Membrane-anchored cyclin A2 triggers Cdc2 activation in *Xenopus* oocyte

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Abstract In *Xenopus* oocyte, the formation of complexes between neosynthesized cyclins and Cdc2 contributes to Cdc2 kinase activation that triggers meiotic divisions. It has been proposed that cytoplasmic membranes could be involved in this process. To investigate this possibility, we have injected in the oocyte two undegradable human cyclin A2 mutants anchored to the endoplasmic reticulum (ER) membrane. They encode fusion proteins between the truncated cyclin A2-Δ152 and a viral or cellular ER-targeting domain. We show that both mutants are fully functional as mitotic cyclins when expressed in *Xenopus* oocytes, bind Cdc2 and activate M-phase promoting factor. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Cyclin; Cdc2; Cytoplasmic membrane; Meiotic maturation; *Xenopus laevis* oocyte

1. Introduction

Cyclins play an essential role in progression through the eukaryotic cell cycle, acting as regulatory subunits of cyclin-dependent kinases (CDKs). CDK–cyclin complexes control cell cycle progression by phosphorylating regulatory proteins. A- and B-type cyclins are more specifically responsible for the onset of mitosis, and, through their degradation, the exit from mitosis. Their activities are mediated by changes in their subcellular localization during successive phases of the cell cycle.

The meiotic maturation of *Xenopus* oocytes represents an excellent model for studying the G2–M transition of the cell cycle, especially the regulation of the mitotic cyclins and their CDK partner, Cdc2. In response to progesterone, prophase-blocked oocytes re-enter the cell cycle by activating the universal mitotic kinase, the cyclin B–Cdc2 complex, also called MPF for M-phase promoting factor. Resting prophase oocytes contain a stockpile of cyclin B–Cdc2 complexes, called pre-MPF, that are maintained inactive by two inhibitory phosphorylations of the Thr14 and Tyr15 residues of Cdc2 [1]. These inhibitory phosphorylations are catalyzed by the Myt1 kinase [2]. The Cdc25 phosphatase acts antagonistically to Myt1 by catalyzing the dephosphorylation of both inhibitory residues of Cdc2 [3,4]. In response to progesterone, MPF is activated by a two-step mechanism involving a positive feedback loop known as auto-amplification [5]. A small amount of active MPF is first generated. It acts as a trigger, contributing to the activation of Cdc25 and consequently to the conversion of the pre-MPF store into active MPF. The mechanism of the formation of the triggering activity of Cdc2 is not clearly elucidated, although it probably involves the synthesis of new Cdc2 partners [6], such as cyclins or the RINGO/Speedy proteins [7–9]. These proteins would recruit monomeric Cdc2 molecules, which are present in large excess over the pre-MPF molecules [10]. The neoformed complexes would escape Myt1 phosphorylation and would be directly active as trigger, to initiate the auto-amplification mechanism. This model is supported by many experimental data, including injection of ectopic mitotic cyclins, A- or B-types, known to induce rapidly Cdc2 activation in the *Xenopus* oocyte [11,12].

MPF activation occurs in the oocyte cytoplasm. All the molecular players involved in Cdc2 activation are mainly, if not exclusively, located in the cytoplasm, and progesterone is able to trigger MPF activation in enucleated oocytes [5]. Several reports support the view that membrane compartments could be involved in the progesterone transduction pathway leading to Cdc2 activation. During progesterone-induced meiotic maturation, protein trafficking is arrested and the Golgi apparatus is disorganized [13]. Moreover, drugs that interact with Golgi membranes and block intracellular membrane trafficking can release the prophase block and trigger MPF activation [14,15], implying that intracellular vesicle traffic is linked with Cdc2 activation. Indeed, several molecules directly involved in Cdc2 regulation are located in the membranes, such as Myt1 [2] and a yet unidentified mitotic Cdc2 inhibitor [16]. Moreover, it has recently been reported that free Cdc2 is recovered in association with the external layer of membrane vesicles, whereas cyclin B2-associated Cdc2 is not [17]. All these data implicate that in *Xenopus* oocyte, the newly formed complexes between free Cdc2 and neosynthesized cyclins could form at the vicinity of a membrane compartment. However, it has never been reported that cyclin–Cdc2 complexes

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Abbreviations: APC/C, anaphase promoting complex/cyclosome; CDK, cyclin-dependent kinase; ER, endoplasmic reticulum; GVBD, germinal vesicle breakdown; MPF, M-phase promoting factor

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can be recovered in association with membranes, and that such complexes can be active, as expected from the threshold activity initiating MPF amplification in the oocyte.

In order to test this hypothesis, we took advantage of our previous identification of a mutant cyclin A2 in a human hepatitis B virus (HBV)-induced hepatoma [18]. The mutant protein, called S2A, consists of the N-terminal 156 residues of the PreS2/St HBV envelope protein fused to the C-terminal 280 residues of cyclin A2, a region that includes the cyclin box but is devoid of the sequence responsible for cyclin degradation through the anaphase promoting complex/cyclosome (APC/C) ubiquitin pathway. S2A is constitutively localized to the endoplasmic reticulum (ER) membranes through its viral PreS2/St HBV portion. Interestingly, it is endowed with a transforming activity [19], suggesting that both the stabilization and the ER location of the cyclin would be sufficient to lead to oncogenic activation. We also constructed another chimeric cyclin A2 protein (PRL-A2), addressed to ER by a cellular targeting domain. PRL-A2 consists of cyclin A2 deleted of its N-terminal 152 amino acids (A2-Δ152) and fused to an ER-targeting domain, PRL, containing the prolactin signal peptide in frame with an IgM stop transfer sequence [20]. We established that cyclin A2-Δ152 targeted to the ER by either the viral (PreS2/St in S2A) or cellular (PRL in PRL-A2) domain is fully functional, and acting as a mitotic cyclin when microinjected in *Xenopus* oocytes. Both proteins bind Cdc2 in ovo and trigger MPF activation.

2. Materials and methods

2.1. Materials

Xenopus laevis adult females (CNRS, Rennes, France) were bred and maintained under laboratory conditions. [γ-^{32}P]ATP and [^{35}S]methionine were obtained from NEN. Reagents, unless otherwise specified, were from Sigma.

2.2. Plasmid constructs

pSP cyclin A2-Δ152 and pSP cyclin A2-Δ152m vectors were obtained by cloning the cyclin A2-Δ152 *Eco*RI/*Sac*I fragment and its corresponding mutant in the MRAIL motif into the *Eco*RI/*Sac*I sites of pSP Sp^{+1}L.ST.gG plasmid, which encodes an ER-targeting domain, allowing proteins to become embedded in the lipid bilayer [20]. It contains the signal peptide and the first amino acid of bovine prolactin, followed by the β-lactamase sequence and the mouse IgM heavy chain stop transfer sequence. All reading frames were verified by direct sequencing.

2.3. Xenopus oocyte treatment and extract preparation

Fully grown *Xenopus* oocytes were isolated and prepared as previously described [1]. Meiotic maturation was induced by 1 μM progesterone. Oocytes were microinjected with 50 ng mRNAs. They were also injected with ^{35}S-labeled A2-Δ152, S2A and S2Am proteins in the presence of 100 μg/ml cycloheximide in the external medium. Germinal vesicle breakdown (GVBD) was assessed by the appearance of a characteristic white spot at the animal pole and oocyte dissection. At various times after injection or addition of progesterone, oocytes were collected and frozen at −80°C. Oocytes were then lysed at 4°C in four volumes of EB (80 mM β-glycerophosphate pH 7.3, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiothreitol) supplemented with protease inhibitors: 25 μg/ml leupeptin and aprotinin, 10 μg/ml pepstatin, 1 mM benzamidine, 1 μM AEBSF (Pentapharm AG). To visualize the electrophoretic shift of Cdc25, 1 mM orthovanadate was included in EB. Lysates were centrifuged at 5000×*g* for 3 min at 4°C (Sigma 302K centrifuge). The supernatant was harvested and analyzed. For subcellular fractionation, oocyte extracts were made in high-salt EB (0.5 M NaCl) and the supernatant obtained at 5000×*g* was further centrifuged at 100 000×*g* for 1 h at 4°C, leading to a supernatant (soluble proteins) and a membrane pellet. The pellet was washed in high-salt EB and ultracentrifuged again. Both fractions were analyzed by immunoblot.

2.4. Histone H1 kinase assay

Extracts (equivalent to five oocytes) were collected for p13^{suc1} binding and the histone H1 kinase activity associated with the beads was assayed as described [1].

2.5. mRNAs preparation and in vitro transcription–translation

Wild-type cyclin A2, cyclin A2-Δ152, S2A, S2Am, PreS2/St, PRL-A2 and PRL-A2m mRNAs containing a 5′,7-methyl-guanosine cap were synthesized using the Riboprobe in vitro Transcription System (Promega). The mRNA pellets were resuspended in RNase-free water at a concentration of 1 μg/μl, and then stored at −20°C until further use. In vitro transcription–translation of pBS plasmids containing human cyclin A2-Δ152, S2A and S2Am cDNAs was performed in the presence of [^{35}S]methionine using the TNT reticulocyte lysate kit (Promega).

2.6. Western blot analysis and antibodies

Equal amounts of oocyte extracts (equivalent of two oocytes per lane) were separated on 12% SDS–PAGE, transferred on nitrocellulose (Schleicher and Schuell) and processed for immunoblotting. The following primary antibodies were used: sheep polyclonal anti-*Xenopus* cyclin B2 [21], rabbit polyclonal anti-*Xenopus* Cdc25 [22], mouse monoclonal anti-*Xenopus* Cdc2 [23] and rabbit polyclonal anti-human cyclin A2 raised against the C-terminal region, CHLA-4 [24]. The appropriate horseradish peroxidase-conjugated secondary antibodies were used (Jackson Immunoresearch). The chemoluminescence reagent was obtained from NEN.

3. Results

3.1. Description of the various cyclin A forms used in this study

In order to investigate whether a membrane-associated cyclin A2 was functional, we used the chimeric proteins PRL-A2 and S2A sketched in Fig. 1. These proteins are deleted from 152 residues in the cyclin A2 N-terminal region, and therefore lack the cyclin A2 N-terminal degradation signal [25]. Both proteins are targeted to the ER membrane, the cyclin A2 portion being on the cytosolic side. The principal difference between S2A and PRL-A2 is the nature of the amino-terminal ER-targeting portion, respectively viral: PreS2/St, or cellular: PRL. The viral and cellular ER-targeting domains contain respectively 156 and 179 amino acids, so the chimeric proteins have a molecular weight similar to the full-length wild-type cyclin A2. So as to determine whether the function of S2A and PRL-A2 relies on binding to a CDK, we also constructed MRAIL to MAAIL point mutants of S2A and PRL-A2 incapable of binding CDKs, and referred to as S2Am and PRL-A2m, respectively. The cyclin A2-Δ152 moiety, which is strictly nuclear and undegradable, was used as a comparison.

3.2. ER-targeted cyclin A2-Δ152 functions as a mitotic cyclin in Xenopus oocytes

We examined whether the expression of cyclin A2-Δ152 targeted to the ER was able to induce the meiotic maturation of *Xenopus* oocytes. Capped mRNAs encoding the different proteins described in Fig. 1 were microinjected into prophase-blocked *Xenopus* oocytes. We then analyzed the percentage of oocytes having undergone GVBD and assayed the histone H1 kinase activity of Cdc2 (Fig. 2). As expected, wild-type cyclin A2 and A2-Δ152 caused GVBD and Cdc2 activation. Both S2A and PRL-A2 induced GVBD with the same time-course as wild-type cyclin A2 and A2-Δ152, within 3 h after injection, in parallel with the induction of a significant increase in the H1 kinase activity of Cdc2, already maximal 2 h after injec-

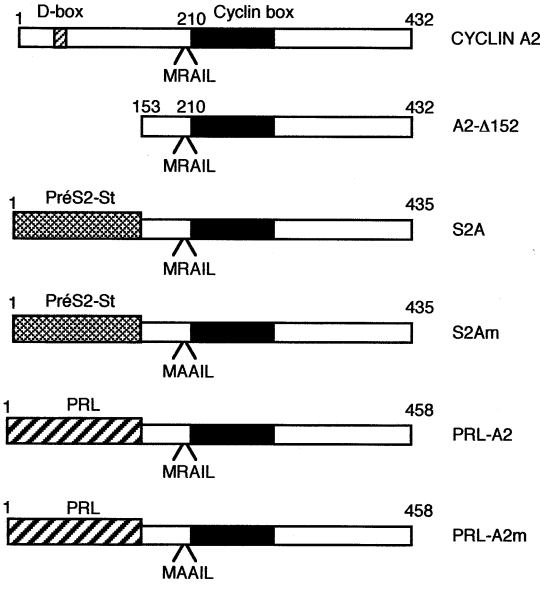

Fig. 1. Sketches of the different constructs used in this study. Black box: highly conserved central cyclin box. Cyclin A2: full-length human cyclin A2 protein. N-terminal degradation signal: hatched. Cyclin A2-Δ152: carboxy-terminal portion of cyclin A2 lacking the 152 amino acids of the N-terminus. S2A: chimeric protein resulting from the fusion of A2-Δ152 to PreS2/St, a truncated middle envelope protein of HBV (residues 1–156), in which the 55-amino acid PreS2 domain is fused to the 101-amino acid truncated S domain (gray). S2Am: MRAIL to MAAIL S2A mutant unable to bind CDKs. PRL-A2: protein resulting from the fusion of A2-Δ152 to the ER-targeting domain PRL (hatched). PRL-A2m: MRAIL to MAAIL PRL-A2 mutant unable to bind CDKs.

tion (Fig. 2). It has to be noted that A2-Δ152 induced a stronger and more stable Cdc2 activity than that of other forms, probably due to a higher stability of the protein (see below). The S2Am and PRL-A2m point mutants were completely inactive, as were the viral moiety of S2A (PreS2/St) and the ER-targeting domain PRL, which are unable to bind to and activate a CDK (Fig. 2 and data not shown). This demonstrates that the activity of S2A and PRL-A2 depends on their binding to Cdc2. We ascertained that all the expressed cyclin A2 proteins, except PRL-A2m and S2Am, were bound to Cdc2 by immunoblotting isolated $p13^{suc1}$-bound Cdc2 with an anti-cyclin A2 antibody (data not shown).

3.3. Ectopically expressed PRL-A2 and S2A cyclins trigger MPF auto-amplification in Xenopus oocytes

The Cdc2 activity generated by the injection of A-type cyclins may be solely due to the formation of ectopic complexes between *Xenopus* Cdc2 and cyclin A2. Alternatively, the neoformed cyclin A2–Cdc2 complexes may be capable of triggering the activation of endogenous cyclin B2–Cdc2 complexes (i.e. pre-MPF), initiating the positive feedback loop known as MPF auto-amplification in *Xenopus* oocytes. To address this question, we monitored the phosphorylation states of Cdc2, its regulatory subunit cyclin B2 and its activating phosphatase Cdc25 by immunoblot after injection of oocytes with the various cyclin A mRNAs (Fig. 2). Cdc2 migrated as a single band, corresponding to the active dephosphorylated form of the kinase, in the oocytes that had matured following progesterone treatment or microinjection with the various active cyclins, namely wild-type A2, A2-Δ152, S2A and PRL-A2 (Fig. 2). In contrast, prophase-blocked oocytes or oocytes microinjected with the inactive mutants S2Am and PRL-A2m exhibited the Cdc2 doublet, representative of the inactive Cdc2 forms (the upper band corresponding to tyrosine-phosphorylated Cdc2 bound to cyclin B, or pre-MPF, and the lower band to unphosphorylated monomeric Cdc2). Accordingly, cyclin B2 phosphorylation, visualized by the transition of a doublet to a single upper band, and mitotic activatory phosphorylation of Cdc25, visualized by a large shift in its electrophoretic mobility, were specifically observed in the samples containing active Cdc2 (Fig. 2). In contrast, phosphorylation of cyclin B2 and Cdc25 was detected neither in oocytes injected with S2Am and PRL-A2m nor in immature oocytes. Taken together, these experiments demonstrate that S2A and PRL-A2 are fully functional in vivo. These constructs recruit and activate Cdc2 in the vicinity of ER membranes, and are able to trigger MPF activation.

3.4. ER-targeted cyclin A2-Δ152 is stable in Xenopus oocytes

In order to analyze the expression levels of ectopically expressed cyclins A2 in *Xenopus* oocytes, batches of oocytes

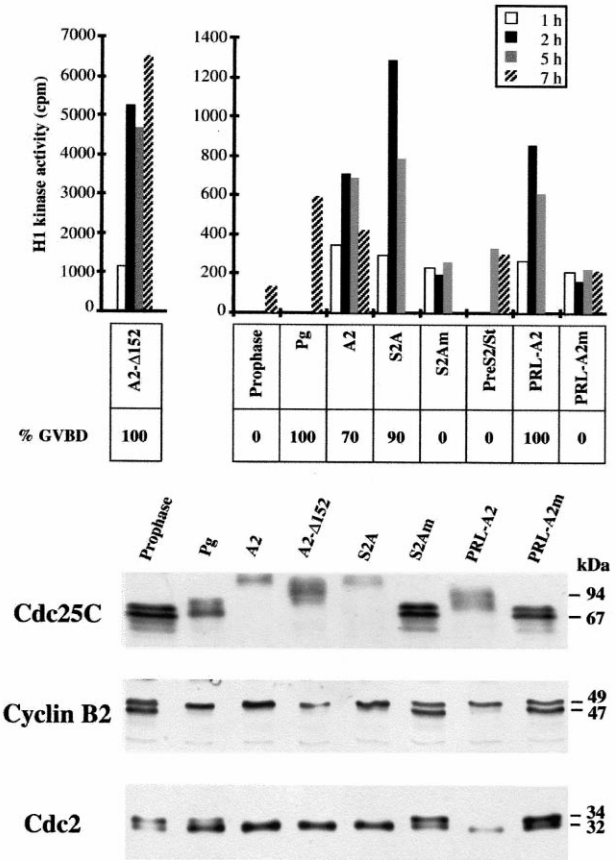

Fig. 2. ER membrane-targeted cyclin A2 induces GVBD and MPF activation in vivo. Capped mRNAs encoding various forms of cyclin A2 were injected into oocytes (30 oocytes per construct). Histone H1 kinase activity of Cdc2 was assayed at the indicated times after injection. GVBD was scored 5 h after injection. At the time of GVBD, mRNA-injected oocytes were lysed, and then centrifuged at $100\,000 \times g$. The supernatants were analyzed by immunoblotting with anti-Cdc2, anti-cyclin B2 and anti-Cdc25C antibodies. Pg: progesterone-treated oocytes; prophase: untreated oocytes. Three separate experiments gave identical results.

were lysed at different times after the microinjection of mRNAs, and cyclin A2 proteins were detected by immunoblot (Fig. 3A). An antibody directed against human cyclin A that does not recognize *Xenopus* cyclin A was used. S2A, PRL-A2, and A2-Δ152 appeared to be translated within the first hour following mRNAs injection, and then accumulated during meiotic maturation – GVBD was initiated at 2 h after injection and completed at 5 h in this typical experiment (Fig. 3A). This observation is consistent with the absence of the N-terminal degradation signal in the three constructs giving rise to stabilized proteins. In vitro translated S2A appeared as a doublet of bands corresponding to the alternative use of two functional AUG codons for translation initiation in the open reading frame. When translated in the oocyte, S2A appeared as a triplet of bands with an increased apparent molecular weight. This is most probably due to glycosylation occurring post-translationally into an asparagine-linked glycosylation site (Asn4) present in the PreS2/St region. The expression levels of the mutant forms S2Am and PRL-A2m were weaker (Fig. 3A and data not shown). As expected, wild-type cyclin A2 containing a degradation signal and representing an APC/C target was transiently accumulated at the time of GVBD (data not shown).

We next addressed the question of the instability of the PRL-A2m and S2Am mutants, which cannot bind Cdc2 and do not possess a degradation signal. In vitro translated ^{35}S-labeled S2A, S2Am and A2-Δ152 proteins were injected into prophase oocytes in the presence of cycloheximide, to prevent protein synthesis. Under these conditions, the amount of microinjected proteins was insufficient to induce meiotic maturation (not shown). Oocytes were lysed at different times after injection, and radioactive proteins were monitored by autoradiography (Fig. 3B). A2-Δ152 was highly stable, exhibiting no degradation during the time-course of the experiment. S2A showed a half-life of 18 h, whereas S2Am was very unstable with a half-life of 15 min. PRL-A2 and PRL-A2m stability was comparable to the stability of S2A and S2Am, respectively (data not shown). Thus, the mutation in the MRAIL motif that prevents Cdc2 binding, targets the protein to degradation through a pathway distinct from the APC/C ubiquitin pathway, which is inactive in prophase oocytes [26,27].

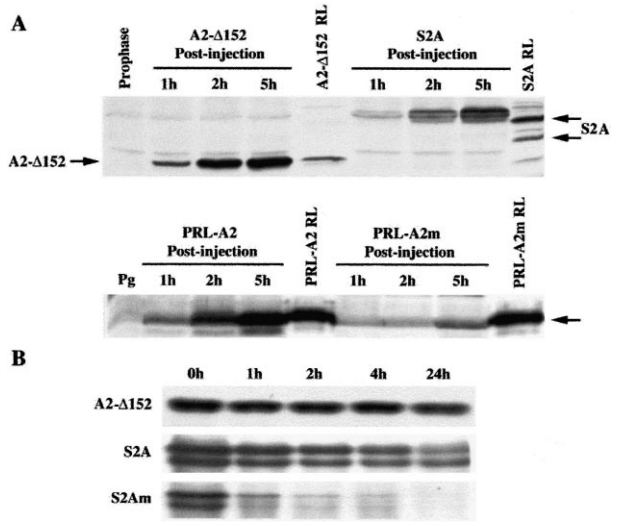

Fig. 3. ER membrane-targeted cyclin A2 forms are stable and accumulate in oocytes whereas the inactive mutants are unstable. A: Capped mRNAs encoding various forms of cyclin A2 were injected into oocytes. Oocytes were lysed at the indicated times after injection of mRNAs. Proteins were analyzed by immunoblot using an anti-cyclin A2 antibody. Arrows: in vitro translated cyclins used as molecular weight markers (crude labeled reticulocyte lysate: RL). B: Prophase oocytes were injected with the indicated in vitro translated ^{35}S-labeled proteins in the presence of cycloheximide, and lysed at the indicated times after injection. Proteins were separated by gel electrophoresis and autoradiographed.

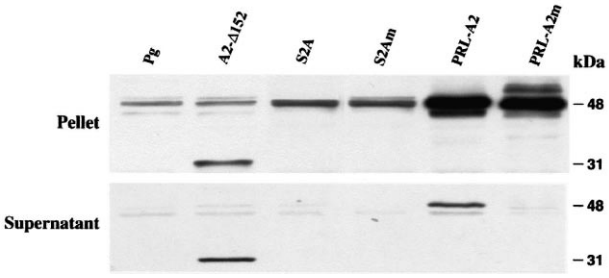

Fig. 4. S2A, PRL-A2 and the corresponding mutants are associated with membranes in *Xenopus* oocytes. Anti-cyclin A2 immunoblot of cytosolic (supernatant) and membrane (pellet) fractions. Oocytes matured by incubation with progesterone (Pg) or injected with the indicated capped mRNAs were lysed in high-salt EB 1 h later. Two oocytes were loaded in each lane.

3.5. *PRL-A2 and S2A proteins are membrane-associated in Xenopus oocytes*

In order to confirm that both PreS2/St and PRL, the ER-targeting signals, are functional in *Xenopus* oocytes, and thus that the observed activities were actually due to cyclin A2 anchored to the ER, subcellular fractionation was performed. Oocytes were microinjected with the various capped mRNAs and lysed 1 h after injection, when in vivo synthesis of the proteins and Cdc2 activation were initiated, but before GVBD. Lysates were centrifuged for 1 h at 100 000 × *g*. The supernatant (soluble proteins) and pellet (membrane proteins) were analyzed by immunoblot using an anti-cyclin A2 antibody. Fig. 4 shows that S2A and PRL-A2 as well as the corresponding mutants were recovered in the membrane fraction although their modes of insertion into the ER membrane are different (cotranslational for PRL-A2 and post-translational for S2A). In contrast to S2A, S2Am and PRL-A2m, which are exclusively recovered with membranes, a minor fraction of the PRL-A2 protein (around 5%) was detected in the supernatant in this particular experiment, probably resulting from a membrane contamination since this was not reproducibly observed. Cyclin A2-Δ152, which was expected to be cytosolic and did not contain any exposed hydrophobic domain, was equally distributed in both the soluble and membrane fractions, in two independent experiments, despite the high ionic strength of the lysate buffer. The presence of cyclin A2-Δ152 in the membrane fraction may be due to its recruitment by free monomeric Cdc2, which exhibits a strong affinity for oocyte membranes [17]. The distribution of the various cyclin A2 forms did not vary from 1 to 7 h after injection (data not shown). According to the presence of S2A and PRL-A2 in the membranes, histone H1 kinase activity can be measured in the membrane pellets starting 2 h after the corresponding mRNAs injection (data not shown).

4. Discussion

We have shown that cyclin A2 anchored to the ER membrane by a cellular or viral signal peptide functions as a mitotic cyclin. PRL-A2, as well as S2A, recruits and activates cyclin A2-dependent kinase in the vicinity of the ER membrane, indicating that folding of the membrane-anchored protein and the biochemical micro-environment existing near the membrane are favorable to the binding and activation of Cdc2, and to subsequent triggering of MPF activation. The presence of active cyclin A-dependent kinase in the cytoplasmic membranes has already been described in the particular case of regenerating liver cells. In such cells, cyclin A–CDK2 complexes have been found not only in the nucleus, but also in the cytoplasm, specifically in the microsomal and endocytic compartments, and exhibited histone H1 kinase activity [28,29].

It has been shown previously that cyclin A microinjected into G2-arrested *Xenopus* oocytes activates MPF [11,30,31]. Our results show that ER-targeted A2 cyclins are also able to induce meiotic maturation and activate MPF through their binding to Cdc2, despite their membrane insertion. In G2-blocked oocytes, Cdc2 exists in two inactive states: a major monomeric form that is not bound to any cyclin, and a Tyr15/Thr14 phosphorylated form that is associated with cyclin B2 and represents inactive 'pre-MPF' [10]. Myt1 kinase is responsible for the inhibitory phosphorylations of Cdc2 in the resting prophase oocyte [2]. The activation of MPF by cyclin injection therefore requires Myt1 down-regulation. In agreement with previous reports, our present work suggests that an excess of cytoplasmic cyclin A2, either cytosolic or membrane-associated, promotes entry into meiotic division by bypassing or suppressing the inhibitory potential of Myt1. A simple explanation is that free Cdc2 binds to the ectopically expressed cyclin A2, leading to an excess of cyclin A2–Cdc2 complexes, which are able to escape the inhibitory activity of Myt1. Moreover, cyclin A2–Cdc2 complexes have been shown to be poorly sensitive to Tyr15 phosphorylation [32]. These observations explain why injection of cyclin A2 can generate active complexes after endogenous monomeric Cdc2 recruitment. Once some Cdc2 kinase activity is generated, it leads to endogenous Cdc2–cyclin B2 activation by inducing the positive feedback loop known in amphibian oocytes as MPF auto-amplification [5]. This complex mechanism relies on both Myt1 inactivation and Cdc25 activation, and can be initiated by a threshold of either cyclin A– or cyclin B–Cdc2 complexes [33]. Our study demonstrates for the first time that active cyclin–Cdc2 complexes can be formed in association with membranes, and are then able to induce all the machinery supporting MPF amplification through the activation of cytoplasmic soluble Cdc25 phosphatase. It has been reported that monomeric Cdc2 exhibits high affinity for oocyte membranes [17]. Moreover, progesterone induces a rearrangement of the ER and Golgi membranes [13]. Interestingly, several drugs known to alter the intracellular membrane trafficking can release the prophase block and trigger MPF activation [14,15], showing the importance of the intracellular membrane turn-over in meiotic maturation. Therefore, it can be proposed that the threshold of MPF activity physiologically generated by progesterone and responsible for entry into the first meiotic division, may form at the ER membrane level. The hormone would favor the association between membrane-located monomeric Cdc2 and cyclin B both by remodeling the intracellular membranes and by promoting cyclin B synthesis independently of MPF, as already reported [7]. Protein fusion using the PRL or PreS2/St domain applied to different molecular players involved in MPF activation in the oocyte in order to target them to the ER may be of value to assessing the physiological relevance of this model.

Another interesting feature of our results came of the use of cyclin A2 proteins point-mutated in the MRAIL motif, so they cannot bind Cdc2. As expected, these mutants are unable to trigger meiotic maturation, demonstrating that the ER-targeted cyclin A2 forms are active by recruiting free Cdc2 in the oocyte. Unexpectedly, despite the deletion of the N-terminal sequences, known to stabilize mitotic cyclins [25], these mutants exhibit a short half-life (about 15 min) when expressed in the prophase oocyte. This implies that a proteolytic machinery, which does not depend on the APC-mediated ubiquitination (the N-terminal degradation signal is missing and APC is inactive in prophase oocytes), degrades free cyclins. Indeed, Cdc2 is stoichiometrically 10 times more abundant than cyclins in the oocyte [10]. An excess of cyclins could represent a pathological situation, corrected by a kind of checkpoint mechanism that recognizes and eliminates by degradation the Cdc2-non-bound cyclin molecules.

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