mechanisms governing the oocyte-specific expression of non-5S genes, such as OAX and $tRNA^{Met1}$ (tMET), are not well understood. In this study, we undertook an analysis of the factors responsible for this developmental regulation. The class III system provides a means to dissect the transcription factors responsible for stage-specific gene expression, in that the polymerase III (pol III) factors can be partially purified and recombined to reconstitute efficient transcription in vitro. At least two components, TFIIIB (factor B) and TFIIIC (factor C), are required to direct correct initiation by RNA pol III on tRNA-type class III genes (43; reviewed in 16, 36, and 45). A third factor, TFIIIA (factor A), is additionally required for transcription of 5S RNA genes (13). There is recent evidence, obtained with the *Bombyx* system, for an additional component of class III transcription complexes, TFIIIR, which is composed of RNA rather than protein (58).

The tRNA-type promoter consists of two separable elements of ~10 bp each, known as the A box and B box (16, 36, 45). Binding by factor C to the B box promoter element permits subsequent association by factor B, which in turn stabilizes the complex (27, 32, 44). For the 5S RNA gene, which lacks a B box sequence element, initial binding by factor A to the 50-bp internal promoter facilitates binding by factor C, apparently through protein-protein contacts (21, 32). Only after binding by both factors A and C does factor B associate with the 5S template (32). The assembly of the pol III factors with the template produces a stable complex which remains associated throughout multiple rounds of transcription initiation in vitro (6, 41). In the yeast system, factors C and A can be removed from these complexes by treatment with heparin or high salt, leaving factor B bound to sequences preceding the initiation site and capable of independently directing transcription (28, 29).

Factor B has been purified from both *Saccharomyces cerevisiae* (30) and human systems (51). In the yeast system, the use of antibodies, photocross-linking studies, and Southwestern (protein blot) analysis has identified several polypeptides which copurify with factor C and specifically bind to DNA sequences in and around the A and B box promoter elements (4, 15, 26, 37). Recently, a gene encoding a 95-kDa DNA-binding subunit of yeast factor C has been cloned (48). Most evidence suggests that HeLa factor C also comprises a complex set of proteins (42, 56, 57), which can be chromatographically separated into two components (56, 57). The equivalent of human and yeast factor C for the *Bombyx* system may be represented by two essential factors, termed IIIC and IIID (35). Little information is as yet available concerning the *Xenopus* homolog.

The differential expression of the somatic and oocyte 5S genes has been studied in detail (5, 39, 40, 52, 54). There is evidence attributing the developmental inactivation of the oocyte 5S genes to a less-effective interaction with factor C (52). Less-stable factor-DNA complexes on the oocyte 5S genes are thought to lead to permanent inactivation through assembly into inactive, late-replicating chromatin (19, 52). Little is known concerning the differential expression of tRNA-type class III genes during development. We set out to examine the role of factors B and C in the regulation of two *Xenopus* class III genes, tMET and OAX, which appear to be oocyte specific. OAX refers to a multigene family, of unknown function, which is inactive in somatic cells yet becomes active when somatic nuclei are microinjected into oocytes (1, 50). In agreement with earlier studies (24, 50), Northern (RNA blot) analysis has shown that OAX transcripts are present in ovaries, are absent in early embryonic stages, reappear transiently at gastrula stage, and are there-

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after absent in neurula and later stages (10). Transient reexpression in blastula-gastrula stage embryos has also been noted for the oocyte-type 5S gene (54) and the oocyte-type $tRNA^{TyrC}$ gene (47). The tMET gene is thought to be oocyte specific, since it is present within the same tandemly repeated gene family as the oocyte-specific $tRNA^{TyrC}$ gene (9, 47). A recent study provides further evidence that both the OAX and tMET genes are inactivated at the gastrula-neurula transition (3).

In an effort to identify the factors which might be responsible for stage-specific expression of OAX and tMET, we prepared extracts of early- and late-stage oocytes and assayed for the ability to activate these genes in vitro. We found that immature oocyte extracts activated OAX and tMET, whereas mature oocyte extracts did not. Fractionation of these extracts revealed that factor C, or components which copurify with factor C by phosphocellulose or DNA affinity chromatography, is responsible for this differential expression. Template exclusion assays indicate that the inability of mature oocyte factor C to activate OAX or tMET correlates with an inability to bind stably to these templates. These findings provide the first evidence for developmental changes in factor C which alter relative gene activities. Moreover, this is the first evidence that factor C may be important for the developmental regulation of tRNA-type genes.

MATERIALS AND METHODS

Plasmid DNAs. The OAX gene present in plasmid pE190 (31) was used in this study. OAX genes contain characteristic A and B box promoter elements and encode a major transcript of approximately 186 nucleotides (nt) (1, 2, 31). In vitro, use of additional termination signals results in additional transcripts of 267, 101, and 88 nt (2). The OAX.2 plasmid used in Fig. 4 contains a 253-bp *Hae*III fragment containing the OAX gene with truncated flanking sequences. The *Xenopus laevis* somatic 5S RNA gene used in this study was pXls11 (38). The *X. laevis* major oocyte-type 5S RNA gene used was pXlo+176, originally subcloned from pXlo31 (14). The *X. laevis* $tRNA^{met1}$ (tMET) gene was described by Clarkson and coworkers (9).

Partial purification of factors B and C. Oocyte S150 extracts were prepared from stage 6 oocytes essentially as described by Worcel and coworkers (18). Ovaries were excised from large adult frogs and incubated with collagenase (0.15%) in modified OR2 buffer (82 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 5 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] · [pH 8.0]) for approximately 1 h or until the mature oocytes could be dislodged from connective tissue by agitation. Large stage 6 oocytes were separated from immature stages by successive washes in OR2 buffer for a period of 1 h in the presence of 1 mM phenylmethylsulfonyl fluoride (PMSF). The intact stage 6 oocytes were centrifuged in 1 additional volume of buffer A (70 mM KCl, 20 mM HEPES [pH 7.9], 0.1 mM EDTA, 0.5 mM PMSF, 1 mM dithiothreitol [DTT], 20% glycerol) in an SW41 rotor for 45 min at 30,000 rpm at 4°C. The S150 supernatant was used directly in transcription reactions (Fig. 1A) or fractionated according to established procedures on phosphocellulose (P11) ion-exchange resin (43). The phosphocellulose was treated with successive base and acid washes according to the manufacturer's instructions (Whatman) and then equilibrated in buffer A containing bovine serum albumin (BSA) (100 μg/ml). Typically, 30 ml of S150 extract was applied to a 10-ml P11 column. The column was washed with 5 column volumes of buffer A containing 100 mM KCl. Fraction B was step eluted with buffer A containing 350 mM KCl, and fraction C was step eluted with buffer A containing 550 mM KCl. All washing and elution buffers contained BSA (100 μg/ml). These fractions were dialyzed against buffer A overnight and frozen in aliquots at −70°C. The total protein concentration of fraction B ranged from 2 to 3 mg/ml, and that of fraction C ranged from 0.5 to 1 mg/ml.

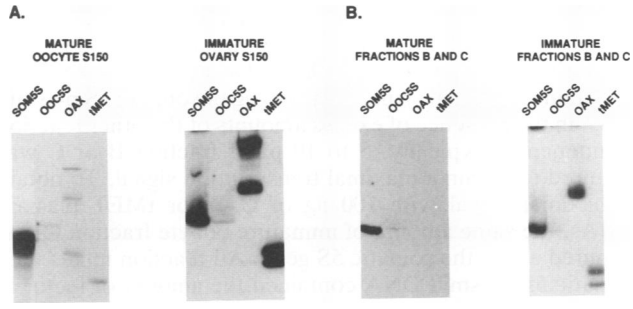

FIG. 1. Differential expression of oocyte-type OAX and tMET genes in vitro. (A) In vitro transcription reaction mixes contained 100 ng of the templates indicated above each lane, 40 μl of S150 extracts of mature oocytes or immature ovaries, and other components as outlined in the text. After a 2-h incubation, reactions were stopped, and the transcription products were purified, electrophoresed in sequencing gels, and exposed to film. An autoradiograph is shown. The somatic 5S gene gives rise to a 120-nt transcript, whereas the oocyte 5S gene gives rise to three transcript lengths owing to termination at the correct termination signal and two downstream termination signals. The OAX gene gives rise to a predominant 186-nt transcript and additional transcripts of 267, 101, and 88 nt, resulting from use of other termination signals (2). The tMET gene gives rise to one or two transcript lengths, representing precursor and processed transcripts (23). (B) Transcription reaction mixes contained 100 ng of indicated plasmid DNAs and saturating amounts of fractions B and C from mature oocyte S150 extracts (first panel) or immature ovary extracts (second panel), along with factor A (50 ng).

To isolate factor C by B box DNA affinity, a synthetic oligonucleotide (gatcAAAGGTTGTGGGTTCGATTCCCA) containing the B box (underlined) was multimerized and coupled to Sepharose. Oocyte S150 extracts (15 ml) were passed over a 0.5-ml DNA affinity column in the presence of poly(dI-dC) (1 μg/ml). The column was then washed with 10 ml of buffer A containing 100 mM KCl, and factor C activity was eluted in 1 ml of buffer A containing 550 mM KCl and BSA (100 μg/ml). This fraction, containing 0.5 to 1 mg of total protein per ml, was dialyzed against buffer A.

To obtain immature oocyte fractions B and C, ovaries were removed from juvenile frogs (3 to 4 in. [ca. 8 to 10 cm] long) and homogenized with five strokes of a Dounce homogenizer in 2 volumes of buffer A. The homogenate was centrifuged at 30,000 rpm for 30 min in an SW41 rotor. The supernatant was removed and passed over phosphocellulose, and fractions B and C were eluted as described above.

Factor A was independently purified from 7S ribonucleoprotein particles as previously described (46). The protein concentration of the factor A preparation was 0.1 mg/ml. HeLa fraction C was prepared by the method of Johnson and coworkers (25).

In vitro transcription assays. Transcription assays were performed for 2 h at 20°C in a reaction volume of 20 to 30 μl, containing 20 mM HEPES (pH 7.9), 70 mM KCl, 10% glycerol, 5 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 0.6 mM each ATP, CTP, and UTP, 20 μM GTP, and 10 μCi of

[α-^{32}P]GTP. Reaction mixes contained 100 ng of each plasmid DNA and saturating amounts of fractions B and C. The saturating concentration was defined as the amount required to obtain a maximal signal from 100 ng of somatic 5S plasmid DNA in the presence of excess amounts of the other reaction components. Typically, 5 to 10 μl of fraction B or C was required to obtain a maximal transcription signal. To obtain a maximal signal with 100 ng of OAX or tMET plasmid DNAs, the same amount of immature oocyte fraction C was required as for the somatic 5S gene. All reaction mixes with somatic 5S plasmid DNA contained the amount of factor A required to obtain a maximal transcription signal (typically 50 ng in a volume of 0.5 μl). Reactions were terminated by addition of 5 volumes of 20 mM EDTA–0.5% sodium dodecyl sulfate (SDS). The transcription products were purified by extraction with phenol-chloroform (1:1), followed by ethanol precipitation. The precipitate was redissolved in 95% formamide–0.05% bromophenol blue–1× TBE (90 mM Tris base, 90 mM boric acid, 2.5 mM EDTA). The samples were heated at 95°C for 3 min and loaded onto 6% polyacrylamide gels containing 1× TBE and 8 M urea. Following electrophoresis, the gel was dried and exposed to X-ray film. Typical exposure times were between 1 and 4 h.

For template exclusion assays, plasmid DNAs (100 ng) were first incubated with fraction C in the presence of other reaction components except fraction B and factor A for 15 min. Following preincubation, a second plasmid DNA (100 ng) was added along with fraction B and factor A, and the reaction continued for 2 h. Except where immature fraction B is specified in the figure legends, reaction mixes contained mature oocyte fraction B. The transcription reaction mixes with crude S150 extracts in Fig. 1 contained 40 μl of extract in buffer A, 100 ng of plasmid DNA, 5 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 0.6 mM each ATP, CTP, and UTP, 20 μM GTP, and 10 μCi of [α-^{32}P]GTP. Reactions were performed for 2 h and then processed as above.

RESULTS

Differential expression of OAX and tMET genes in vitro in crude extracts of immature and mature oocytes. As an initial step towards identifying the factors which might be responsible for the stage-specific expression of the OAX and tMET genes, S150 extracts were prepared from mature oocytes and immature ovaries, as described in Materials and Methods. These S150 extracts were assayed for their ability to support transcription in vitro of the oocyte-type OAX and tMET genes, as well as the somatic and oocyte-type 5S RNA genes (Fig. 1A). Mature oocyte S150 extracts supported transcription of the somatic 5S gene to high levels, but OAX, tMET, and the oocyte 5S gene were expressed at very low levels. In contrast, in immature ovary extracts, OAX and tMET genes were expressed at high levels, as was the somatic 5S gene, whereas the activity of the oocyte 5S gene remained low. These findings indicate that the immature and mature oocyte extracts contain all components necessary for reproducing the differential expression of OAX and tMET in vitro.

Fractions B and C are sufficient to reproduce the expression pattern seen in crude extracts of mature and immature oocytes. To identify the components responsible for the differential transcription of OAX and tMET, the mature and immature oocyte extracts were fractionated to separate transcription factors B and C by established techniques with phosphocellulose (P11) ion-exchange resin (43). The material which eluted from P11 with 350 mM KCl (fraction B) contained factor B, along with most of the RNA pol III activity, whereas factor C eluted with 550 mM KCl (fraction C). Factor A was separately purified to homogeneity from immature oocytes as described previously (46). Fractions B and C derived from mature or immature oocytes, when combined with saturating concentrations of factor A, were able to reconstitute transcription of the somatic 5S gene in vitro (Fig. 1B). Separately, either fraction in combination with factor A did not support transcription (data not shown). As shown in Fig. 1B, reconstitution of mature oocyte fractions B and C with factor A produced a pattern of expression like that obtained with crude extracts of mature oocytes; the somatic 5S gene was expressed at high levels, whereas tMET, OAX, and the oocyte 5S gene were relatively inactive. In contrast, with fractions B and C from immature ovaries, tMET and OAX were expressed at high levels, as was the somatic 5S gene, while the oocyte 5S gene was expressed at low levels. This demonstrated that fractions B and C contain all the components necessary to reproduce the expression pattern seen with crude extracts of either immature or mature oocytes. The OAX and tMET genes were differentially expressed with fractions B and C from immature versus mature oocytes. In contrast, the somatic and oocyte 5S genes were not differentially expressed by immature versus mature fractions B and C; the somatic 5S gene produced the same maximal signal with either reaction mixture, whereas the oocyte 5S gene was approximately 10-fold less active in either reaction mixture. With fraction C from HeLa cells as well, the oocyte 5S gene was approximately 10-fold less active than the somatic 5S gene (data not shown).

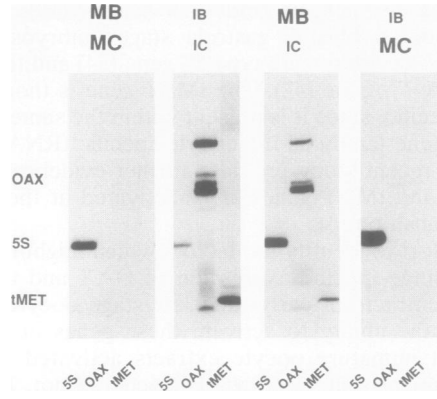

FIG. 2. Transcription reactions with fractions B and C isolated from mature oocytes or immature ovaries. Plasmid DNAs containing the somatic 5S RNA (5S), OAX, or tMET gene were transcribed in reaction mixes containing P11 fractions B and C from mature oocyte S150 extracts (first panel) or immature ovary extracts (second panel), a mixture of mature B and immature C (third panel), or a mixture of immature B and mature C (fourth panel). All reaction mixes contained 50 ng of purified factor A, which was the saturating concentration for 100 ng of somatic 5S plasmid DNA. All four panels are from the same autoradiographic exposure.

Fraction C is the primary determinant for the differential activity of OAX and tMET. To determine which fraction was responsible for the differential expression of OAX and tMET, reactions were performed with heterologous mixtures of mature and immature fractions B and C. In the first two panels of Fig. 2, OAX and tMET, along with the somatic 5S gene, were transcribed in reaction mixes containing mature or immature fractions B and C. OAX and tMET were active with the immature fractions and virtually inactive with

mature fractions B and C. The somatic 5S gene was included as a control template to demonstrate that the mature fractions B and C, which do not express OAX and tMET, do contain viable transcription factors and polymerase. Combining mature fraction C with immature fraction B produced the mature pattern of expression (fourth panel); the somatic 5S gene was expressed at high levels, whereas OAX and tMET were inactive. In contrast, combining mature fraction B with immature fraction C produced the immature pattern (third panel); OAX and tMET were expressed at high levels, as was the somatic 5S gene. This showed that the source of fraction C was the primary determinant of activity for OAX and tMET in these reactions. The activities of OAX and tMET were as much as 50- to 100-fold higher with immature fraction C than with mature fraction C (compare second and fourth panels). There was also a twofold variation in the activities of OAX and tMET genes with fraction B (compare second and third panels); however, this effect was small compared with the effect of fraction C. The activity of the somatic 5S gene also varied by two- to fourfold in these several reaction mixtures; however, this variation was not attributable to either fraction B or C alone (compare third and fourth panels).

Since the factor C activity is only partially purified in P11 fraction C, this fraction could contain additional activities which modulate class III gene expression. In an attempt to separate essential factor C activity from negative regulators potentially present in mature oocyte fraction C, this activity was eluted from P11 by a linear salt gradient instead of by step elution. None of the fractions were found to support OAX or tMET gene expression, indicating that P11 chromatography does not separate factor C from putative negative regulators of OAX or tMET. Analogous results were obtained when factor C from immature ovary extracts was eluted with a linear salt gradient from P11. Those fractions which supported somatic 5S gene expression also supported expression of OAX and tMET. This indicated that P11 chromatography does not separate factor C from other activities which might be present in immature oocyte fraction C and required for high-level expression of OAX and tMET (data not shown).

In a further attempt to separate factor C from potential activities which might copurify by P11 chromatography and bring about the differential expression of OAX and tMET, factor C was isolated from immature and mature oocyte extracts by B box DNA affinity chromatography as described in Materials and Methods. With B box affinity-purified factor C isolated from mature oocyte extracts, the somatic 5S gene had much higher activity than either OAX or tMET, whereas B box affinity-purified factor C from immature ovary extracts activated all three genes to similar levels (Fig. 3). Thus, using both phosphocellulose linear salt gradient chromatography and B box affinity chromatography, we were unable to resolve an additional component responsible for the stage-specific expression of the OAX and tMET genes. These results suggest that factor C, or a factor which may be associated with it, is responsible for the differential expression of OAX and tMET in immature and mature oocyte extracts.

In order to determine whether the difference in activities between fractions C from immature and mature oocytes might reflect a difference in factor concentration, we performed transcription reactions with various concentrations of each fraction. Increasing or decreasing the amount of each fraction in transcription reaction mixes did not alter the relative activities of OAX and tMET compared with that of

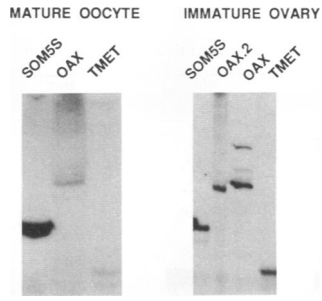

FIG. 3. Transcription reactions containing factor C isolated by B box DNA affinity chromatography. Transcription reaction mixes contained 100 ng of the templates indicated and factor C isolated by B box DNA affinity chromatography from either mature oocyte S150 extracts (first panel) or immature ovary extracts (second panel), mature oocyte fraction B, and purified factor A (50 ng). The OAX.2 plasmid differs from the OAX plasmid in the extent of 5′- and 3′-flanking sequences, as described in the text.

the somatic 5S gene (data not shown). Thus, the inability of mature oocyte fraction C to significantly activate OAX or tMET is not explained by low concentrations of factor C. This finding is consistent with the observation that the somatic 5S gene attains the same maximal level of expression with mature oocyte fraction C as with immature oocyte fraction C, indicating that saturating concentrations of factor C are present in both cases. This suggests that these fractions differ in a qualitative rather than a quantitative sense. As an argument against differing levels of RNA pol III contributing to the differential expression of OAX and tMET, we found that supplementation with a partially purified fraction containing RNA poly III (11) but not containing factor B or C did not alter the activities of any template in reconstituted reactions (data not shown).

Factor C from mature oocytes is unable to bind stably to tMET. To test whether the inability of mature oocyte factor C to activate tMET is due to its inability to bind stably to this template, template exclusion assays were performed. In these assays, saturating concentrations of one template are incubated with mature oocyte fraction C for 15 min, and then a second template is added along with fraction B and other components of the reaction mix. If the first template stably binds and depletes factor C, the second template will not be transcribed. Preincubation of tMET with mature oocyte fraction C resulted in a very weak tMET signal and no significant inhibition of the second template, the somatic 5S gene (Fig. 4A, first panel). In contrast, preincubation of tMET with immature oocyte fraction C resulted in a strong tMET signal and complete inhibition of somatic 5S transcription (second panel). Thus, a high level of expression for tMET correlates with stable binding by immature oocyte factor C, whereas a low level of tMET expression correlates with an inability of mature oocyte factor C to bind stably to that template.

The fact that tMET was differentially bound and activated by factor C from immature and mature oocytes suggested that these might represent distinct forms of factor C. Alternatively, *Xenopus*-specific modulatory factors might exist which alter the binding affinity of factor C for the tMET gene. To investigate the latter possibility, we assayed the ability of fraction C from HeLa cells, as a heterologous source, to bind and activate tMET. Preincubation of tMET with HeLa fraction C resulted in a high level of expression and complete inhibition of the second template, the somatic

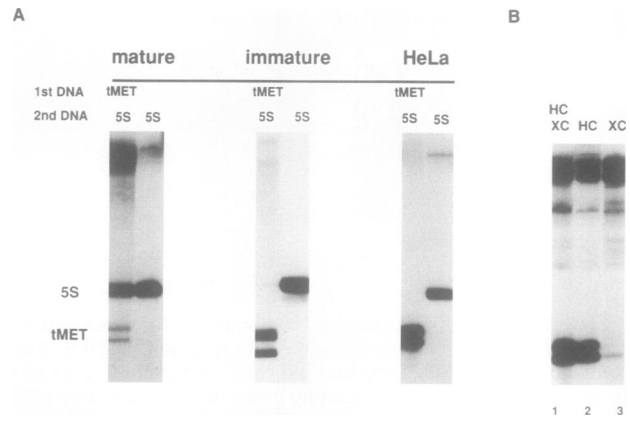

FIG. 4. Mature oocyte factor C does not bind tMET in template exclusion or mixing assays. (A) In the first panel, first lane, saturating amounts (100 ng) of tMET DNA were preincubated with mature oocyte P11 fraction C for 15 min, and then 100 ng of somatic 5S DNA was added along with fraction B and factor A (50 ng). The reaction was then continued for 2 h. In the second lane, the reaction mix contained only somatic 5S DNA; tMET was omitted from the preincubation reaction. In the second and third panels, the same protocol was followed, substituting immature ovary fraction C and HeLa fraction C, respectively. (B) Reaction mixes contained a mixture of mature oocyte fraction C (XC) and HeLa fraction C (HC) (lane 1), HeLa C (lane 2), or mature oocyte C (lane 3). All reaction mixes contained *Xenopus* fraction B.

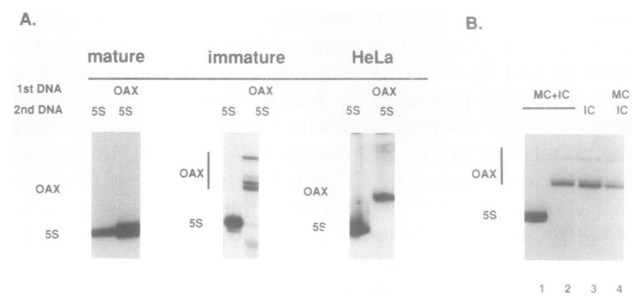

FIG. 5. Mature oocyte factor C does not bind OAX in template exclusion or mixing assays. (A) In the first panel, second lane, saturating amounts (100 ng) of OAX plasmid DNA were preincubated with mature oocyte P11 fraction C for 15 min, and then 100 ng of somatic 5S DNA was added along with fraction B and factor A (50 ng). The reaction was then continued for 2 h. In the first lane, the reaction mix contained only somatic 5S DNA; OAX DNA was omitted from the preincubation reaction. In the second and third panels, the same protocol was followed, substituting immature ovary fraction C and HeLa fraction C, respectively. (B) Reaction mixes contained a mixture of mature oocyte fraction C (MC) and immature ovary fraction C (IC) (lanes 1 and 2) or immature ovary fraction C (lane 3), in addition to fraction B and factor A (50 ng). In lane 4, OAX DNA was preincubated with mature fraction C for 1 h prior to addition of immature fraction B and the remaining reaction components. Plasmid DNAs (100 ng) were somatic 5S (lane 1) or OAX (lanes 2 through 4).

5S gene (Fig. 4A, third panel). This finding argues against *Xenopus*-specific or immature-oocyte-specific modulatory factors which are required for binding or activation of tMET.

The inability of mature oocyte factor C to bind tMET could be attributable to a deficiency inherent to the factor or, alternatively, to its copurification with a competitor protein which binds to the tMET promoter, thereby blocking binding by the factor. To assay for the presence of a competitor protein, transcription reactions were performed with a mixture of HeLa fraction C and *Xenopus* mature oocyte fraction C (Fig. 4B). tMET was inactive with *Xenopus* mature fraction C yet highly active with HeLa fraction C or with a mixture of both fractions. Thus, the positive effect of HeLa fraction C overrides the negative effect of *Xenopus* mature oocyte fraction C. This argues against a repressorlike protein in mature oocyte fraction C which binds to the tMET promoter and blocks binding by factor C. This finding instead suggests the absence in mature oocyte fraction C of a positive effector required for high-level expression of tMET. One possibility is that factor C in that fraction is modified so as not to bind stably to the tMET gene.

Results analogous to those obtained with tMET were obtained with the OAX gene. In template exclusion assays, preincubation of OAX DNA with mature oocyte C fraction resulted in a very weak OAX signal and no decrease in somatic 5S transcription (Fig. 5, first panel). Preincubation of OAX DNA with either immature fraction C or HeLa fraction C resulted in expression of OAX and prevented transcription of the somatic 5S gene (second and third panels). In mixed reactions containing both immature and mature oocyte C fractions, OAX was transcribed at a high level, similar to that of the somatic 5S gene, indicating that immature oocyte C fraction overrides the negative effect of mature oocyte C fraction (Fig. 5B, lanes 2 and 3). This argues against a competitor B box-binding protein in mature oocyte fraction C. As further evidence against a competitor B box-binding protein, preincubation of OAX DNA with mature oocyte C fraction for 1 h prior to addition of immature oocyte C fraction and the rest of the reaction components did not impair OAX transcription (lane 4). The fact that the OAX signal is not diminished in mixed reactions further argues against the presence of specific nucleases in mature oocyte fraction C which might otherwise account for the low transcriptional signal of OAX. To assay for the ability of OAX and tMET to form stable complexes in the presence of both factor B and mature factor C, we preincubated either template in the presence of mature fractions B and C for 30 min prior to addition of the somatic 5S gene. This resulted in no inhibition of somatic 5S transcription (data not shown).

DISCUSSION

These results demonstrate that fractions C isolated from immature and mature oocytes have different properties with respect to transcriptional activation of the OAX and tMET genes. Fraction C isolated from extracts of immature ovaries activated OAX and tMET to high levels of expression. In sharp contrast, fraction C isolated from mature oocyte extracts did not significantly activate OAX or tMET. Both of these fractions, however, were able to activate the somatic 5S gene to high levels. The source of fraction B, whether from immature or mature oocyte extracts, had relatively little effect on the activities of OAX and tMET. The same results were obtained with fractions C isolated by linear salt gradient elution from phosphocellulose or by B box DNA affinity chromatography, indicating that the ability to express or not express OAX and tMET copurifies with the B box-binding component of factor C. These findings suggest that factor C, or some other component which copurifies with factor C, is different in early- and late-stage oocytes, so that mature oocyte factor C is unable to support transcription of the OAX and tMET genes. The inability of mature

oocyte factor C to activate OAX and tMET correlates with its inability to bind stably to those templates. In contrast, immature oocyte factor C activated and bound stably to the OAX and tMET genes. Mixing experiments indicate that the positive effect of the immature oocyte C fraction is dominant, which argues against a B box-binding competitor protein in mature oocyte C fraction which blocks binding by factor C. These findings raise the interesting possibility that developmental changes in factor C are responsible, at least in part, for the selective inactivation of oocyte-type OAX and tMET genes during oocyte maturation.

One possible explanation for the results reported here is that factor C in mature oocytes is modified so that it has reduced ability to bind stably to the B box promoter element. This would explain why mature oocyte factor C lacks the ability to stably associate with the OAX or tMET gene, which contain B box elements, while retaining the ability to bind the 5S gene, which lacks a B box and interacts with factor C primarily through protein contacts with factor A. There are two ways in which such modification of factor C could play a role in the developmental inactivation of the oocyte-type tRNA and OAX genes. First, such modification could lead to loss of transcription complexes from all tRNA-type genes active during oogenesis. Since the majority are likely to be oocyte-type, due to their higher copy numbers, the simplest mechanism might be to inactivate all tRNA-type genes in mature oocytes and thereafter, in late-stage embryos, to selectively reactivate the relatively few somatic-type tRNA genes by a separate mechanism. Alternatively, factor C might be modified in maturing oocytes so that it loses the ability to stably associate with oocyte-type tRNA class genes, such as OAX and tMET, while retaining the ability to activate somatic-type tRNA genes owing to sequence elements which identify those genes as somatic-type. For example, the 5′-flanking sequences of *Xenopus* somatic-type $tRNA^{Tyr}$ genes result in higher transcriptional activity than oocyte-type $tRNA^{Tyr}$ genes in somatic cell extracts (20). Future experiments will address the possibility that mature oocyte C factor associates less effectively with the OAX and tMET genes because of sequence elements which define those genes as oocyte specific.

These results are consistent with a model in which factor C in mature oocyte S150 extracts is altered so as to bind less stably to tMET and OAX genes. There are several ways in which modification of factor C could alter the ability to form stable complexes with tRNA-type genes. Since vertebrate factor C appears to consist of a number of subunits, mature factor C might lack a subunit necessary for effective binding to tRNA-type promoters or for effective protein-protein interactions with factor B. A second means by which to alter activity is postsynthetic modification, such as phosphorylation; the activation of RNA pol III transcription by the adenovirus E1A protein was shown to correlate with phosphorylation of HeLa factor C, which increased DNA-binding affinity and transcriptional activity (22). In contrast to the increase in factor C activity brought about by adenovirus infection of HeLa cells, poliovirus infection results in a decrease in factor C activity (7, 8). This loss of activity has been attributed to proteolysis of factor C by a virally encoded protease, 3Cpro. These are two examples of virus-induced changes in factor C which dramatically alter levels of pol III transcriptional activity. The inactivation of the large families of oocyte-type tRNA class genes during oocyte maturation is an example of a naturally occurring event in which pol III transcription decreases dramatically. The findings reported here suggest that this event might also involve a change in factor C activity.

There are previous reports of dual forms of factor C and of modulatory agents that alter factor C activities. Active and inactive forms of factor C have been isolated from HeLa cell extracts, both of which bind the VA I gene (12, 22, 49). The active form was found to be present in both nuclear and cytoplasmic (S100) extracts, whereas the inactive form was predominantly found in nuclear extracts (49). In *Xenopus* oocyte extracts, an inhibitory agent was identified which results in loss of DNA-binding activity for factor C and loss of transcriptional activity for both the 5S and tRNA genes (17). A second activity was also identified which reversed the effects of the inhibitory agent. It is unclear at present what relationship these modulatory activities have to the findings reported here, since those activities affect transcription of both 5S and tRNA genes, whereas the change in factor C which we report apparently affects only tRNA-type genes.

The inability of mature oocyte factor C to form stable complexes with the OAX and tMET genes may not reflect a change in DNA-binding function directly, but instead may reflect an ineffective interaction with factor B on tRNA-type templates. A fully stable complex on tRNA-type genes requires binding by both factors B and C (32, 44). Since factor B lacks the ability to independently associate with DNA, this interaction presumably requires protein-protein contacts between these factors. A change in subunit composition or modification state for mature factor C could conceivably interfere with correct contacts with factor B on the tRNA-type promoter.

Much is known about the developmental switch from transcription of oocyte-type to somatic-type 5S genes. The oocyte and somatic 5S genes have equal affinity for factor A (33), but factor C is less efficient at stabilizing binding by factor A to the oocyte 5S gene (52). Instability of factor complexes on oocyte 5S genes is thought to lead to eventual displacement by histones and formation of inactive chromatin (19, 54). Since inactive chromatin is late replicating, the oocyte 5S genes would be at a disadvantage for binding declining amounts of transcription factors during embryonic development. In this regard, it is important to note that the oocyte 5S gene was approximately 10-fold less active than the somatic 5S gene with either mature or immature fraction C or with HeLa fraction C. Thus, the property of mature oocyte factor C which prevents the activation of tMET and OAX appears to be distinct from properties which affect the relative activities of the two 5S genes. The latter property may be a general feature of factor C, common to immature and mature oocyte factor C.

Our results indicate that OAX and tMET genes, when transcriptionally active, compete for factors commonly required by the somatic 5S gene; however, when these genes are inactive, as with mature oocyte C fraction, they do not compete for factors. These findings are consistent with a report by Andrews and coworkers (2) that OAX genes microinjected into oocyte nuclei are active and compete for factors with a 5S gene. Wolffe (53) obtained somewhat different results with crude oocyte nuclear extracts; OAX and tMET were transcriptionally active yet did not compete with each other or with the somatic 5S gene. This discrepancy presumably reflects differences between crude nuclear extracts and a reconstituted system with partially purified factors B and C; crude extracts contain histones and other components which could compete for factor binding. His-

tone H1 is known to contribute to the inactivation of OAX genes in crude extracts (53).

Oocyte S150 extracts and oocyte nuclear extracts exhibit markedly different transcriptional properties (34, 39, 40, 53). Oocyte-type genes are typically expressed at higher levels in oocyte nuclear extracts than in whole-oocyte S150 extracts; for example, the oocyte 5S gene is 5- to 10-fold less active than the somatic 5S gene in oocyte nuclear extracts (53), compared with a 50- to 100-fold difference in activities in whole-oocyte S150 extracts (34, 39, 53). Similarly, OAX and tMET are expressed at high levels in oocyte nuclear extracts (53) but have low activities in oocyte S150 extracts, as shown here. The reduced activity of the oocyte 5S gene in the S150 extracts has been attributed to low levels of factor C, coupled with the relative instability of factor A/C complexes on that template (52). However, the low activity of OAX and tMET with mature oocyte fraction C is not due to low concentrations of factor C; the somatic 5S gene attained the same maximal level of activity in our reconstituted system with mature oocyte C fraction as with immature oocyte C fraction, indicating that saturating concentrations of factor C were present in each case. Moreover, varying the concentration of fraction C or B in the reconstituted system did not alter the relative activities of tMET and OAX and the somatic 5S gene (data not shown).

The observed differences between oocyte nuclear and oocyte S150 extracts may stem from the fact that the latter are largely composed of cytoplasmic components. The factors present in the nuclear and cytoplasmic compartments of oocytes may differ in activities. Such compartmentalization could explain why oocyte-type class III genes are highly expressed in oocyte nuclear extracts and in microinjected oocyte nuclei (34, 39) but are poorly expressed in oocyte S150 extracts. This might also explain the variation reported between different oocyte S150 extracts with respect to the relative activities for the somatic and oocyte 5S genes (5) or OAX and tMET (34; this study); depending on the method of S150 extract preparation, the contribution from the nuclear contents may vary.

In summary, these results provide the first evidence that the activity of factor C is altered during development in *X. laevis*. Changes in factor C or associated components result in loss of the ability to activate or bind stably to the tRNA-type genes OAX and tMET. This inability to bind stably to oocyte-type OAX or tMET is likely to play a role in the selective inactivation of these genes during development in vivo. Our future studies will assess specific changes in the transcriptional machinery as well as sequence elements within oocyte-type class III genes which might dictate their regulation by factor C during development.

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