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The tyrosine phosphatase cdc25 selectively inhibits transcription of the Xenopus oocyte-type tRNAtyrC gene

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ABSTRACT

The Xenopus tyrosine tRNAtyrC (TyrC) genes are developmentally regulated. These multicopy genes are expressed in early oocytes and inactivated as oocytes reach maturity (1). As shown here, this developmental regulation can be reproduced in vitro in extracts of early and late stage oocytes: the TyrC gene is transcribed in early oocyte extracts but is virtually inactive in mature oocyte extracts. The inability to transcribe the TyrC gene is not due to the lack of functional pol III transcriptional components, since the somatic-type TyrD gene is fully active in mature oocyte extracts. Instead, the loss of TyrC transcription appears to be due to a change in the template specificity of transcription factor TFIIIC: addition of TFIIIC from immature extracts restores TyrC transcription in mature extracts. In mixtures of immature and mature extracts, the transcriptional activity of the TyrC gene is reduced. The presence of sodium vanadate, an inhibitor of tyrosine phosphatases, increases the level of TyrC transcription in the extract mixtures. Also, cdc25 phosphatase treatment of immature extracts causes a decrease in TyrC transcription which is reversed by addition of exogenous TFIIIC. These findings indicate that changes in phosphorylation state alters the template specificity of TFIIIC leading to the selective inactivation of oocyte type TyrC genes.

INTRODUCTION

In the early stages of oogenesis in Xenopus, multicopy oocyte-type tRNA genes are expressed (1), allowing the accumulation of product RNAs for use during embryogenesis. As the oocytes approach maturity, these genes are gradually and stably inactivated. In adult somatic cells, the oocyte type tRNA genes are in a quiescent state, while a different set of somatic type tRNA genes are expressed. The mechanism underlying the developmental inactivation of oocyte type tRNA genes appears to involve a change in the template specificity of transcription factor TFIIIC (2). Factors TFIIIC and TFIIIB, along with TATA-binding protein (TBP), are required for accurate initiation by RNA polymerase III on the two major subtypes of class III genes, tRNA and 5S RNA genes (3; reviews: 4 –7). The 5S RNA genes require, in addition, factor TFIIIA (3,8). TFIIIC is a multisubunit factor which binds independently to two promoter elements internal to tRNA genes, known as the A and B boxes. Crosslinking studies in the yeast system indicate that the various subunits of TFIIIC contact DNA sequences extending from 40 bp preceding the start site to the B box region at +80–90 of a tRNA gene (9,10). TFIIIB does not independently bind DNA (11,12); protein-protein interactions with TFIIIC are necessary to direct TFIIIB to bind upstream of the initiation site (10,13).

This report compares the regulation of two Xenopus tRNAtyr genes, oocyte-type TyrC and somatic-type TyrD (14). The oocyte-type TyrC gene is a member of a tandemly repeated cluster of eight tRNA genes, present in ~150 copies at a single chromosomal locus (15). Hybridization studies, using a unique intervening sequence, indicated the TyrC genes are expressed in early, stage I oocytes; there is a marked decline in expression as the oocytes approach maturity (stages V and VI)(1). In early embryos and adult somatic cells, the TyrC genes are virtually inactive, except for a transient burst of expression at gastrula stage, which is also observed for the oocyte-type OAX gene (tRNA-subclass) (16). Hybridization studies using a distinct intron present in the somatic-type TyrD gene detected transcripts in gastrula and later stage embryos, and in adult somatic cells, but not in oocytes, perhaps due to the low copy number of this gene (1). The coding regions of these two tRNA genes differ only in a single base conversion within the B box element, by distinctive 12–13 bp introns situated between the A and B boxes, and by highly distinctive 5′ flanking sequences. In somatic cell extracts, the higher transcriptional activity of TyrD, relative to TyrC, was attributable to the upstream sequence differences (14).

The regulation of oocyte type tRNA genes can be studied in vitro with extracts of early and late stage oocytes which reproduce in vivo patterns of gene expression. In an earlier study, we showed that oocyte-type $tRNA^{met}1$ (tMET) and OAX genes are transcribed in immature, but not mature oocyte extracts (2). The inability to transcribe tMET and OAX genes is not attributable to the absence of viable pol III transcriptional components in mature oocyte extracts, since the somatic 5S RNA gene is transcribed with high efficiency. Instead, the inability to transcribe tMET and OAX genes correlates with a change in TFIIIC activity: TFIIIC from early oocytes supports transcription of tMET and OAX genes, but TFIIIC from mature oocytes fails

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to activate or form a stable complex with these oocyte type templates. That previous study did not address the question of whether mature TFIIIC specifically lacks ability to activate oocyte type tRNA genes, or simply lacks ability to activate all tRNA type genes, including somatic type.

The experiments described here demonstrate that soluble components in mature oocytes are able to differentially activate oocyte type and somatic type tRNA genes. Mature oocyte extracts fully support transcription of the somatic type TyrD gene while failing to transcribe oocyte type TyrC. The loss of TyrC transcriptional activity appears to be due to a phosphatase in mature extracts: sodium vanadate, an inhibitor of tyrosine phosphatases, increases the transcriptional activity of TyrC in mixtures of immature and mature extracts. Moreover, addition of bacterially expressed cdc25, a tyrosine phosphatase, leads to preferential loss of TyrC transcription in early oocyte extracts, which is restored by addition of exogenous TFIIIC.

METHODS

Xenopus DNAs

The plasmid pTyrC* (TyrC) contains the oocyte-type tRNAtyrC* gene and 5′ flanking sequences joined to the 3′ flanking sequences of the TyrD gene to take advantage of a stronger termination signal (14). Plasmid pTyrD contains the somatic-type tRNAtyrD gene (14). Plasmid tMET contains the $tRNA^{met}1$ gene (15).

Oocyte extract preparation

For preparation of immature oocyte extracts, ovaries were removed from intermediate sized frogs (3−4 inches), containing a mixture of oocyte stages with relatively few mature (Dumont stage VI). The ovaries were homogenized in buffer A (100 mM KCl, 20 mM HEPES (N-2-hydroxyethylpiperazine-N′-2 ethanesulfonic acid) (pH 7.8), 0.1 mM EDTA, 0.5 mM phenylmethylsulfonylfloride (PMSF), 1 mM dithiothreitol (DTT), 20% glycerol), and centrifuged for 30 minutes in an SW41 rotor at 35,000 RPM. For preparation of mature oocyte extracts, ovaries were removed from large, adult frogs and incubated in modified OR2 buffer (82 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 5 mM HEPES (pH 7.9) with collagenase (0.15%) for 1 hour or until the oocytes could be separated by agitation. The largest (stage VI) oocytes were separated from smaller oocytes by successive washes in modified OR2 buffer in the presence of 1mM PMSF. The oocytes were then centrifuged in an equal volume of buffer A for 30 minutes at 35,000 RPM in an SW41 rotor at 4°C, essentially as described by Glikin *et al.* (17). There is some variation in transcriptional properties between mature oocyte extracts, possibly due to variable contribution of nuclear and cytoplasmic compartments or variable amounts of phosphatase/kinase regulators of pol III gene expression. For example, the cdc25 protein is localized in the oocyte cytoplasm (29). The extracts chosen for use in this study were most typical and exhibited low transcriptional efficiency for the TyrC and tMET genes.

DNA affinity purification of TFIIIC

A double-stranded synthetic oligonucleotide containing the B box promoter element (underlined) and surrounding sequences (TA GGTCGCTGGTTCAATTCCGGCTC) from the TyrD gene was multimerized and coupled to CNBr-activated Sepharose (Pharmacia). Immature oocyte S150 extracts were incubated with the B box coupled Sepharose in the presence of 1 μg/ml poly (dI-dC) for 2 hours at 4°C. After washing in buffer A, the bound protein was eluted with buffer A containing 0.8M KCl, and dialyzed against buffer A. Total protein concentration was 0.5 to 1.0 mg/ml.

Isolation of phosphocellulose fractions TFIIIB and TFIIIC

Immature oocyte extracts (30 mls) were incubated with phosphocellulose (Whatman P11) (5 mls) prepared according to manufacturer's instructions in buffer A containing bovine serum albumin (BSA) (100 μg/ml). The column was washed with 5 column volumes of buffer A, and fraction TFIIIB was step-eluted with buffer A containing 0.35 M KCl, and dialyzed against buffer A, according to the method of Segall *et al.* (3). The total protein concentration was 2 mg/ml. HeLa fractions TFIIIB and TFIIIC were prepared by the method of Johnson *et al.* (18).

Production of cdc25 in bacteria

The mouse cdc25M2 protein was expressed as a glutathione-S-transferase fusion protein (GST-cdc25M2) from the construct pGEX-K288 (19)(kindly provided by B. Sebastian and T. Hunter) in *E.coli* strain BL21. Bacteria were grown in 1 liter of L broth at 37°C to mid-log phase and treated with 0.4 mM IPTG for 2 hours at 25°C. The cells were harvested, washed in TD (150mM NaCl, 1 mM DTT, 10 mM Tris−HCl, pH 7.5), resuspended in TD with 1% Triton X-100 and sonicated for 30 seconds on ice. The extract was centrifuged at 10,000 RPM for 15 minutes, and the supernatant was collected and incubated with 1 ml of glutathione-coupled agarose (Sigma) for 2−3 hours at 4°C. The protein bound beads were collected by centrifugation for 60 seconds at 3000 RPM and washed extensively in 500 mM NaCl, 10 mM DTT, 10 mM Tris−HCl (ph 8.0), followed by TD. The GST-cdc25M2 protein was eluted from the beads with 10 mM glutathione, dialyzed against TD, and added to oocyte extracts at a concentration of 10 μg/ml. In some experiments, the GST-cdc25M2-bound beads were added directly to oocyte extracts, using amounts of beads equivalent to 10 μg/ml eluted protein. Protein concentration was determined by SDS gel electrophoresis and the Bio-Rad protein assay kit. To assay phosphatase activity, dephosphorylation of *p*-NPP was measured according to the protocol described by Kakizuka *et al.* (19).

Bacterially expressed Drosophila cdc25 protein ($p61^{cdc25}$) and the corresponding pET expression construct were kindly provided by B. Dunphy and A. Kumagai (Caltech). Expression and isolation of the Drosophila cdc25 protein was performed as previously described (20). Protein concentration and phosphatase activity was determined as described above. The cdc25 protein was ~90% pure and was added to oocyte extracts at a concentration of 10 μg/ml.

Transcription reactions

Transcription assays were performed for 2 hr at 22°C in a reaction volume of 25 μl containing 20 μl oocyte extract, 5mM $MgCl_2$, 1 mM DTT, 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 template plasmid DNA. 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 minutes and loaded onto 10% polyacrylamide gels

containing 1× TBE and 8M urea. Following electrophoresis, the gel was dried and exposed to X-ray film. Typical exposure times were between 1 and 4 hours. The relative levels of expression was quantitated by radioanalytic scanner (AMBIS company, San Diego).

RESULTS

Somatic type TyrD is transcribed in mature oocyte extracts, while oocyte type TyrC is inactive

In vitro transcription assays were carried out in S150 extracts of immature ovaries or mature oocytes. The immature ovaries contained mixed populations of oocytes with relatively few mature stage VI oocytes. The mature oocyte extracts were prepared from stage VI oocytes, obtained by collagenase treatment of adult ovaries. The templates included oocyte-type tRNA genes, TyrC and tMET, and somatic-type TyrD. The tMET gene is presumed to be oocyte-type since it is present within the same repeated, eight gene family as oocyte-type TyrC (21), and it exists in a transcriptionally inactive state in chromatin isolated from late stage embryos (22). As shown in figure 1, and consistent with earlier findings (2), the oocyte-type and somatic-type genes were transcribed with equal efficiency in immature extracts; however, in mature extracts, the oocyte-type TyrC and tMET genes were ten fold less active than somatic-type TyrD. This result is consistent with earlier findings that TyrD is six fold more active than TyrC in Xenopus somatic cell extracts (14), suggesting that the transcriptional properties of mature oocytes and somatic cells are similar.

The TFIIIC activity in oocyte extracts is altered through the action of a phosphatase

Mixtures of immature and mature oocyte extracts have transcriptional properties resembling mature extracts; the transcriptional activity of TyrC is 5−10 fold below that of TyrD (Fig. 2A, lanes 1 and 2). The TyrC signal decreased further if the mixed extracts were allowed to preincubate prior to template addition (Fig.2A, lanes 3 and 4), indicating a time-dependent change in the transcriptional components. One possible mechanism for such change in transcriptional properties is protein phosphorylation (23−25). Our earlier findings have indicated that a change in properties of factor TFIIIC is responsible for the loss of oocyte type tRNA gene expression in mature oocyte

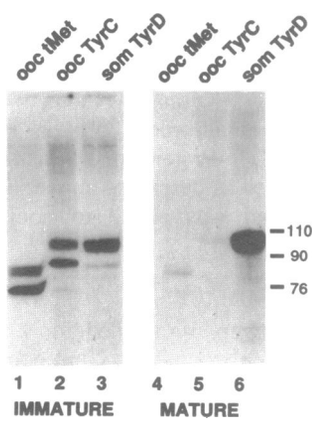

Figure 1. Differential transcription of oocyte-type and somatic-type tRNA genes in mature oocyte extracts. Transcription reactions contained 20 μl of immature (lanes 1−3) or mature (lanes 4−6) oocyte extracts, and 100 ng of plasmid DNAs containing oocyte-type tMET, TyrC, or somatic-type TyrD genes, as indicated. The two transcript sizes for tMET represent precursor and processed RNAs. The upper transcripts for TyrC and TyrD contain 12−13 base intervening sequences, while the lower transcripts are processing intermediates. DNA size markers are indicated at right.

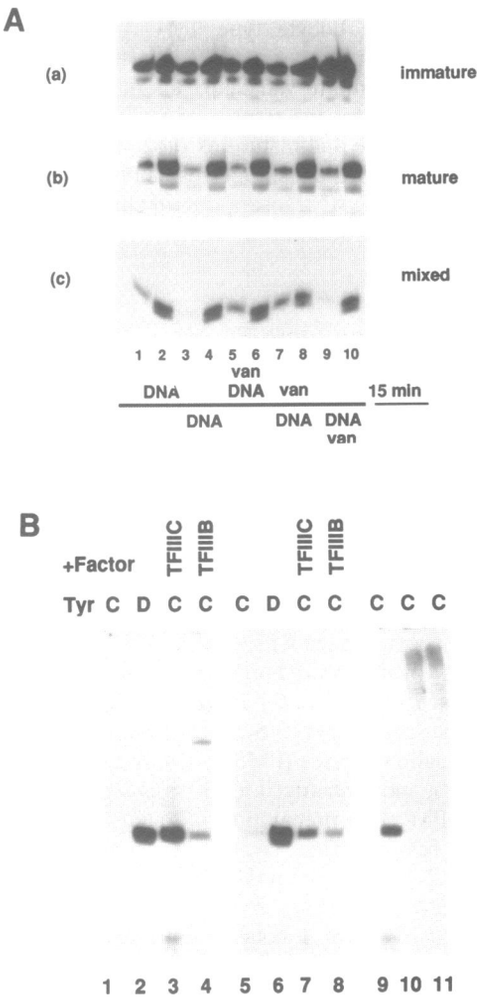

Figure 2. A tyrosine phosphatase leads to a selective decrease in TyrC transcription in mixtures of immature and mature oocyte extracts. **A.** Transcription reactions contained 20 μl each of immature oocyte extracts (panel a), mature oocyte extracts (panel b), or a mixture of 10 μl of each extract (panel c). The template DNAs were TyrC (odd numbered lanes) and TyrD (even lanes). For all three panels, in lanes 1 and 2, the template DNAs were added to the extracts at the start of the two hour incubation period. In lanes 3 and 4, the extracts were preincubated for 15 minutes prior to addition of template DNAs. In lanes 5 and 6, 2mM sodium vanadate and template DNAs were added at the start of the reaction. In lanes 7 and 8, vanadate was present during the 15 minute preincubation, followed by template DNAs. In lanes 9 and 10, the extracts were preincubated for 15 minutes, followed by addition of vanadate and template DNAs. **B.** Transcription reactions in lanes 1−4 contained mature oocyte extracts (20 μl), and 100 ng of TyrC or TyrD plasmid DNAs as indicated above each lane. In lane 3, DNA affinity purified TFIIIC (5 μl) and 2 mM vanadate were included in the reaction. In lane 4, phosphocellulose fraction TFIIIB (5 μl) and 2 mM vanadate were included. The reactions in lanes 5−8 were the same as in lanes 1−4 except that 2 mM vanadate was omitted from reactions. The reactions in lanes 9 −11 contained TyrC DNA, along with affinity purified TFIIIC and phosphocellulose fraction TFIIIB (lane 9), only TFIIIC (lane 10), or only fraction TFIIIB (lane 11). Oocyte extracts were not added to these three reactions.

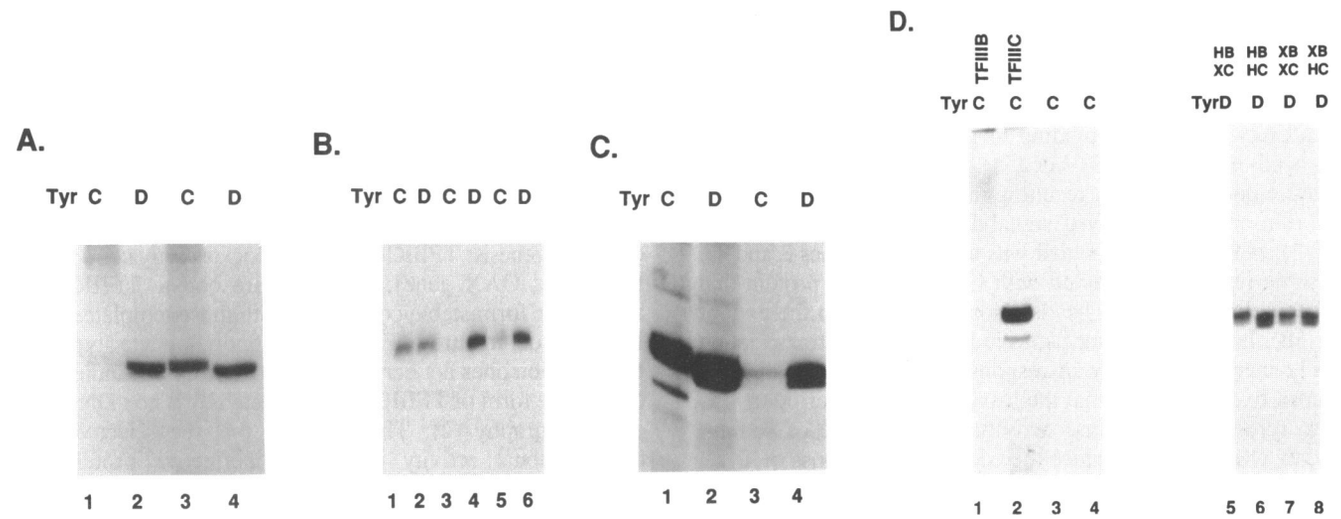

Figure 3. The tyrosine phosphatase cdc25 preferentially inhibits TyrC transcription. **A.** Transcription reactions in lanes 1 and 2 contained 20 μl of immature oocyte extract that had been preincubated for 60 min with the fusion protein GST-cdc25M2 bound to glutathione-agarose beads (equivalent to 0.25 μg cdc25 protein). Reactions in lanes 3 and 4 contained nontreated extracts. Plasmid DNAs (100 ng) were TyrC (lanes 1 and 3) or TyrD (lanes 2 and 4).**B.** Reactions contained 20 μl of untreated immature oocyte extract (lanes 1 and 2), extract that had been preincubated with 0.25 μg of GST-cdc25M2 protein that had been eluted from glutathione-agarose (lanes 3 and 4), or extract that had been preincubated with GST-cdc25M2 in the presence of 2 mM sodium vanadate (lanes 5 and 6). Reactions contained 100 ng of TyrC (lanes 1,3,5) or TyrD (lanes 2,4,6) plasmid DNAs. **C.** Transcription reactions contained 20 μl of immature oocyte extract and 100 ng of either TyrC (lanes 1 and 3) or TyrD (lanes 2 and 4) plasmid DNA. Reactions in lanes 3 and 4 also contained 0.25 μg of Drosophila cdc25 protein. **D.** HeLa TFIIIC restores TyrC transcription in cdc25M2 treated extracts. Transcription reactions contained 100 ng of TyrC plasmid DNA and 20 μl of immature oocyte extract that had been treated with GST-cdc25M2 (same treated extract as in panel A, lanes 1 and 2). Reactions also contained 5 μl of Hela phosphocellulose fraction TFIIIB (lane 1), 5 μl of HeLa fraction TFIIIC (lane 2), or no additives (lane 3). The control reaction in lane 4 contained Hela fraction TFIIIC with 20 μl of buffer A (no oocyte S150 extract). Reactions in the second panel (lanes 5–8) did not contain oocyte S150 extracts. These contained 100 ng of TyrD plasmid DNA, and 20 ml each of HeLa TFIIIB and Xenopus affinity purified TFIIIC (lane 5), HeLa TFIIIB and TFIIIC (lane 6), Xenopus TFIIIB and TFIIIC (lane 7), and Xenopus TFIIIB and HeLa TFIIIC (lane 8).

extracts (2). To investigate the possibility that a change in the phosphorylation state of Xenopus TFIIIC, or other transcriptional components, is responsible for the observed decrease in TyrC transcription, the effects of phosphatase inhibitors were examined. Sodium vanadate, an inhibitor of tyrosine phosphatases, was found to increase TyrC transcription in mixtures of immature and mature extracts if added at the start of incubation(Fig.2A, lanes 5–8). but not if added after a 15 minute preincubation (lanes 9 and 10). Interestingly, the addition of vanadate to the separate mature or immature extracts had no significant effect on transcription of TyrC or TyrD (panels a and b, lanes 5–10). Similarly, preincubation of the separate extracts had no significant effect on transcription levels (lanes 3 and 4). These results are consistent with a model whereby a tyrosine phosphatase, present only in mature oocytes, alters a transcription factor(s) which is active for TyrC only in immature oocytes. The inability of vanadate to rescue TyrC transcription in mature oocyte extracts suggests the relevant transcription factor has already been modified by the phosphatase. The inability of vanadate to alter transcription patterns in immature oocyte extracts suggests the phosphatase is not present at early stages of oogenesis.

To determine which transcriptional component was affected by the vanadate sensitive phosphatase, factors TFIIIB and TFIIIC were partially purified and added back to mature extracts in the presence or absence of vanadate. In the presence of 2 mM sodium vanadate, DNA affinity purified TFIIIC from early oocytes restored TyrC transcription to the same level as somatic-type TyrD, an increase of 15 fold (Fig. 2B, lanes 1–3). In the absence of vanadate, the addition of TFIIIC resulted in significantly less rescue of TyrC transcription (lanes 5–7). Addition of phosphocellulose fraction TFIIIB, containing factor TFIIIB, TBP, and pol III (3,7), had relatively little effect on the level of TyrC transcription, either in the presence or absence of 2mM vanadate (lanes 1 and 4, or 5 and 8). These findings suggest that in mature extracts, an endogenous vanadate-sensitive tyrosine phosphatase causes a change in TFIIIC, resulting in selective inability to support TyrC transcription.

The tyrosine phosphatase cdc25 selectively inhibits TyrC transcription in oocyte extracts

The cdc25 protein is a vanadate-sensitive tyrosine phosphatase which is present in oocytes and increases markedly in activity during the final stages of progesterone-induced maturation (29,30). The primary target of cdc25 is cdc2 kinase which, in association with cyclin B, comprises maturation (or mitosis) promoting factor (MPF)(31,32). The correlation of cdc25 hyper-activation with the final stages of oocyte maturation suggested this phosphatase might be responsible for the vanadate-sensitive loss of TyrC transcription. Moreover, its substrate, cdc2 kinase, as part of MPF, is known to completely repress pol III transcription when added to interphase-arrested egg extracts (33) and presumably is the cause of the transcriptional arrest seen in vivo following oocyte maturation into eggs (34). If the oocyte-specific tRNA genes, such as TyrC, are more highly susceptible to a cdc25-triggered change in transcriptional components, this could explain the early, selective inactivation of this subset of genes prior to the final stages of oocyte maturation. To test this idea, mouse and Drosophila cdc25 proteins were expressed in bacteria, and added to oocyte extracts. Other studies have demonstrated that human and Drosophila cdc25 proteins are active in Xenopus extracts, in that these are able to dephosphorylate Xenopus cdc2 kinase and the related cdk2

kinase, indicative of a high degree of cross-species, functional conservation (20,35). In the experiment shown in figure 3A, mouse cdc25 phosphatase (cdc25M2)(19) was expressed in bacteria as a glutathione-S-transferase (GST) fusion protein and then affinity purified by binding to glutathione-agarose beads. The bead-associated GST-cdc25M2 protein was added to immature oocyte extracts, resulting in the selective inhibition of TyrC transcription; the TyrC signal decreased seven fold (lanes 1 and 3), while the TyrD signal was unchanged (lanes 2 and 4). The same result was obtained with GST-cdc25M2 protein that had been eluted from the beads prior to addition to the extract (Fig. 3B). In some experiments, as in fig. 3B, TyrD transcription actually increased slightly in response to cdc25 treatment. As a control to demonstrate that the loss of TyrC transcription was due to tyrosine phosphatase activity, the presence of sodium vanadate was shown to reduce the cdc25-mediated loss of TyrC signal (compare lanes 3 and 5). As a further control, the GST protein alone was expressed in bacteria, using the pGEX-2X vector, and affinity purified; when added to oocyte extracts, the GST protein had no effect on TyrC transcription (data not shown).

Analogous results were obtained using bacterially-expressed Drosophila cdc25 protein (Fig.3C). Full length Drosophila cdc25 was expressed as a nonfusion protein and isolated from the insoluble inclusion body fraction as previously described (20). Addition of Drosophila cdc25 protein to immature oocyte extracts resulted in a dramatic, ten fold decrease in TyrC transcription with a relatively small, two fold decrease in TyrD transcription. Since it is not possible to affinity purify the nonfusion cdc25 protein, the two fold reduction in TyrD transcription could be due to nonspecific inhibitors; the inclusion body fraction isolated from control bacteria, lacking the cdc25 expression construct, similarly inhibited transcription by two fold, but had no selective effect on the TyrC signal (not shown).

Factor TFIIIC restores TyrC transcription in cdc25 treated oocyte extracts

To determine which transcriptional component is altered in cdc25-treated extracts, TFIIIB and TFIIIC activities were partially purified from early oocyte extracts and added back to the treated extracts. Affinity purified TFIIIC increased TyrC transcription by two to three fold, while addition of phosphocellulose fraction TFIIIB had no effect (data not shown). The effect of adding HeLa TFIIIC was also tested since this heterologous factor was previously shown to support transcription of Xenopus oocyte type tMET (2). HeLa TFIIIC is compatible with Xenopus TFIIIB in supporting transcription of tRNA templates (Fig.3D, lane 8), as is HeLa TFIIIB and Xenopus TFIIIC (lane 5). Addition of HeLa TFIIIC to cdc25-treated extracts resulted in a dramatic restoration of TyrC transcription, while addition of HeLa fraction TFIIIB had no effect (lanes 1−2). These findings indicate that TFIIIC is the transcriptional component altered in cdc25-treated extracts.

DISCUSSION

These findings suggest a tyrosine phosphatase is part of the mechanism leading to the selective inactivation of the oocyte type TyrC gene. The data further suggest that the change in phosphorylation state leads to altered TFIIIC activity. Extracts of mature stage VI oocytes were shown to support transcription of the somatic type TyrD gene but not the oocyte type TyrC gene. In mixtures of early and late stage extracts, sodium vanadate, an inhibitor of tyrosine phosphatases, preserves TyrC transcription. Addition of the cdc25 phosphatase to early oocyte extracts results in the selective loss of TyrC transcription. Addback experiments suggest that a change in TFIIIC properties underlies the loss of TyrC transcription in mature oocyte extracts and in cdc25 treated extracts; TFIIIC from early oocytes restores TyrC transcription in mature oocyte extracts, and HeLa TFIIIC restores TyrC transcription in cdc25 treated extracts. These results are consistent with earlier findings that TFIIIC activity changes during oogenesis; TFIIIC from early oocytes activates oocyte type tMET and OAX genes, while mature oocyte TFIIIC fails to activate or form stable complexes with those templates (2). The inability of mature TFIIIC to support oocyte type tRNA transcription does not derive from a loss of DNA binding ability; the mature form of TFIIIC can be isolated by B box DNA affinity chromatography (2). This suggests that the selective loss of transcriptional activity is due to ineffective protein-protein interactions with other components, presumably TFIIIB-TBP, on oocyte type templates.

Most eukaryotic transcription factors which have been examined have been found to be phosphorylated (review, 23). While in most cases the physiological effects are unknown, there are many cases in which phosphorylation has been shown to either positively or negatively regulate transcription factor activity. One example is c-Jun; phosphorylation at one set of sites within c-Jun increases transactivation activity (24), whereas phosphorylation at another set of sites negatively regulates DNA binding (25). In another example with relevance to this study, phosphorylation of c-Myb results in differential binding to different subsets of Myb responsive genes (26). In some cases, such as serum response factor (SRF), phosphorylation results in a conformation change (27). Such allosteric effects could interfere with protein-protein contacts between transcription factors, such as between TFIIIC and TFIIIB. In support of this model: phosphorylation of HeLa TFIIIC was shown to alter the level of factor activity, possibly by affecting the interaction between TFIIIB and TFIIIC (28).

A vanadate sensitive phosphatase in mature extracts could act directly to dephosphorylate TFIIIC, or indirectly through another effector. Specifically, since cdc25 phosphatase activates at least two kinases (cdc2 and cdk2)(29,36), it is possible that modification of TFIIIC is through these kinases. It is the hyperphosphorylated form of cdc25 which activates cdc2 kinase in vivo, leading to meiosis and a generalized shutdown of gene expression. The cdc25 protein present in stage VI oocytes is not hyperphosphorylated and has basal phosphatase activity, as does the bacterially-expressed form of cdc25. Since bacterially expressed cdc25M2 is sufficient to selectively inhibit oocyte type TyrC transcription in oocyte extracts, the basal levels of cdc25 activity in maturing oocytes may similarly act to selectively inhibit TyrC gene expression in vivo. These oocyte type tRNA genes may be more sensitive than somatic type tRNA genes to the negative effects of the cdc25-induced regulatory pathway.

Sequence differences preceding the TyrC and TyrD genes are responsible for differential expression in somatic cell extracts (14) as well as in mature oocyte extracts (manuscript in preparation). Studies in the yeast system indicate the upstream sequences include binding sites for TFIIIB-TBP as well as TFIIIC (10,13). Crosslinking studies revealed contacts between the 135K subunit of yeast TFIIIC and DNA sequences within a 40 bp region preceding a tRNA gene (10). These upstream sequences also contact the two subunits of yeast TFIIIB, and to a lesser extent, TBP. The interaction between the 135K subunit of TFIIIC and

TFIIIB is thought to convert the latter into a DNA binding factor, possibly through a conformational change (13). Curiously, there are no obvious DNA sequence requirements for binding to the upstream region, although several studies have shown that sequences preceding certain tRNA and 5S RNA genes are responsible for tissue-specific gene expression (14,37,38,39); the TyrC and TyrD genes are one example. The upstream sequences presumably affect factor binding only in particular circumstances, as in the presence of the mature form of TFIIIC. Modifications to TFIIIC could interfere with protein contacts with TFIIIB, such that TFIIIB does not assume DNA binding capability. The particular sequences preceding the somatic type TyrD gene may provide an advantageous binding site, thereby counteracting mature TFIIIC, allowing formation of the TFIIIC-TFIIIB-TBP complex.

Highly purified HeLa TFIIIC is composed of five subunits ranging in approximate size from 60K to 230K (40,41). This factor has been isolated in active and inactive forms which differ in the apparent size of one subunit, possibly due to phosphorylation state (41). The DNA binding properties of the two forms are equivalent, suggesting the inactive form is deficient in protein-protein interactions with other transcriptional components. These active and inactive forms of HeLa TFIIIC appear to be functionally distinct from the immature and mature forms of Xenopus TFIIIC: mature oocyte TFIIIC fails to activate the oocyte type TyrC gene but retains full activity for somatic type TyrD or the somatic 5S gene (2). Thus, the mature form of Xenopus TFIIIC is selectively inactive for a subset of tRNA genes. The dual forms of Xenopus TFIIIC and HeLa TFIIIC are presumably the products of distinct modification pathways. Eventual elucidation of the biochemical distinctions between the mature and immature versions of Xenopus TFIIIC will require biochemical purification of the factor or isolation of the subunit cDNAs.

There are likely to be key differences as well as similarities between the mechanisms of inactivation for the oocyte-specific tRNA genes, such as TyrC, and the oocyte-type 5S RNA gene. These two subclasses differ in promoter structure as well as factor requirements. The factor TFIIIA is specifically required for 5S gene expression (3,8). It is thought that the reduced transcriptional activity of the oocyte 5S gene in mature oocyte and egg extracts is due to a less stable interaction between TFIIIA and TFIIIC (42), or to a slower rate of complex formation (43). TFIIIA is not part of the transcription complex on tRNA-type templates, suggesting the loss of TyrC gene expression is due to altered TFIIIC binding or ineffectual interactions between TFIIIC and TFIIIB-TBP.

In summary, these findings point to changes in the phosphorylation state of transcriptional components, or regulators thereof, as being instrumental in the selective loss of oocyte type TyrC transcription. A suggested hypothesis is that cdc25 phosphatase leads to a change in TFIIIC activity, resulting in a nonproductive interaction with factor TFIIIB-TBP on the sequences preceding the TyrC gene.

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