**Xenopus Cdc6 confers sperm binding competence to oocytes without inducing their maturation**

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**ABSTRACT** Amphibian eggs normally require meiotic maturation to be competent for fertilization. A necessary prerequisite for this event is sperm binding, and we show that under normal physiological conditions this property is acquired at, but not before, meiotic maturation. Immature oocytes do not bind sperm, but injection of total egg poly(A)* mRNA into immature oocytes confers sperm binding in the absence of meiotic maturation. Using an expression cloning approach we have isolated a single cDNA from egg poly(A)* mRNA that can induce sperm binding in immature oocytes. The cDNA was found to encode Xenopus Cdc6, a protein that previously has been shown to function in meiotic maturation step in inducing sperm binding, providing that the mRNA obviates the necessity of the progesterone-induced maturation step in inducing sperm binding, providing that the oocytes have passed through the oviduct. Based on this finding, we developed an expression cloning approach and identified a mRNA that can induce sperm binding to oocytes while not inducing their complete maturation.

The transformation of the CE to the sperm-receptive vitelline envelope (VE) of the oviposited egg occurs when the coelomic egg passes through the oviduct. This process involves ultrastructural changes in the envelope and processing of a major 43-kDa glycoprotein (gp43) in the CE to 41 kDa by a protease secreted from the oviduct (8, 16, 17). After several hours the coelomic egg passes through the oviduct and emergses as an oviposited egg. Subsequent fertilization releases the egg from its metaphase arrest and allows it to complete meiosis II and enter mitosis.

The detailed mechanism of fertilization in *Xenopus* remains to be elucidated (reviewed in refs. 18 and 19). As a first step in identifying at the molecular level the components involved in this process, we have identified a pair of related glycoproteins in the egg vitelline envelope, gp69/64, that play a key role in sperm binding (20). We also have found that although gp69/64 is present in the envelopes of oocytes and coelomic eggs, it is not accessible to polyclonal anti-gp69/64 antibodies or to sperm. Therefore, sperm do not bind to oocytes or coelomic eggs. Instead, sperm only bind to oviposited eggs, in which gp69/64 is exposed on the surface (21). Evidence that a disintegrin on *Xenopus* sperm interacts with the plasma membrane of the egg has been reported very recently (22).

In the present study, we found that without meiotic maturation passage through the oviduct is not sufficient to induce immature oocytes to bind sperm, suggesting that the earlier surface changes that take place during meiotic maturation are a prerequisite to sperm binding. Assuming that some of these changes are caused by new protein synthesis, we sought to identify these factors that conferred sperm binding ability to the oocyte. We found that microinjection of total egg poly(A)* mRNA obviates the necessity of the progesterone-induced maturation step in inducing sperm binding, providing that the oocytes have passed through the oviduct. Based on this finding, we developed an expression cloning approach and identified a mRNA that can induce sperm binding to oocytes while not inducing their complete maturation.

**MATERIALS AND METHODS**

**Oocyte Manipulations.** *Xenopus laevis* was purchased from Nasco, and segments of ovaries were surgically removed from unstimulated females (females not injected with human chorionic gonadotropin for at least 4–6 weeks) under hypothermic anesthesia. Oocytes were isolated and defolliculated by a brief (0.5-hr) treatment of the ovary segments with 0.5% collagenase (type I, Sigma) in OR2 medium (23), followed by manual removal of the follicular layer with forceps. Isolated oocytes were transferred to a fresh Petri dish and washed with fresh OR2 medium on a shaker for 3 × 1 hr to remove the remaining follicle cells. Isolated oocytes were incubated in OR2 medium supplemented with 0.1 mM spermine and 200 μM calcium. Immature oocytes do not bind sperm, but injection of total egg poly(A)* mRNA into immature oocytes confers sperm binding in the absence of meiotic maturation. Using an expression cloning approach we have isolated a single cDNA from egg poly(A)* mRNA that can induce sperm binding in immature oocytes. The cDNA was found to encode Xenopus Cdc6, a protein that previously has been shown to function in meiotic maturation step in inducing sperm binding, providing that the mRNA obviates the necessity of the progesterone-induced maturation step in inducing sperm binding, providing that the oocytes have passed through the oviduct. Based on this finding, we developed an expression cloning approach and identified a mRNA that can induce sperm binding to oocytes while not inducing their complete maturation.

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mg/ml of gentamycin (GIBCO) at 18°C overnight before being used in sperm binding assay. Oocytes after these steps were free of follicle cells. To induce maturation, oocytes were exposed to progesterone (Sigma) at 1 μg/ml for ~6 hr until GVBD had occurred.

**Sperm Binding Assay and in Vitro Fertilization Assay.** Sperm-oocyte binding assays and in vitro fertilization assays were performed as previously described (20).

**Polyclonal Antibodies.** Rabbit antisera were raised against purified gp69 and gp64. Antibodies were partially purified by ammonium sulfate precipitation and DEAE-matrix chromatography. This antibody was named anti-VE antibody because it reacts, in addition to gp69/64, with gp120/112, gp41, and weakly with gp37. After preadsorption with gp120/112, gp41, and gp37 that had been covalently linked to Affigel-15 (Bio-Rad), the resulting antibody, named anti-gp69/64 antibody, specifically recognizes only gp69 and gp64 glycoproteins in both their denatured form and their native form in the VE (20).

**Western Blot and Immunofluorescent Staining.** Immunological procedures were performed as previously described (21).

**Egg cDNA Library Construction and mRNA Transcription.** Total RNA was isolated from unfertilized, oviposited eggs as described by Evans and Kay (24). Poly(A)+ mRNA was purified from total RNA using Dynabeads and reagents from Dynal (Oslo, Norway). An unidirectional egg cDNA library was constructed starting from 5 μg of egg poly(A)+ mRNA using a ZAP express cDNA synthesis and cloning kit (Stratagene). The primary lambda phage library was amplified once, and an aliquot of the library was transferred into a phagemid library by mass in vivo excision. Plasmid DNAs purified from pools of clones from the phagemid library were linearized by NotI restriction digestion and used as templates for mRNA synthesis using T7 promoter. Capped mRNAs were synthesized by using an Ambion (Austin, TX) in vitro transcription kit.

**mRNA Microinjection and Expression Cloning.** Oocytes were microinjected at the equatorial zone with 10–40 ng of mRNA (40 nl) dissolved in RNase-free ddH2O and incubated in oocyte culture medium (14) at 18°C for ~36 hr. Cultured oocytes were directly prepared for host transfer (14) without progesterone maturation. To distinguish groups of oocytes injected with different pools of mRNA from each other and from host eggs as many as four groups (~40 oocytes per group) were stained with vital dyes of different colors for 15 min before being transferred aseptically into the coelomic cavity of a host female frog that had started to lay her own eggs. Approximately 3 hr after the transfer, the host frog was gently squeezed to help release of its eggs and the injected oocytes. After removing jelly with 45 mM 2-mercaptoethanol, the oocytes and eggs were used for sperm binding assays.

The expression cloning procedure involved injecting mRNA synthesized from pools of egg cDNA clones into oocytes and then subdividing the pools that positively promoted sperm binding to injected oocytes. This process was repeated until a single clone was isolated. To clone a full-length cDNA, a ~230-bp fragment from the original cDNA was amplified by PCR, radiolabeled, and used to screen the egg cDNA library by colony hybridization. A full-length cDNA clone was selected and sequenced from both directions.

**Northern Blot Analysis.** Total RNA (10 μg/lane) was resolved by electrophoresis on a 1% agarose formaldehyde gel. To remove the poly(A) tail, the egg total RNA was hybridized with oligo(dt) and treated with RNase H (25). After electrophoresis, the gel was treated with 50 mM NaOH/10 mM NaCl for 40 min, neutralized in 0.2 M Tris-HCl (pH 7.8) and 18% standard saline citrate (SSC) for 2 × 20 min, blotted onto a Nylon membrane (Hybond, Amersham), and UV-crosslinked. The membrane was prehybridized at 60°C for 6 hr in a buffer containing 1 M EDTA, 0.5 M Na2HPO4 (pH 7.2), 7% SDS, and 1% BSA, and hybridized under the same condition for 24 hr with a radiolabeled, PCR-amplified ~1-kb cDNA fragment. The blot was washed for 3 × 15 min in 2× SSC, 0.2% SDS, followed by 2 × 15 min in 0.2× SSC, 0.2% SDS, and exposed to film for 24 hr.

**RESULTS**

**Xenopus Oocytes Acquire Sperm Binding Activity After Meiotic Maturation and Oviduct Passage.** Using a quantitative sperm binding assay, we previously showed that sperm do not bind to isolated stage 6 oocytes, nor to coelomic eggs. Rather, sperm bind only to dejellied, oviposited eggs (21). In the current study, we found that when isolated stage 6 oocytes were reintroduced into the coelomic cavity of a host female and allowed to pass through the oviduct without preexposure to progesterone, the recovered oocytes, after removal of their jelly, did not bind sperm (Table 1). This observation suggested that without meiotic maturation passaged through the oviduct is not sufficient to induce sperm binding. The results summarized in Table 1 indicate that for the stage 6 oocytes to acquire sperm binding activity, two sequential events are required: (a) maturation of oocytes by exposure to progesterone; and (b) passage of the mature oocytes through oviduct. If either step is omitted, the oocytes do not bind sperm.

As shown by electron microscopic studies, meiotic maturation causes major structural alterations in the oocyte envelope and the plasma membrane (7, 8). We asked if these alterations could contribute to the acquisition of sperm binding capacity during oocyte maturation. Because the proteolytic processing of gp43 in the oviduct is essential for unmasking the putative sperm binding sites in coelomic eggs (8, 16, 17, 21), initially we determined if this processing can occur in immature oocytes. By analyzing the envelope proteins isolated from the mature stage 6 oocytes that have been forced to pass through the oviduct, we found that gp43 was cleaved to gp41 (Fig. 1A), a process that normally occurs with coelomic eggs. This result indicates that the proteolytic processing step does not depend on the maturation status of the oocytes. If this is the case, it is possible that the putative sperm-binding site itself on immature oocytes is different from that on mature eggs. To test this hypothesis, we used the polyclonal anti-gp69/64 antibody as a probe to determine if there was a difference in the extent of exposure of gp69/64 in the envelope of mature and immature oocytes after they have passed through the oviduct. Oocyte surface staining experiments with polyclonal anti-gp69/64 (antibody and fluorescein isothiocyanate-conjugated secondary antibody (Fig. 1B) revealed that after passing through the oviduct and removal of their jelly coats, the progesterone-matured oocytes (Fig. 1Bc) exhibited much brighter staining than the immature oocytes (Fig. 1Bb). The immature oocytes, which did not show any staining before passing through the oviduct (Fig. 1Ba), exhibited a very low, but detectable, amount of staining after oviduct passage (Fig. 1Bb). Probably this is due to the cleavage of gp43 and ultrastructural alterations that occur to the envelope during oviduct passage. It is noteworthy that the antibody cannot penetrate the intact envelope (Fig. 1Bd); it can only recognize and bind the determinants on gp69/64 that are exposed on the egg surface. From these experiments we conclude that,

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Stage 6 oocytes that had not been exposed to progesterone or had not passed through the oviduct did not bind sperm (rows 1–3); only after progesterone-induced meiotic maturation followed by passage through the oviduct was sperm-binding observed (row 4). Standard deviations are indicated with n = 20 oocytes. See Materials and Methods for details on oocyte manipulations.
To test this hypothesis, we asked if injection of total egg poly(A) mRNA is likely that this process would require synthesis of new proteins.

Having determined that meiotic maturation results in changes at the oocyte surface that affect the accessibility of the sperm binding sites, we assumed that it was crucial to determine whether the injection of total egg poly(A) mRNA into oocytes that had been injected with total egg poly(A) mRNA, incubated in oocyte culture medium for 36 hr, host transferred without exposure to progesterone, and then tested for sperm binding.

**Functional Cloning and Characterization of a Xenopus Cdc6 Gene.** To identify protein(s) that can promote sperm binding to oocytes, a cDNA library was prepared from total poly(A) mRNA isolated from unfertilized eggs. A mixture of capped mRNAs was synthesized from a pool of 10,000 cDNA clones, and 40 ng of this mRNA mixture was injected into the oocytes. After in vitro culture and host transfer, the injected oocytes showed an approximately 2.5-fold increase in sperm binding activity compared with control oocytes. With the objective of isolating a single clone(s) that could promote sperm binding, the initial 10,000 clones were subdivided into five pools. Capped mRNAs were made from the five subpools, and each pool was tested for its ability to induce sperm binding to injected oocytes. It has been documented that injection of many different mRNAs or proteins into stage VI oocyte can induce oocyte maturation, although some of the molecules may not be actually used in the endogenous pathway of progesterone-stimulated maturation (26, 27). To avoid recloning known inducers of oocyte maturation, we used a relatively long oocyte culture time (36 hr) after injection. A small fraction (<10%) of the mRNA-injected oocytes became mature (as evidenced by the appearance of a white spot in the animal pole) and died during this incubation period. This long incubation step presumably helped to eliminate the mRNA molecules that caused oocyte maturation. Of the five subpools, two were positive in the sperm binding assay, and the pool that induced the highest level of sperm binding was further divided into smaller pools of clones and screened as described above. After a total of 6–7 rounds of subdivision and screening, a single clone was isolated that could induce sperm binding to oocytes injected with its mRNA. Because only the pool that exhibited the highest activity in inducing sperm binding was chosen for further screening, this potentially could result in loss of some other candidates. A ~230-bp fragment of this cDNA was amplified by PCR, radiolabeled, and used to screen the same egg cDNA phagmid library by colony hybridization. Under conditions of high stringency, five clones of various sizes were positive in duplicate lifts and in a secondary screen. The largest clone had an insert of ~2.4 kb in length. Capped poly(A) mRNA was synthesized from the 2.4-kb insert, and various amounts of this mRNA were injected into groups of stage 6 oocytes. A dose–response relationship was observed between the quantity of the injected mRNA and the level of sperm binding (Fig. 3). As in the case of injection of total poly(A) mRNA, it was observed that oocytes injected with this mRNA could be cultured in oocyte culture medium for 3–4 days without maturation or deterioration.

The nucleotide sequence of this cDNA (GenBank accession no. U76834) was obtained by sequencing in both directions and
the largest predicted ORF contained 554-aa residues (Fig. 4). The deduced ORF was believed to be full length because stop codons were found in all three different reading frames immediately flanking the start and the stop codons that defined the ORF. The Mr of the bacterially expressed Xcdc6 protein was approximately 61–62 kDa as determined by SDS-PAGE (data not shown), which is consistent with the size of the deduced ORF. A BLASTP search of the GenBank indicated that the ORF was identical to that recently reported by Coleman et al. (28), and it encoded the 61-kDa Xenopus Cdc6 protein. As shown in Fig. 4, in addition to a ATP/GTP binding site and two nuclear localization signals, Xcdc6 protein has a putative leucine zipper motif with the sequence LPNRMVIGIALALDTRIL (29) and five consensus phosphorylation sites (S/T-P-X-Z, where X is any amino acid and Z is a basic amino acid) for the p34cdc2/cyclin B protein kinase (30). It also has a potential N-linked glycosylation site. The leucine zipper motif and the first three p34cdc2 kinase phosphorylation sites were found to be conserved in the human Cdc6 protein (31).

The cloned Xcdc6 cDNA includes a 5'-untranslated region (UTR) of 125 nucleotides and a 3'-UTR of 532 nucleotides that have not been reported previously. A long poly(A) tail was found attached to the 3'-UTR of this clone. As shown in Fig. 4, multiple copies of two cis-acting sequences that regulate polyadenylation of mRNA during oocyte maturation were found in the 3'-UTR. The two sequences include the cytoplasmic polyadenylation element (CPE) with the consensus sequence UUUUUAU (32, 33) and the hexanucleotide polyadenylation signal AAUAAA, which is also necessary for nuclear pre-mRNA polyadenylation (33). Another type of CPE, with a minimum of dodecauridine that is used to direct polyadenylation during embryogenesis (34, 35) also was found in the 3'-UTR of Xcdc6 (U6GCU12G).

The presence of these sequences suggested that the Xcdc6 mRNA may be subjected to cytoplasmic polyadenylation during oocyte maturation and development. Northern blot analysis agreed with this hypothesis. The Xcdc6 transcripts (~2.4 kb) were found in all the early developmental stages tested, including the stage 6 oocyte, unfertilized egg, fertilized egg (0.5 hr after fertilization), and dividing embryos at two-cell and eight-cell stages.

**Fig. 3.** Injection of Xcdc6 mRNA induces sperm binding to injected oocytes. (A) Quantitation of sperm binding to oocytes injected with 40 ng of fibronectin mRNA, or 10, 30, and 40 ng of Xcdc6 mRNA. Bars represent SD with n = 20 oocytes. (B) Fluorescence micrographs showing sperm binding to oocytes injected with 30 ng of Xcdc6 mRNA (Right) and to control oocytes injected with water (Left). The bright dots on the oocyte surface are sperm heads stained with Hoechst 33342. (Bar = 0.2 mm.)

**Fig. 4.** Nucleotide sequence of Xcdc6 cDNA and deduced amino acid sequence. In the 3'-UTR of the nucleotide sequence, the CPE sequence (TTTT TAT) and the poly(A) signal sequence (AATAAA) are indicated. The embryonic-type CPE sequence is boxed. In the amino acid sequence, the two nuclear localization signals (NLS), leucine zipper motif, and ATP/GTP binding domains are underlined and labeled. The N-linked glycosylation site is double underlined. The five p34cdc2 kinase consensus sites are boxed.

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shown). The level of the mRNA remains essentially constant. However, the size of the transcripts increases during oocyte maturation. When the poly(A) tails of the total egg mRNA mixture is removed by hybridization with oligo(dT) and RNase H treatment, the Xcde6 transcript (Fig. 5, lane 0) was reduced to approximately the same size as the cloned cDNA without its poly(A) tail, indicating that the cloned Xcde6 cDNA is likely to be full length.

### Injection of Xcde6 mRNA Does Not Block Normal Progesterone-Induced Oocyte Maturation nor Fertilization.

To determine if injection of Xcde6 mRNA has any effects on the normal progesterone-induced oocyte maturation process or on fertilization or embryonic development, we incubated the oocytes that had been injected with 15 ng of Xcde6 mRNA in OR2 medium containing 3 μM progesterone. After 6–8 hr of incubation, more than 85% (n = 40) of the oocytes became mature as scored by their ability to undergo GVBD. As compared with noninjected control oocytes, no significant increase or decrease in time to reach GVBD was noted. These mature oocytes then were introduced into the ooplasm cavity of a host frog and allowed to pass through the oviduct. The recovered mature oocytes immediately were mixed with sperm and tested for fertilization efficiency. It was found that the fertilization rate of the mature oocytes that had been injected with Xcde6 mRNA was close to control oocytes, no significant increase or decrease in time to reach GVBD was noted. This result suggested that injection of exogenous Xcde6 mRNA alone induced changes that resulted in sperm binding, these changes did not have adverse effects on the normal process of progesterone-induced oocyte maturation or fertilization. However, it was found that the embryos resulted from fertilization of the progesterone-matured oocytes that had been injected with Xcde6 mRNA did not develop normally. They typically showed severe defects in gastrulation and greater than 80% of the embryos did not survive beyond the gastrulation stage (data not shown).

### DISCUSSION

The molecular mechanism of fertilization in *X. laevis* has just begun to be elucidated, with many important questions remaining to be answered. In the current study, we investigated the development of fertilizable ability in *Xenopus* oocytes and focused on one aspect of this process: the acquisition of sperm-binding ability. It was found that for the immature stage 6 oocyte to develop the ability to bind sperm at fertilization, two steps are essential. First, the oocyte has to become meiotically mature by exposure to progesterone; second, the mature oocyte must pass through the oviduct where the oocyte surface is modified and sperm binding sites on the VE are unmasked. If either of the two steps is omitted, the oocyte cannot bind sperm. Ultrastructural studies using special electron microscopic techniques have revealed significant differences between the surface of the immature stage 6 oocyte and that of the mature oolemic egg (7, 8). Based on these studies, it is believed that the plasma membrane of stage 6 oocytes is highly folded, with long microvilli extending into the oocyte envelope, which consists of tight bundles of long, thick protein filaments. After meiotic maturation, the plasma membrane flattens and the microvilli become shortened, but more ordered. At this stage, the entire CE is elevated above the microvilli tips, but probably still makes contacts with them. In addition, the filaments in the CE become more loosely bundled, creating large spaces in the envelope.

To understand how these ultrastructural changes may affect the sperm-binding property of the oocyte surface, we introduced stage 6 oocytes either before or after incubation with progesterone to induce meiotic maturation into the oolemic cavity of the frog and allowed them to pass through the oviduct. By analyzing the envelope proteins isolated from these two groups of oocytes, we found that the proteolytic processing of gp43, a major component of the oocyte envelope, occurred in both mature and immature oocytes. Based on previous studies (8, 16, 17), we believe that cleavage of gp43 to gp41 is a prerequisite to subsequent formation of functional sperm binding site consisting of the VE glycoprotein, gp69/64, and possibly other components (21). By comparing the intensity of polyclonal anti-gp69/64 antibody staining on the surface of the mature and immature oocytes after both had passed through the oviduct, we searched for changes in the sperm binding sites that might affect the conformation or accessibility of gp69/64. The result of this experiment showed that the antibodies stained the surface of the mature oocytes much more intensely than that of the immature oocytes. This result suggests that independent to the processing of gp43, additional changes in the sperm binding sites caused by meiotic maturation process also contribute to the acquisition of sperm binding ability. These changes may involve either ultrastructural alterations or addition of new components. However, we have not yet been able to detect compositional changes in the envelope or in the plasma membrane caused by oocyte maturation using detection methods not targeted to any specific component (data not shown). In any case what is clear from these studies is that two stages are involved in formation and exposure of functional sperm binding sites. In the first stage, meiotic maturation results in unknown changes that are required for subsequent binding of sperm by gp69/64; in the second stage, the gp69/64 molecules are exposed in functional form due to proteolytic processing of gp43 to gp41 and corresponding ultrastructural changes in the envelope.

To determine if new protein synthesis might be essential in the acquisition of sperm binding ability, we injected egg poly(A)+ mRNA into oocytes and found that the injected oocytes showed higher levels of sperm binding than control oocytes after passage through the oviduct. The rationale of this experiment was that by introducing purified total poly(A)+ mRNA from unfertilized egg into stage 6 oocyte, we might mimic the unmasking and translational activation processes that occur to the maternal mRNAs during oocyte maturation, because the injected egg mRNAs are devoid of RNA binding or masking factors and are likely to have undergone cytoplasmic polyadenylation. Although this approach is only an approximation of what happens during oocyte maturation and could produce nonspecific effects, the result confirmed that expression of certain mRNAs present in the unfertilized egg can induce changes that result in acquisition of sperm binding. In an effort to identify a protein(s) that might be responsible for acquisition of sperm binding, we designed an expression cloning strategy and used it to screen an egg cDNA library. The
Based on the current understanding of the mechanism of Cdc6 action, it is not yet clear how this protein is involved in the induction of sperm binding. The Cdc6 protein in yeast Saccharomyces cerevisiae and its related protein Cdc18 in Schizosaccharomyces pombe both are required for initiation of DNA replication and for ensuring that DNA replication is complete before mitosis (36–44). Lack of Cdc6/18 causes cells to enter mitosis without DNA replication. On the other hand, overexpression of Cdc18 results in repeated rounds of DNA replication in the absence of mitosis. Studies on the recently cloned Xenopus and human Cdc6 homologs indicate that these proteins also play an essential role in the initiation of DNA replication (28, 31). Currently, it is believed that interactions of Cdc6/18 with a variety of other protein complexes, such as the origin recognition complex, the minichromosome maintenance protein complex, and cyclin-dependent kinases are important in coordinating chromosome replication and mitotic cell cycle control (reviewed in ref. 45). However, little is known about the regulation and mechanism of Xc6 function during oocyte meiotic maturation.

Although we are still not certain if the induction of sperm binding by Xc6 represents a specific physiological process that actually takes place during normal Xenopus oocyte maturation, several possibilities can be considered. One is that the observed effect could be the result of interaction between Xc6 protein with the maturation promoting factor (MPF). The Xc6 protein contains five p34cdc2/cyclin B protein kinase consensus sites, and three of them are conserved in the human Cdc6 protein. Yeast Cdc6 protein also has five such sites and recently has been demonstrated to be an in vitro substrate of the Cdc28 kinase (homolog to Cdc2 in fission yeast). In addition, Cdc6 can inhibit Cdc28 histone H1 kinase activity and can be coimmunoprecipitated with Cdc28 (46). Cdc18 also interacts with cyclin-dependent kinase in fission yeast (47). In Xenopus, the MPF (also called the mitosis and meiosis promoting factor) activity is essential for entry into meiosis and mitosis (48, 49). If Xc6 physically interacts with MPF, we suggest that overexpression of Xc6 protein somehow may affect a subset of MPF activities that turn on certain cell cycle events and lead to alterations on the cell surface that affect sperm binding.

Alternatively, microinjection of Xc6 mRNA with a long poly(A) tail may mimic the effect of translational activation of the message during oocyte maturation. The maternal Xc6 mRNA was found to undergo cytoplasmic polyadenylation during oocyte maturation. The presence of multiple copies of the maturation-type CPE sequence (UUUUUAUU) and the conserved polyadenylation signal (AAUAAA) in the 3′-UTR also predict that Xc6 mRNA can be translationally activated during meiotic maturation. Because Xc6 protein presumably can interact with a variety of factors important in chromosome replication and cell cycle control (28, 45), the elevated Xc6 mRNA and protein levels in the injected oocytes could perturb the complex cell-cycle control system, which might result in initiation of events that normally occur during meiotic maturation, such as new protein synthesis required for appearance of a functional sperm receptor.

It has been observed that in fission yeast overexpression of Cdc6 can trigger DNA replication (data not shown). Similarly, in vitro studies have shown that addition of His6-Xc6 protein to egg extracts does not stimulate replication to normal levels (28). We also observed that injection of Xc6 mRNA into oocytes that then are subjected to host transfer does not seem to affect normal maturation of these oocytes or subsequent fertilization. However, it may be noteworthy that the embryos developed from the oocytes injected with Xc6 mRNA showed severe defects during early embryogenesis. This could reflect a difference in the ability of eggs and embryonic cells to cope with high concentrations of the Xc6 protein. Analysis of the effect of overexpression of Xc6 in frog oocytes and embryos remains a subject for future studies.

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