Mitogen-activated Protein Kinase and Cyclin B/Cdc2 Phosphorylate Xenopus Nuclear Factor 7 (xnf7) in Extracts from Mature Oocytes

IMPLICATIONS FOR REGULATION OF xnf7 SUBCELLULAR LOCALIZATION*

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Xenopus nuclear factor 7 (xnf7) is a maternally expressed putative transcription factor that exhibits phosphorylation-dependent changes in subcellular localization during early Xenopus development. Xnf7 is localized to the germinal vesicle (nucleus) of immature oocytes in a hypophosphorylated state. Xnf7 is phosphorylated during oocyte maturation and released to the cytoplasm. The protein is retained in the cytoplasm during early embryonic cleavage stages but returns to nuclei at the mid-blastula transition. Xnf7 is phosphorylated at two sites during oocyte maturation, designated P1, consisting of one threonine at position 103, and P2, consisting of three clustered threonines at positions 209, 212, and 218. Phosphorylation of both sites is important in regulating xnf7 localization. The P1 site can be phosphorylated by cyclin B/Cdc2 in vitro. To further understand the mechanisms regulating subcellular localization of xnf7 during early development, kinases capable of catalyzing phosphorylation of the P2 site were purified from mature oocyte extracts. We found that mitogen-activated protein kinase phosphorylated Thr²¹² and cyclin B/Cdc2 phosphorylated Thr²⁰⁹ and Thr²¹². No other kinase in mature oocyte extracts phosphorylated the xnf7 P2 site to a significant extent. These results implicate mitogen-activated protein kinase and cyclin B/Cdc2 in regulating xnf7 localization during oocyte maturation. This also suggests that localization of xnf7 may be regulated by multiple kinase activation pathways.

Control of subcellular localization is an important mechanism for regulating activities of biomolecules. In particular, the regulation of localization of nuclear proteins is a powerful mechanism for controlling their activities, essential for proper cell growth and differentiation of eukaryotic cells (reviewed in Refs. 1–3). Protein phosphorylation is a primary mechanism for controlling regulatable nuclear protein transport and is also responsive to activation of signal transduction pathways, providing a sensitive link between intracellular and extracellular events.

One protein that exhibits differential subcellular localization during early embryonic development, Xenopus nuclear factor 7 (xnf7), is a maternally expressed zinc finger protein of the RING finger-B box family (4–7). Xnf7 is localized to the nucleus, or germinal vesicle, of developing oocytes and is released to the cytoplasm upon germinal vesicle breakdown during oocyte maturation. Xnf7 is retained in the cytoplasm after fertilization and throughout the rapid, synchronous cell divisions of early Xenopus embryonic development. Xnf7 reenters all nuclei at approximately the 4000-cell stage, a time corresponding to the mid-blastula transition (MBT). MBT is characterized by a series of significant cellular and molecular changes including changes in cell cycle rate and synchrony, onset of cell movement, and activation of zygotic gene transcription (reviewed in Ref. 8). The correlation between entry of xnf7 into nuclei and MBT suggests a potential role for xnf7 in activating zygotic gene expression. Xnf7 possesses several characteristics of an activator of gene expression. It associates with DNA (5) and contains an acidic domain capable of transactivating transcription (9, 32). It also contains a region that shares homology with the chromodomain of proteins involved in chromatin remodeling (10). Taken together, these observations suggest that xnf7 may function in regulating the expression of at least a subset of zygotic genes at MBT.

Proper subcellular localization of xnf7 is dependent on its phosphorylation state and the functions of specific cytoplasmic retention and nuclear localization domains (5–7, 9, 11, 32). Xnf7 is phosphorylated during oocyte maturation, resulting in a decrease in its electrophoretic mobility (5). It is maintained in a hyperphosphorylated state until MBT, when it is dephosphorylated. Xnf7 is phosphorylated at two sites, designated P1 and P2. P1 contains one threonine residue (Thr¹⁰⁳), which can be phosphorylated by cyclin B/Cdc2 in vitro (9). P2 contains three threonines (Thr²⁰⁹, Thr²¹², and Thr²¹⁸). Substitution of alanines for the P1 and P2 site threonines results in xnf7 protein that cannot be phosphorylated and enters embryonic nuclei before MBT (6). Glutamate-substituted xnf7, mimicking a permanently phosphorylated protein, is retained in the cytoplasm after MBT (5). Thus, the phosphorylation states of both P1 and P2 regulate the subcellular localization of xnf7.

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† The abbreviations used are: xnf7, Xenopus nuclear factor 7; MBT, mid-blastula transition; MAPK, mitogen-activated protein kinase; cyc, cyclin; MOE, mature oocyte extract; MBF, myelin basic protein; AS, ammonium sulfate; WT, wild type; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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The importance of proper subcellular localization of xnf7 has been illustrated in experiments involving overexpression of the glutamate-substituted protein in developing embryos. Xnf7 is capable of homodimerization (5), and the glutamate-substituted form prolongs cytoplasmic retention of endogenous xnf7, presumably preventing it from performing its normal nuclear functions. These results indicate that glutamate-substituted xnf7 acts as a dominant negative (dnxnf7). Prolonged cytoplasmic retention of xnf7 by dnxnf7 results in defects in axial development and alterations in expression of mesodermal patterning genes in developing embryos. These results suggest that xnf7 is involved in regulating expression of genes required for axial patterning.

The ability of xnf7 to function as a modulator of gene expression is regulated by its subcellular localization, which is dependent on its phosphorylation state. To gain an understanding of the biochemical mechanisms underlying xnf7 localization, it was important to identify kinases that catalyze phosphorylation of xnf7 during oocyte maturation. In previous studies, it was shown that the P1 site is phosphorylated by phosphorylation of xnf7 during oocyte maturation. In previous studies, it was shown that the P1 site is phosphorylated by phosphorylation of xnf7 during oocyte maturation. In previous studies, it was shown that the P1 site is phosphorylated by phosphorylation of xnf7 during oocyte maturation. Mitogen-activated protein (MAP) kinase and cyclin B/Cdc2 were identified as the predominant kinases that identify kinases that phosphorylate the P2 site during oocyte maturation. Mitogen-activated protein (MAP) kinase and cyclin B/Cdc2 were identified as the predominant kinases that catalyze phosphorylation of xnf7. Furthermore, it was found that MAP kinase predominantly phosphorylates Thr212, and Cyclin B/Cdc2 is capable of phosphorylating Thr209 or Thr212. These results suggest that multiple kinase activation pathways are involved in the phosphorylation events controlling the subcellular localization of xnf7.

MATERIALS AND METHODS

Peptides—Peptides representing wild-type and alanine-substituted versions of the xnf7 P2 phosphorylation site (Fig. 1A) were synthesized by the University of Texas M.D. Anderson Cancer Center Synthetic Antigen Laboratory.

Kinase Assays—P2 phosphorylation assays were performed in a mixture consisting of 25 mM Tris-HCl (pH 8), 200 mM NaCl, 10 mM MgCl2, 0.1 mM ATP, 1 μCi of [γ-32P]ATP, 1 μg of peptide, and 1–3 μl of egg or oocyte extract or partially purified kinase in a final volume of 20 μl. After incubation for 20 min at 25 °C, reactions were terminated by addition of an equal volume of 2× SDS-polyacrylamide gel electrophoresis sample buffer and heating for 5 min at 90 °C. Reaction products were then resolved by electrophoresis on a 17% polyacrylamide gel. Immunoblotting was performed using a PhosphorImager and Imagequant software (Molecular Dynamics).

Casein kinase I, casein kinase II, glycogen synthase kinase 3, and protein kinase A were obtained commercially (New England Biolabs) and assayed for the ability to phosphorylate P2 peptide according to manufacturer’s directions. Appropriate synthetic substrates were obtained (New England Biolabs) and used as positive controls for casein kinase I and glycogen synthase kinase 3 activity. Assays for cyclin-dependent kinases and MAP kinase were performed as described elsewhere, using histone H1 and myelin basic protein (MBP) as substrates, respectively (13, 14).

Extract Preparation and Fractionation—Extracts were prepared from Xenopus laevis immature or mature oocytes as described previously (14). Mature oocyte extracts (MOE) were fractionated sequentially as outlined in Fig. 2. Fractionations were performed as described previously (14). At each step, fractions were assayed for the ability to catalyze phosphorylation of P2 peptide as described above.

Immunodepletions—Antibodies used were raised against Xenopus MAP kinase (a gift from J. Farrell, University of California, San Francisco) or a 1:1 mixture of antibodies raised against Xenopus cyclins B1 and B2 antibodies (a gift from J. Maller, University of Colorado Health Sciences Center). To prepare immunoprecipitation beads, protein G-agarose (Life Technologies, Inc.) was equilibrated with Tris-buffered saline (50 mM Tris-HCl (pH 7.5), 150 mM NaCl) and incubated with an equal volume of anti-MAP kinase or anti-cyclin B antibodies for 1 h at room temperature on a rotator. As a negative control, protein G-agarose beads were similarly incubated with serum from an immunized rabbit (referred to as “nonimmune antibodies”). Immunodepleted beads were then collected by centrifugation and washed twice with an equal volume of extraction buffer (EB, Ref. 14). A 0–40% ammonium sulfate precipitate of MOE or pooled column fractions were immunodepleted by two consecutive 2-h incubations with immunodepleted beads. The effectiveness of the depletions was determined by H1 kinase or MBP assays using immunodepleted enzymes (see Figs. 3C, 4C, and 5) and by immunoblotting (not shown). The immunoprecipitation step was omitted in assays involving immunodepletion of partially purified enzymes.

RESULTS

A Synthetic Peptide Spanning the P2 Phosphorylation Site Is Phosphorylated by Extracts of Mature but Not Immature Oocytes—To facilitate the biochemical characterization of P2 site phosphorylation, peptides containing the wild-type or various mutated P2 site sequences were synthesized (Fig. 1A) and used in in vitro kinase activity assays. The wild-type peptide (P2WT) was phosphorylated by extracts prepared from mature X. laevis oocytes (MOE) but not by extracts prepared from immature oocytes (IOE, Fig. 1B). These results indicate that a kinase activity capable of catalyzing phosphorylation of the P2 peptide appeared during oocyte maturation, correlating to the in vivo situation where xnf7 is phosphorylated during oocyte maturation (5). A peptide in which all three P2 site threonines were replaced by alanines (P2MUT) was not phosphorylated by either extract. Therefore, the band observed when P2WT was used in the assay (Fig. 1B, lane 6) resulted only from phosphorylation of threonines contained in P2WT. Additional bands of lesser mobility were also observed. These bands result from phosphorylation of proteins endogenous to each extract since their
appearance does not depend on the presence of P2 peptide substrate (Fig. 1B, compare lane 1 with lanes 2 and 3 or compare lane 4 with lanes 5 and 6). Since phosphorylation of P2WT in this system was specific and paralleled the in vivo phosphorylation of xnf7 with respect to oocyte maturation, this assay was utilized to identify candidate kinases that phosphorylate the P2 site.

MOE Contains Multiple Kinases That Can Phosphorylate P2 Peptide—To identify kinases capable of phosphorylating the P2WT peptide, MOE was fractionated by a four-step procedure, summarized in Fig. 2. At each step, fractions were assayed for the ability to phosphorylate the P2 peptide as discussed above. MOE was first fractionated by ammonium sulfate (AS) precipitation. P2 kinase activity was detected in the 0–40% AS precipitate but not in the 40–65% AS precipitate (Fig. 3A). Furthermore, the level of P2 phosphorylation catalyzed by the 0–40% AS fraction was at least as great as that of MOE, suggesting that most of the P2 phosphorylation activity was preserved during fractionation. Finally, reconstitution of the two AS fractions did not result in an increase in the level of P2 peptide phosphorylation (Fig. 3A), suggesting that no kinase activity was lost as a result of dissociation of multiple subunit kinases into the different ammonium sulfate fractions. In fact, a slightly higher activity was observed in the MOE or the combination of the two AS fractions, than in MOE or the combination of the two AS fractions, a slightly higher activity was observed in the 0–40% AS precipitate than in MOE or the combination of the two AS fractions, and a slightly higher activity was observed in the 0–40% AS precipitate than in MOE or the combination of the two AS fractions.

To purify further kinases capable of phosphorylating the P2 peptide, ACA34 column fractions exhibiting P2 kinase activity were pooled and subjected to additional column chromatography. Q-Sepharose, an anion exchange resin, was chosen as the next column since it has been previously used to separate CycB/Cdc2 and MAP kinase (14). The pooled ACA34 column fractions were loaded onto the Q-Sepharose column, and the column was developed by step elution with 0.2 and 0.4 M NaCl, and each fraction was assayed for P2 phosphorylation activity (Fig. 3C). P2 kinase activity was detected in the column flow-through and 0.2 M NaCl eluate (designated QF and QE1, respectively). No P2 kinase activity was found in the 0.4 M NaCl eluate (QE2 in Fig. 3C). Furthermore, mixtures of QF, QE1, and QE2 in various combinations resulted in additive increases in P2 phosphorylation activity (data not shown). No evidence of cooperativity was observed, suggesting that QF and QE1 contained kinases capable of individually phosphorylating the P2 peptide. Finally, no P2 phosphorylation activity was observed in a 0.4–2 M NaCl column eluate (not shown), suggesting that the kinases contained in QF and QE1 were the only kinases from the ACA34 column material capable of catalyzing P2 peptide phosphorylation. CycB/Cdc2 and MAP kinase are known to elute in QE1 (14), respectively, reinforcing the idea that they are candidate P2 kinases, as discussed above.

Cyclin B/Cdc2 Is the P2 Phosphorylating Activity in QF—To identify the P2 phosphorylation activities eluted from the Q-Sepharose column, the P2 kinases in QF and QE1 were further purified by Superose 6 gel filtration column chromatography (Figs. 4 and 5). P2 peptide phosphorylation activity eluted from the column in a single peak (Fig. 4A), indicating that there was probably only a single P2 phosphorylation activity in QF. Next, a series of results showed that CycB/Cdc2 accounted for the P2 phosphorylation activity of QF, as hypothesized above. First, the elution profile of cyclin-dependent kinase activity was determined by assaying these fractions for the ability to phosphorylate histone H1. The P2 and H1 phosphorylation profiles were essentially superimposable (Fig. 4A), suggesting that these activities co-fractionated from the column. Next, these fractions were subjected to immunoblotting using an antibody raised against Cdc2 (Fig. 4B). It was confirmed that fractions exhibiting peak P2 kinase activity also contained peak amounts of CycB-Cdc2 complexes (Fig. 4B, fractions 28–30). A second peak of Cdc2 protein was observed on the blot (Fig. 4B, fractions 34–38), corresponding to the elution of uncomplexed Cdc2 protein. Finally, fractions exhibiting peak P2 phosphorylation activity were pooled and subjected to depletion of CycB-containing complexes using antibodies raised against Xenopus cyclins B1 and B2. Depletion with anti-CycB antibodies resulted in a significant reduction in P2 phosphorylation activity as compared with depletion using non-immune antibodies (Fig. 4C, lanes 1 and 2). This reduction in P2 phosphorylation activity correlated with the reduction in CycB-associated H1 kinase activity in the samples depleted using anti-cyclin B antibodies (Fig. 4C, lanes 3 and 4). Taken together, these results confirm that CycB/Cdc2 is the major component of QF that catalyzes P2 peptide phosphorylation.

MAP Kinase Is the P2 Phosphorylating Activity in QE1—To explore the possibility that the major P2 peptide phosphorylation activity in QE1 was due to MAP kinase, QE1 was fractionated by Superose 6 column chromatography. P2 phosphorylation activity eluted from the column in a single major peak (Fig. 5A), indicating that there was probably only a single P2 phos-

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3 J. Kuang, unpublished data.
phosphorylation activity in QE1. It was then determined that MAP kinase was the major component of QE1 capable of supporting phosphorylation of the P2 peptide since 1) MAP kinase activity, as assayed by myelin basic protein (MBP) phosphorylation, co-eluted with P2 phosphorylation activity (Fig. 5A); 2) MAP kinase protein co-eluted with P2 kinase activity, as determined by immunoblotting using antibodies raised against MAP kinase (Fig. 5B); and 3) immunodepletion of MAP kinase from pooled Superose 6 column fractions exhibiting peak P2 phosphorylation activity resulted in significant reduction of both activities (Fig. 5C). These results suggest that MAP kinase accounts for the P2 phosphorylation activity of QE1.

Cyclin B/Cdc2 and MAP Kinase Are the Major P2 Kinases Found in MOE—The identification of cyclin B/Cdc2 and MAP kinase as P2 kinases led to the question of whether or not MOE contained other kinases capable of phosphorylating the P2 site phosphorylation activity in QE1. It was then determined that MAP kinase was the major component of QE1 capable of supporting phosphorylation of the P2 peptide since 1) MAP kinase activity, as assayed by myelin basic protein (MBP) phosphorylation, co-eluted with P2 phosphorylation activity (Fig. 5A); 2) MAP kinase protein co-eluted with P2 kinase activity, as determined by immunoblotting using antibodies raised against MAP kinase (Fig. 5B); and 3) immunodepletion of MAP kinase from pooled Superose 6 column fractions exhibiting peak P2 phosphorylation activity resulted in significant reduction of both activities (Fig. 5C). These results suggest that MAP kinase accounts for the P2 phosphorylation activity of QE1.
Thr212 are both potential candidates for phosphorylation by immunoblotting using antibodies raised against nase activity. A peptide under our conditions. major kinases found in MOE capable of phosphorylating P2 enzyme activity. Thus CycB/Cdc2 and MAP kinase were the C). These controls show that immunodepletion of MOE results or the reciprocal antibody did not result in significant decreases eliminated by depletion with either the cognate antibody or the 6). Levels of CycB/Cdc2 or MAP kinase activity were nearly eliminated by depletion with either the cognate antibody or the mixture of both antibodies, whereas depletion with non-immune or the reciprocal antibody did not result in significant decreases in levels of CycB/Cdc2 or MAP kinase activities (Fig. 6, B and C). These controls show that immunodepletion of MOE results in the specific removal of these kinase activities and, in general, does not result in significant nonspecific inhibition of enzyme activity. Thus CycB/Cdc2 and MAP kinase were the major kinases found in MOE capable of phosphorylating P2 peptide under our conditions.

Thr209 and Thr212 Can Be Phosphorylated by Cyclin B/Cdc2 and Thr212 Can Be Phosphorylated by MAP Kinase—Thr212 is Thr212 is a part of a consensus MAP kinase site, whereas Thr209 and Thr212 are both potential candidates for phosphorylation by cyclin-dependent kinases (see Ref. 15 and Table I). To deter-

mine the residues phosphorylated by each of these kinases, synthetic peptides containing threonine-to-alanine substitutions were used as substrates for phosphorylation by MAP kinase and cyclin B/Cdc2 (Fig. 7). MAP kinase was unable to phosphorylate a P2TAT, where an alanine is substituted for Thr212 (Fig. 7A, lane 4). Furthermore, MAP kinase did not significantly phosphorylate peptides P2TAA or P2AAT, containing single threonines at positions 209 or 218 (Fig. 7B, lanes 6 and 8), but was able to efficiently phosphorylate a peptide P2ATA containing a single threonine at position 212 (Fig. 7B, lane 7). Therefore, MAP kinase predominantly phosphorylates Thr212 of the P2 peptide. CycB/Cdc2 phosphorylated P2TAT to a lesser degree than P2WT (Fig. 7A, lanes 5 and 6), suggesting that it could phosphorylate Thr212 and either Thr209 or Thr218. In experiments involving peptides containing only single threonines, CycB/Cdc2 could phosphorylate both P2TAA and P2ATA (Fig. 7B, lanes 10 and 11), suggesting that cyclin B/Cdc2 could phosphorylate Thr209 and Thr212.

For unknown reasons, MOE, MAP kinase, and cyclin B/Cdc2 phosphorylated P2ATA to a much greater degree than P2WT (Fig. 7B, compare lanes 3, 7, and 11 to lanes 1, 5, and 9). These differences were not attributable to different peptide concentrations since phosphorylation levels were essentially unchanged by normalizing to the amount of peptide in each reaction (Fig. 7C). Binding of CycB/Cdc2 to Thr209 may interfere with interaction of CycB/Cdc2 at Thr212. MAP kinase may also be able to interact with Thr209 and interfere with productive phosphorylation of Thr212. Substitution of alanines for Thr209 may render P2ATA a better substrate for CycB/Cdc2 and MAP kinase by eliminating interference from enzymes interacting with Thr209. Interestingly, Thr218 was not phosphorylated by either MAP kinase, cyclin B/Cdc2, or any kinase in MOE, suggesting that it may not be phosphorylated during oocyte maturation, at least in the context of peptide P2ATA.

The P2 Site Is Not Phosphorylated by Other Potential P2 Site Kinases—Analysis of the P2 phosphorylation site revealed potential recognition sites for several protein kinases in addition to MAP kinase and CycB/Cdc2, including casein kinase I, casein kinase II, cAMP-dependent kinase, and glycogen synthase 3 (Table I; see Ref. 15). Each of these potential P2 site kinases was obtained commercially and tested for the ability to phosphorylate the P2 peptide. None of these kinases was capable of phosphorylating P2WT. Casein kinase I and glycogen synthase kinase 3 have been reported to preferentially recognize amino acid motifs containing phosphoserine or phospho-
threonine residues (15) and may catalyze phosphorylation of the P2 peptide if it is first phosphorylated by another kinase. However, pre-phosphorylation of P2 WT peptide using MAP kinase or CycB/Cdc2 did not render it a substrate for phosphorylation by either casein kinase I or glycogen synthase kinase 3 (data not shown).

**DISCUSSION**

Phosphorylation is a common mechanism of regulating subcellular localization of nuclear proteins (1–3). We sought to identify kinases capable of catalyzing phosphorylation of xnf7 during oocyte maturation, necessary for its cytoplasmic retention during early embryonic development. Here we have shown that cyclin B/Cdc2 and MAP kinase are the primary components of mature oocyte extracts capable of phosphorylating xnf7 in vitro.

**Involvement of Cyclin B/Cdc2 and MAP Kinase in Oocyte Maturation and xnf7 Phosphorylation**—The data presented here suggest that xnf7 is phosphorylated by two very different types of kinases. CycB/Cdc2 is well known for its cycles of activation and deactivation during mitotic cell divisions, and it is well established that MAP kinase is involved in activation of quiescent cells in response to mitogenic signals (reviewed in Refs. 16 and 17). However, CycB/Cdc2 and MAP kinase are also activated during oocyte maturation, where they are involved in a complex co-regulatory network. Induction of maturation by progesterone leads to synthesis of the proto-oncogene product, Mos (18–20), resulting in activation of both MAP kinase and CycB/Cdc2 (21–24). Blocking of Cdc2 activation by overexpression of a dominant negative form of Cdc2 or introduction of anti-Cdc2 antibodies results in an inhibition of progesterone-induced MAP kinase activation (25). However, inactivation of MAP kinase by overexpression of MAP kinase phosphatase (MKP1) also inhibits Mos-induced activation of Cdc2 (26). Interestingly, MAP kinase adopts specialized roles during meiosis and meiotic maturation, phosphorylating proteins that are CycB/Cdc2 targets during mitotic cell cycles (27). Thus, the activities of Cdc2 and MAP kinase are closely connected in maturing oocytes. Phosphorylation of xnf7 by both CycB/Cdc2 and MAP kinase is not unexpected in light of the complex relationship that exists between the two kinases during oocyte maturation.

**Phosphorylation of xnf7 after Fertilization and Dephosphorylation of xnf7 at MBT**—The data presented here raise the question of how xnf7 is dephosphorylated at MBT, resulting in its release from the cytoplasm and its transport to the nucleus. Two possible mechanisms are discussed below and outlined in Fig. 8. One possibility is that xnf7 is stably phosphorylated by cyclin B/Cdc2 and MAP kinase during oocyte maturation and...
then actively dephosphorylated by a phosphatase activated or synthesized at MBT. A second possibility is that phosphorylation of xnf7 is relatively unstable and its hyperphosphorylated state requires constant re-phosphorylation of the protein. In support of the latter, it has been observed that treatment of early embryos with inhibitors of protein synthesis, preventing production of cyclin B, results in pre-MBT transport of xnf7 to nuclei. This suggests that cytoplasmic retention of xnf7 may require continual renewal of cyclin B/Cdc2 activity. Contiguous re-phosphorylation of xnf7 during early development may require that Thr212 be phosphorylated by a kinase other than MAP kinase since MAP kinase activity decreases markedly after fertilization (28, 29). Although Thr209 and Thr212 were both phosphorylated by cyclin B/Cdc2 in vitro (Fig. 7), these residues may also be potential sites of phosphorylation by other cyclin-dependent kinases, such as cyclin E1/Cdk2 and cyclin A/Cdc2 or Cdk2, which are active in early embryos (13, 30, 31). This would be consistent with the suggestion that some meiotic substrates for MAP kinase may be phosphorylated by cyclin-dependent kinases during mitotic cell cycles (27). Interestingly, early Xenopus embryonic cell cycles support two rounds of cyclin E1/Cdk2 activation and deactivation (13), which, when combined with cyclin B- and cyclin A-dependent kinase activities, could result in a nearly constant level of xnf7 phosphorylation. Finally, changes in cell cycle length and synchrony at MBT would result in delays between peaks of different cyclin members5 that may be required for its cytoplasmic retention and nuclear functions. These interactions may also be mediated by the phosphorylation state of xnf7 and its interacting proteins.

Phosphorylation of threonine residues may alter the charge and conformation of xnf7, affecting interactions between it and other cellular biomolecules. Interestingly, a change in electrophoretic mobility of xnf7 is observed upon hyperphosphorylation of xnf7 (5). This change in mobility is also observed with glutamate-substituted xnf7 but only when glutamate replaces multiple threonine residues (7). Further characterization of the biochemistry of xnf7 phosphorylation is necessary for understanding mechanisms by which it functions.

Xnf7 is phosphorylated at different sites (Thr103, Thr209, Thr212, and possibly Thr218), by MAP kinase and cyclin B/Cdc2 during oocyte maturation and possibly other kinases during early development. It is interesting to speculate that phosphorylation of xnf7 by different kinases would allow for changes in xnf7 behavior and function in response to a variety of stimuli.

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