Across the meiotic divide – CSF activity in the post-Emi2/XErp1 era

Judy Qiju Wu and Sally Kornbluth*

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

*Author for correspondence (e-mail: Kornb001@mc.duke.edu)

Accepted 11 September 2008
Journal of Cell Science 121, 3509-3514 Published by The Company of Biologists 2008
doi:10.1242/jcs.036855

Summary

Vertebrate eggs are arrested at the metaphase stage of meiosis II. Only upon fertilization will the metaphase-II-arrested eggs exit meiosis II and enter interphase. In 1971, Masui and Markert injected egg extracts into a two-cell-stage embryo and found that the injected blastomere arrested at the next mitosis. On the basis of these observations, they proposed the existence of an activity present in the eggs that is responsible for meiosis-II arrest and can induce mitotic arrest, and named this activity cytostatic factor (CSF). Although the existence of CSF was hypothesized more than 35 years ago, its precise identity remained unclear until recently. The discovery of the Mos-MAPK pathway and characterization of the anaphase-promoting complex/cyclosome (APC/C) as a central regulator of M-phase exit provided the framework for a molecular understanding of CSF. These pathways have now been linked by the discovery and characterization of the protein Emi2, a meiotic APC/C inhibitor, the activity and stability of which are controlled by the Mos-MAPK pathway. Continued investigation into the mechanism of action and mode of regulation of Emi2 promises to shed light not only on CSF function, but also on the general principles of APC/C regulation and the control of protein function by MAPK pathways.

Key words: Anaphase-promoting complex, CSF, Cdc2–cyclin-B, Emi2, Mos

Introduction

Maturation of vertebrate oocytes results in the production of mature eggs, which are arrested at the metaphase-II stage of meiosis (Fig. 1). Upon receipt of an appropriate hormonal signal (e.g. progesterone), the G2-arrested oocyte enters and then rapidly exits meiosis I (MI), transiting without an intervening interphase directly into meiosis II (MII). This process is influenced by multiple cellular factors, but the unique features of the meiotic cycle largely reflect the tightly controlled dynamics of Cdc2–cyclin-B kinase activity. High Cdc2–cyclin-B activity drives entry into MI. Exit from this phase, at the MI-to-MII transition, is typified by an unusual partial degradation of cyclin B, which prevents the interphase that would otherwise intervene between MI and MII, allowing production of haploid gametes. Finally, eggs are arrested at metaphase II with constant levels of cyclin B and relatively high Cdc2 kinase activity (Fig. 1).

The metaphase-II arrest of vertebrate eggs can be extended for prolonged periods of time. Only upon fertilization will the metaphase-II-arrested eggs exit MII and enter interphase, a process that is mediated through the activation of calcium/calmodulin-dependent kinase II (CaMKII) in response to fertilization-induced elevations in cytoplasmic Ca^{2+} (Lorca et al., 1993). Following fertilization, the early embryonic divisions ensue, with oscillations in Cdc2–cyclin-B activity driving each division. These characteristic waves of Cdc2–cyclin-B kinase activity result primarily from the repeated synthesis and degradation of cyclin-B protein with each division (Fig. 1).

In 1971, Masui and Markert injected egg extracts into a two-cell-stage embryo and found that the injected blastomere arrested at the next mitosis (Masui and Markert, 1971). Careful examination of these M-phase-arrested cells revealed that they contained a metaphase spindle of similar morphology to that seen in a metaphase-II-arrested egg, prompting them to postulate the existence of an activity present in eggs that is responsible for inducing meiosis-II arrest. This activity was named cytostatic factor (CSF). Although the existence of CSF was initially proposed in 1971, its precise molecular identity remained unclear for more than three decades. During the intervening years, much effort has been devoted to understanding the nature of CSF. The recent identification and characterization of Emi2 (also known as FBX43, and as XErp1 in *Xenopus*), a meiotic inhibitor of the anaphase-promoting complex/cyclosome (APC/C), has provided considerable insight into CSF and its regulation. Nonetheless, there remain many interesting and crucial questions concerning the molecular nature and regulation of CSF activity. We review here the history, current state of knowledge and unresolved questions that surround the mechanisms underlying CSF-mediated arrest of vertebrate eggs in MII.

CSF in the pre-Emi2 era

Discovery of the Mos pathway

At the time that they postulated the existence of CSF activity, Masui and Markert (Masui and Markert, 1971) proposed that this activity should meet several criteria, as described most recently in several excellent, extensive reviews on the topic by James Maller and colleagues (Liu et al., 2007; Tunquist and Maller, 2003). First, the activity should emerge during oocyte maturation and peak at metaphase II. Second, it should induce M-phase arrest upon injection into embryonic blastomeres. Finally, this activity should be inactivated by the enhanced levels of cytoplasmic Ca^{2+} that are induced by fertilization or egg activation. Almost two decades after CSF was initially proposed, Sagata et al. identified Mos as a kinase that is synthesized during *Xenopus laevis* oocyte maturation

(Sagata et al., 1988). Exogenous introduction of Mos into one blastomere of a two-cell-stage embryo promoted a CSF-like arrest (Sagata et al., 1989). Importantly, both Mos protein and mRNA were rapidly degraded after fertilization (Lorca et al., 1991). Because Mos nicely satisfied the criteria proposed by Masui and Markert for CSF, Mos was proposed as the factor responsible for CSF arrest in vertebrate eggs. However, the detailed molecular mechanism linking Mos expression to metaphase-II arrest was, at that time, entirely undefined.

The biochemical characterization of Mos revealed that it could act as a mitogen-activated protein kinase (MAPK) kinase kinase (Posada et al., 1993). Furthermore, constitutively active MAPK was found to cause M-phase arrest in the dividing blastomere. In that the ability of Mos to confer CSF arrest was abrogated if MAPK kinase activity had been inactivated by either neutralizing antibody, MAPK phosphatase or pharmacological MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor, it was concluded that the MAPK-stimulatory activity of Mos accounted for its ability to promote MII arrest (Abrieu et al., 1996; Cross and Smythe, 1998; Gotoh and Nishida, 1995; Haccard et al., 1993; Kosako et al., 1994a; Kosako et al., 1994b). In 1999, this pathway was extended with the discovery of ribosomal S6 kinase (Rsk; also known as KS6A3), which acts downstream of MAPK (Bhatt and Ferrell, 1999; Gross et al., 1999). Similar to MAPK, constitutively active Rsk that was injected into one blastomere of a two-cell-stage embryo caused M-phase arrest, and Rsk depletion from egg extracts prevented the in vitro development of CSF activity (Bhatt and Ferrell, 1999; Gross et al., 1999). Thus, the Mos-MEK-MAPK-Rsk pathway was generally accepted to be responsible for the metaphase-II arrest in eggs (Fig. 2A). How Rsk, as the terminal kinase in this cascade, was able to communicate with the cell-cycle machinery that controls cyclin-B stability and M-phase arrest would remain unclear for almost a decade.

Control of the APC/C during CSF arrest

As the machinery driving the somatic cell cycle was defined, it became clear that Cdc2–cyclin-B stabilization could promote mitotic arrest and that this stabilization was important for maintaining a CSF arrest. Cyclin B is ubiquitylated by APC/C, which is an E3 ligase complex, and is subsequently degraded by the proteasome. During mitosis, prior to formation of the metaphase plate, APC/C activity is inhibited by the spindle-assembly checkpoint, until all chromosomes are properly attached to the spindle. This APC/C inhibition holds cyclin-B degradation in check to maintain the mitotic state. On the basis of these observations in the mitotic cycle, it was hypothesized that the APC/C might also be a primary target of CSF to maintain cyclin-B levels during CSF arrest. Consistent with this idea, it was found that individual overexpression of several proteins that are implicated in spindle-checkpoint operation, including Mad2, Bub1 and Mps1, could block Ca^{2+}-induced release from CSF arrest in *Xenopus* eggs (Grimison et al., 2006; Schwab et al., 2001; Tunquist et al., 2003). However, when the same spindle-checkpoint proteins were depleted from eggs, APC/C inhibition was not affected, nor did the ablation affect Ca^{2+}-induced CSF release (Abrieu et al., 2001; Sharp-Baker and Chen, 2001; Tunquist et al., 2003; Tunquist et al., 2002).

These results strongly suggested the presence of an additional CSF-specific APC/C inhibitor that is distinct from the spindle-checkpoint proteins (Fig. 2B). In retrospect, this is not surprising because CSF-arrested eggs contain an intact metaphase plate that would not be expected to trigger spindle-checkpoint activation

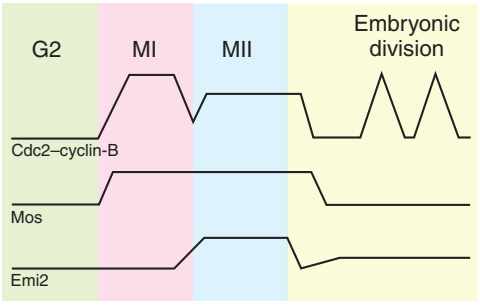

Fig. 1. The process of oocyte maturation. The maturation process is driven by multiple factors, including Cdc2–cyclin-B, Mos protein and Emi2 protein, the relative levels of which throughout the maturation process are illustrated. *Xenopus* oocytes are arrested in G2. Upon progesterone treatment, oocytes enter meiosis I (MI). Oocytes then transit directly from meiosis I to meiosis II (MII), without an intervening interphase, and arrest at MII (when they are termed eggs), often for prolonged periods of time. Only upon fertilization will the eggs exit MII and commence rapid embryonic cell divisions.

(Masui and Markert, 1971). Moreover, the fact that Mos can induce an M-phase arrest in interphase egg extracts in the absence of any genomic DNA strongly indicates that spindle-checkpoint proteins are not required for CSF arrest. Nonetheless, it has been reported that some spindle-checkpoint proteins are activated during maturation and some of them could potentially be targets of the Mos pathway (Schwab et al., 2001). Thus, spindle-checkpoint proteins might play an as-yet unidentified role during metaphase-II arrest.

The relationship between Mos and the APC/C

The recognition that the Mos-MEK-MAPK-Rsk pathway was responsible for CSF arrest and that this arrest relied on APC/C inhibition begged the question of whether these two pathways worked independently or cooperatively. In seeking to address this issue, Gross et al. injected radiolabeled cyclin B1 into *Xenopus* eggs and monitored cyclin-B degradation by the APC/C in the presence or absence of the MEK inhibitor U0126. This treatment reduced the half-life of cyclin B, indicating that Mos might be working through APC/C inhibition (Gross et al., 2000). When endogenous cyclin B was examined in the presence of U0126 in mouse eggs, this treatment caused cyclin-B degradation and pathenogenetic activation (Phillips et al., 2002). However, the same inhibitor did not cause CSF release in *Xenopus* eggs (Reimann and Jackson, 2002; Tunquist et al., 2002). This failure might have resulted from insufficient inhibition of the Mos pathway. Indeed, more recently it was found that treating *Xenopus* eggs with U0126 at higher doses for longer time periods could promote endogenous cyclin-B degradation (Wu et al., 2007a). These observations are consistent with a report showing that immunological neutralization of Mos function caused spontaneous CSF release in *Xenopus* eggs (Yamamoto et al., 2005). Collectively, these findings established the APC/C as a mediator of Mos-induced CSF arrest (Fig. 2C).

Upon fertilization, CaMKII activity is elevated and consequent APC/C activation typically results in cyclin-B degradation within ~10 minutes. By contrast, Mos protein can persist for at least 30 minutes following egg activation or fertilization (Lorca et al., 1991). It is possible that Mos activity is at least partially inhibited (although not degraded) following fertilization. However, another interesting possibility is that Mos works through an intermediary

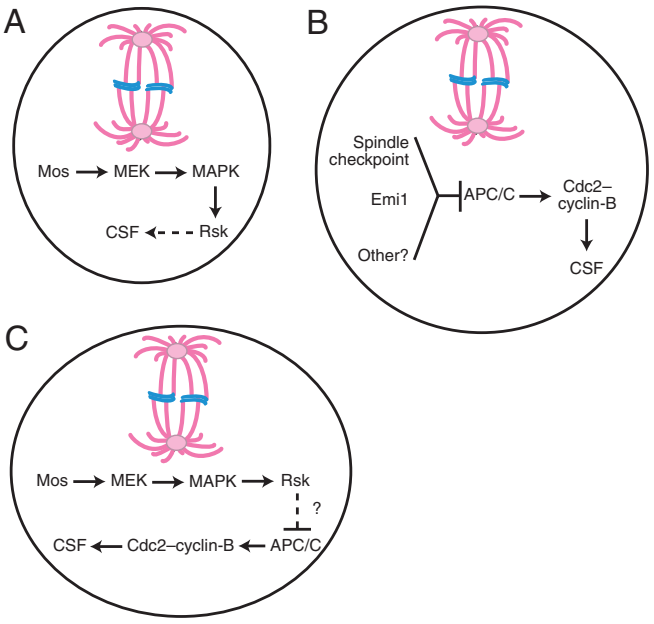

Fig. 2. Mos and the APC/C in the regulation of CSF activity. (A) The Mos pathway, which includes the apical kinase Mos and the downstream kinases MEK, MAPK and Rsk, was shown to be crucial for CSF arrest. However, the link between Rsk and CSF required clarification. (B) CSF was manifested as an inhibition of the APC/C and stabilization of Cdc2–cyclin-B. (C) Taking A and B together, it was postulated that Rsk might work through the APC/C to regulate CSF. However, the link between Rsk and the APC/C was initially unclear.

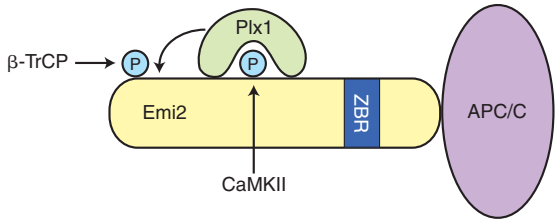

Fig. 3. Fertilization-mediated Emi2 degradation and CSF release. CSF arrest is controlled by Emi2-mediated APC/C inhibition. Upon fertilization, CaMKII kinase activity increases, leading to Emi2 phosphorylation, which creates a Plx1-binding motif on Emi2. Emi2-bound Plx1 then phosphorylates Emi2, allowing Emi2 to be recognized by the β-TrCP E3 ubiquitin ligase. Emi2 ubiquitylation primes Emi2 for degradation, leading to APC/C activation and, eventually, exit from CSF arrest.

to inactivate the APC/C and it is the inactivation of the intermediary, rather than of Mos itself, that induces APC/C activation and cyclin-B degradation following fertilization. In 2002, Emi1 (also known as FBX5) was proposed as the CSF-specific APC/C inhibitor (Reimann and Jackson, 2002). Not only can recombinant Emi1 induce mitotic arrest, but depletion of Emi1 from CSF-arrested egg extracts led to APC/C activation and cyclin-B degradation.

Despite these interesting findings, the physiological relevance of Emi1 to CSF activity was soon called into question. First, Emi1 is not stable under CSF arrest and endogenous Emi1 concentrations in eggs appear to be below the concentration that is necessary to induce CSF arrest. Second, Emi1 does not require active MAPK to induce mitotic arrest. Moreover, Emi1-induced arrest is distinct from CSF in its ability to promote stabilization of both cyclin A and cyclin B, whereas CSF arrest promotes the stabilization of only cyclin B. Finally, Emi1-induced arrest is not sensitive to Ca^{2+} (Geley et al., 2001; Ohsumi et al., 2004; Tung et al., 2005). Thus, despite its undisputed ability to act as an APC/C inhibitor, Emi1 was unlikely to be relevant to CSF arrest, leaving the identification of the CSF-specific APC/C inhibitor open (Fig. 2C).

Connecting Mos to the APC/C – filling the gap with Emi2

The discovery of Emi2

Because the Polo-like kinase Plx1 was reportedly required for CaMKII-induced APC/C activation, in seeking to identify the CSF-specific APC/C inhibitor, the Mayer laboratory performed a yeast two-hybrid screen for proteins that interacted with catalytically inactive Plx1 (Schmidt et al., 2005). In so doing, they successfully identified a crucial missing component of CSF, the Emi2 protein. Given these findings and the differences between CSF activity and Emi1 function described above, Jackson and colleagues proposed that their Emi1 antibody might cross-react with a related protein that was actually responsible for CSF arrest (Tung et al., 2005), because immunodepletion using their antibody promoted activation of APC/C in CSF-arrested egg extracts. In characterizing their anti-Emi1 antibody, they also identified Emi2, which, as described below, met the criteria for a component of CSF much more satisfyingly [note that this work was also expertly reviewed by Thomas Mayer and colleagues (Schmidt et al., 2006)].

Both groups reported that Emi2 could act as an APC/C inhibitor. Emi2 was found to be stable in CSF egg extracts and to be required for CSF arrest (because immunodepletion of Emi2 led to CSF release). Additionally, Emi2 is quickly degraded in a Plx1-dependent manner upon egg activation. Further characterization of Emi2 degradation upon egg activation revealed that Emi2 itself is a CaMKII substrate. Specifically, CaMKII-mediated Emi2 phosphorylation provides a Plx1 docking site on Emi2. A phosphodegron is created through Plx1-mediated phosphorylation of Emi2, and is recognized by the β-TrCP E3 ubiquitin ligase, promoting Emi2 degradation (Hansen et al., 2006; Liu and Maller, 2005; Rauh et al., 2005) (Fig. 3). These findings strongly suggested that Emi2 was the long-sought CSF-specific APC/C inhibitor that is responsible for mediating metaphase-II arrest. Upon egg activation, CaMKII and Plx1 coordinately promote Emi2 degradation, thereby relieving APC/C inhibition and promoting exit from MII.

Emi2 as an APC/C inhibitor

Spindle-checkpoint proteins can strongly interact with Cdc20, and this interaction enables them to inhibit the catalytic activity of the APC/C (Sudakin et al., 2001). Because mouse Emi2 appears to interact with Cdc20 as well, Shoji et al. proposed that Emi2, similar to the spindle-checkpoint proteins, works through Cdc20 to inhibit the APC/C (Shoji et al., 2006). However, in our laboratory, we found that *Xenopus* Emi2 can interact with the core APC/C directly through its C-terminus and that this interaction is key to its APC/C-inhibitory ability (Wu et al., 2007b), raising the possibility that simple binding of Emi2 in some way blocks APC/C activity. However, it does not appear that binding of Emi2 to the APC/C is the only determinant of APC/C-inhibitory activity. It has been reported that Emi2 contains a zinc-binding region (ZBR) and that mutating a single amino acid within this region will lead to the

complete inactivation of Emi2 (Schmidt et al., 2005). We have found, however, that ZBR-mutant Emi2 retains its ability to interact with the APC/C, suggesting that this interaction is not sufficient for the APC/C-inhibitory activity of Emi2 (Wanli Tang, J.Q.W. and S.K., unpublished data; and see below).

A feedback loop controlling Emi2

Although Emi2 can bind directly to the APC/C, this interaction can be controlled by the phosphorylation status of Emi2: Cdc2-mediated phosphorylation of Emi2 at two residues in the C-terminal region of the protein, T545 and T551, weakens the interaction between Emi2 and the APC/C, thus allowing activation of the APC/C (Wu et al., 2007b). Because cyclin B is continually synthesized in MII-arrested eggs, this Cdc2-mediated Emi2 inactivation is used by the eggs to prevent a significant accumulation of cyclin B during the prolonged CSF arrest. Such cyclin-B accumulation might otherwise disrupt normal egg activation and the sharp transition into the first embryonic interphase. The Cdc2–cyclin-B-mediated phosphorylation of the T545 and T551 residues is antagonized by protein phosphatase 2A (PP2A) (Wu et al., 2007b). Thus, when cyclin-B levels rise sufficiently for Emi2-directed Cdc2 kinase activity to exceed Emi2-directed PP2A activity, Emi2 is phosphorylated.

The consequence of phosphorylation at Cdc2–cyclin-B sites within the Emi2 C-terminus is to dissociate Emi2 from the APC/C, whereas similar phosphorylation of a cluster of sites within the Emi2 N-terminus shortens the half-life of Emi2 (Wu et al., 2007a; Wu et al., 2007b). Together, these alterations in Emi2 alleviate APC/C inhibition, allowing some level of cyclin-B degradation to occur, although the egg remains arrested in MII. This degradation is, in turn, held in check because falling cyclin-B levels reduce Cdc2 kinase activity, allowing PP2A-mediated Emi2 dephosphorylation to predominate. This re-stabilizes Emi2 and allows its re-association with the APC/C, thereby preventing the complete degradation of cyclin B that would otherwise result in MII exit. By controlling Emi2 stability and activity, the balance between Cdc2 and PP2A allows homeostatic control of cyclin-B levels to both maintain the CSF arrest and allow rapid M-phase exit upon fertilization (Fig. 4).

The Mos pathway and Emi2

Even after Emi2 was shown to be a relevant CSF-specific APC/C inhibitor, it remained unclear whether Emi2 provided a missing link between the Mos pathway and the APC/C. In 2007, two reports demonstrated that Emi2 was required for the CSF activity of Mos (Inoue et al., 2007; Nishiyama et al., 2007a). Moreover, Rsk, a downstream kinase in the Mos pathway, was shown to phosphorylate Emi2 directly, and this phosphorylation promoted Emi2 stabilization and activation. Because Cdc2 could promote Emi2 degradation and inactivation, we speculated that Rsk-mediated Emi2 phosphorylation would antagonize the function of Cdc2–cyclin-B. Consistent with this, our laboratory found that Rsk-mediated Emi2 phosphorylation could recruit the protein phosphatase PP2A to Emi2 (Wu et al., 2007a). Emi2-bound PP2A promotes Emi2 dephosphorylation at T545 and T551, thus promoting the Emi2-APC/C interaction and allowing APC/C inhibition. Moreover, as alluded to above, we discovered four additional S/T-P phosphorylation sites on Emi2, the Cdc2-mediated phosphorylation of which would lead to Emi2 instability. The same PP2A that promotes Emi2 T545 and/or T551 dephosphorylation was also shown to enhance dephosphorylation of these four sites, thus stabilizing Emi2 during CSF arrest (Fig. 4).

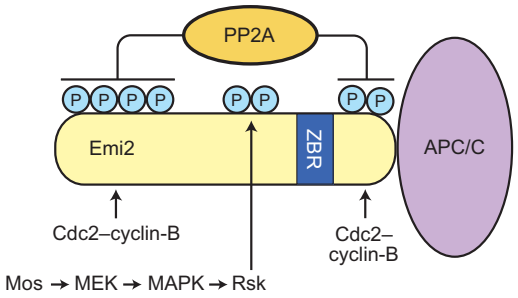

Fig. 4. Emi2 links the Mos pathway and the APC/C to regulate CSF. The ability of Emi2 to bind and inhibit the APC/C is modulated by Cdc2–cyclin-B-mediated phosphorylation. Specifically, phosphorylation at the Emi2 C-terminus weakens the Emi2-APC/C interaction, promoting dissociation of the Emi2-APC/C complex and activation of APC/C. During CSF arrest, Cdc2–cyclin-B-mediated Emi2 phosphorylation is antagonized by the Mos-MAPK pathway. The Mos pathway promotes recruitment of PP2A to Emi2, keeping Emi2 dephosphorylated. This allows Emi2 activation and APC/C inhibition. Bound PP2A can also promote Emi2 stabilization by dephosphorylating Emi2 at its N-terminus – otherwise, Cdc2–cyclin-B-mediated phosphorylation at this terminus would trigger Emi2 ubiquitylation and degradation. The Mos pathway therefore works through Emi2 to modulate APC/C activity, providing a link between Mos and the APC/C to regulate CSF arrest.

Collectively, these findings suggest that Mos, through its downstream kinases, promotes CSF arrest through the regulation of Emi2 and that Emi2 is the CSF-specific APC/C inhibitor that links the Mos pathway to the APC/C. Although this is clearly the case in vertebrates, the target of the Mos pathway in other systems is yet to be identified. For example, in starfish, the Mos pathway was also reported to be responsible for a G1 arrest (Mori et al., 2006). However, the target of the Mos kinase cascade in this arrest is currently unknown.

CSF – unresolved questions

The molecular mechanism of Emi2-mediated APC/C inhibition

Although Emi2 can interact with the core APC/C in the absence of APC/C activators (such as Cdc20 and Cdh1), and this Emi2-APC/C interaction is crucial for the APC/C-inhibitory activity of Emi2, it is not clear whether this physical interaction is sufficient to mediate inhibition. This is of particular interest in light of the fact that several APC/C inhibitors (e.g. MAD3, Acm1, Mes1 and Emi1) have been shown to block APC/C-mediated substrate degradation through a pseudosubstrate inhibitory mechanism (Burton and Solomon, 2007; Enquist-Newman et al., 2008; Kimata et al., 2008; Miller et al., 2006).

Emi2 has a sequence that is related to the proposed Emi1 pseudosubstrate region, raising the possibility that Emi2 could also exert its effects via a pseudosubstrate mechanism. Similar to Emi2, Emi1 also contains a ZBR. A single amino-acid mutation in this domain, which is distant from the pseudosubstrate domain, has been reported to completely disrupt the ability of Emi2 to inhibit the APC/C (Schmidt et al., 2005), suggesting that Emi2 employs a mechanism that is entirely distinct from pseudosubstrate inhibition to prevent APC/C activation. Consistent with this notion, we have observed considerable Emi2-APC/C interactions in the ZBR mutant, suggesting that APC/C-binding alone is not sufficient for APC/C inhibition (Wanli Tang, J.Q.W. and S.K., unpublished data). It is interesting to note that another APC/C inhibitor that was previously identified in our laboratory, Xnf7, also contains a ZBR (Casaletto

et al., 2005), raising the possibility that Xnf7 and Emi2 share a common mechanism for inhibiting the APC/C. For any models of inhibition that require stable binding of Emi2 to the APC/C, it seems problematic that the levels of Emi2 in the egg are sub-stoichiometric to the APC/C. This can only be overcome by invoking the inhibition of particular APC/C pools at distinct sub-cellular locales, or by inhibiting distinct pools of the APC/C by individual, distinct inhibitors (Nishiyama et al., 2007a; Yamano et al., 2004).

Mos-dependent and -independent Emi2 dephosphorylation

Mos accelerates the dephosphorylation of Emi2 on both its N- and C-terminal Cdc2–cyclin-B sites (to control both the stability and APC/C-binding activity of Emi2); this action of Mos resides, at least in part, in the ability of Rsk to promote PP2A-Emi2 interactions. It has been reported that the eggs from a triple Rsk-knockout mouse still maintain a CSF arrest (Dumont et al., 2005). This might be due to a redundant pathway that is responsible for Emi2 phosphorylation and thus PP2A binding. In starfish, it was discovered that Myt1, a Rsk substrate in *Xenopus*, can be inactivated by Akt (Okumura et al., 2002). This suggests that Akt or a related kinase is responsible for Emi2 phosphorylation in the absence of Rsk.

Substrate recognition by PP2A is typically mediated through a substrate-targeting subunit (B subunit). For Emi2 dephosphorylation, the identity of the relevant B subunit has not yet been established. Moreover, it is not clear whether Rsk-mediated phosphorylation of Emi2 directly promotes PP2A B-subunit binding, C-subunit binding or both. It is also possible that Mos (and, by extension, Rsk) directly affects PP2A subunits in addition to acting directly at the level of Emi2. In this regard, it would be of interest to determine whether Mos affects dephosphorylation of PP2A targets other than Emi2.

In interphase extracts that have been prepared from *Xenopus* eggs (which lack Mos protein), a background Emi2-directed phosphatase activity has been observed (Wu et al., 2007b), suggesting the existence of a Mos-independent phosphatase activity that is directed towards Emi2. Moreover, the treatment of CSF-arrested *Xenopus* egg extracts with okadaic acid can induce a release from CSF arrest. We have found that, even at concentrations of okadaic acid that are known to completely inhibit PP2A, a rapid Emi2 gel-mobility downshift is seen as egg extracts entered interphase, suggesting the presence of a phosphatase that is distinct from PP2A and can promote Emi2 dephosphorylation. We would speculate that this phosphatase is PP1 because the gel-mobility downshift of Emi2 was prevented when higher concentrations of okadaic acid that were inhibitory to PP1 were used to induce CSF release (J.Q.W. and S.K., unpublished data). PP2B has been recently reported to be crucial for fertilization and CSF release (Mochida and Hunt, 2007; Nishiyama et al., 2007b); this Mos-independent phosphatase, however, is unlikely to be PP2B because the dephosphorylation of Emi2 was studied in the context of okadaic-acid-induced release from CSF arrest (in the absence of Ca^{2+}) and PP2B is not an okadaic-acid-sensitive phosphatase.

Extra-CSF roles of Emi2 – regulation of embryonic divisions and control of oocyte maturation

As eggs enter interphase and commence embryonic divisions, Emi2 protein is translated and accumulates (Liu et al., 2006). Although levels of Emi2 in embryos are only at 20% of those found in eggs, this amount of Emi2 would be sufficient to induce metaphase arrest if Mos were also present (Liu et al., 2006); thus, the absence of Mos in embryos is crucial for allowing the early embryonic cell divisions to proceed (Fig. 1). The introduction of exogenous Mos into embryos or in vitro cell-cycle extracts most probably induces M-phase arrest by enhancing endogenous Emi2 activity, because Emi2 is stable during the short mitoses of embryogenesis and Mos does not modulate Emi2 translation.

It has been noted that MAPK is activated at mitosis in early embryos even in the absence of Mos. This raises the interesting question, why does this MAPK not cause Emi2 activation and thus mitotic arrest? Because there is a lag between Cdc2 activation and MAPK activation (Bhatt and Ferrell, 1999; Guadagno and Ferrell, 1998), it is possible that Cdc2 promotes Emi2 inactivation before Rsk would phosphorylate, and thus activate, Emi2. This temporally controlled regulation of Emi2 could, therefore, be crucial for embryonic divisions.

During oocyte maturation, Mos translation begins soon after progesterone stimulation and Mos protein persists throughout the entire maturation process (Fig. 1) (Sagata et al., 1988). So why do eggs arrest at MII but not MI? It has been reported that Emi2 translation starts immediately after entry into MI; however, no significant accumulation of Emi2 is observed until MII by immunoblotting (Liu et al., 2006; Madgwick et al., 2006; Ohe et al., 2007; Tung et al., 2007). Therefore, it was postulated that the slow translation of Emi2 in MI is responsible for the smooth transition from MI to MII. Surprisingly, we observed a significant amount of Emi2 accumulation in MI, providing that Emi2 degradation was inhibited by treatment with the proteasome inhibitor MG132 (Tang et al., 2008). In addition, ^{35}S-labeled Emi2 injected into oocytes was quickly degraded in MI. Furthermore, expression of a non-degradable Emi2 at levels comparable with endogenous Emi2 in oocytes under circumstances in which endogenous Emi2 translation had been prevented resulted in MI arrest (Tang et al., 2008). These observations strongly suggest that a smooth MI-to-MII transition relies on Emi2 degradation in MI. These observations raised the question of why Emi2 is stable only in MII and not in MI, despite the presence of Mos-pathway activity in both phases. In this regard, we found that Cdc2 activity in MI is twice as high as that in MII, which promotes Emi2 instability in MI, thus ensuring a smooth MI-to-MII transition (Fig. 1) (Tang et al., 2008). Nonetheless, it still remains to be determined why Cdc2 exhibits higher activity in MI than in MII.

Conclusions and perspectives

Since the initial characterization of CSF in 1971, significant progress has been made towards understanding the nature of CSF and its regulation. Fundamental contributions included the discovery of the Mos pathway and the realization that Mos could indirectly modulate APC/C activity. However, the gap between the Mos pathway and APC/C regulation remained open until the discovery of Emi2. Nonetheless, many questions remain to be answered and, although important for understanding CSF arrest, these answers may have far broader implications. For example, the elucidation of mechanisms that underlie the inhibition of the APC/C by Emi2 might well provide information that is pertinent to the control of the APC/C by its many other regulators – the investigation of PP2A under CSF arrest will not only further our understanding of PP2A regulation, but might provide a paradigm for the targeting of MAPK-pathway substrates by PP2A. Finally, the complex mutual-feedback loops that are seen in the Emi2 regulation of the APC/C are emblematic of cell-cycle-control pathways. Therefore, a thorough understanding of Emi2-APC/C regulation should shed further light on the way in which such regulation allows sharp transitions between cell-cycle states.

This work was supported by a National Institutes of Health grant to S.K. We would like to thank Christopher D. Freel and Wanli Tang for their helpful contributions.

References

Abrieu, A., Lorca, T., Labbe, J. C., Morin, N., Keyse, S. and Doree, M. (1996). MAP kinase does not inactivate, but rather prevents the cyclin degradation pathway from being turned on in Xenopus egg extracts. *J. Cell Sci.* **109**, 239-246.

Abrieu, A., Magnaghi-Jaulin, L., Kahana, J. A., Peter, M., Castro, A., Vigneron, S., Lorca, T., Cleveland, D. W. and Labbe, J. C. (2001). Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint. *Cell* **106**, 83-93.

Bhatt, R. R. and Ferrell, J. E., Jr (1999). The protein kinase p90 rsk as an essential mediator of cytostatic factor activity. *Science* **286**, 1362-1365.

Burton, J. L. and Solomon, M. J. (2007). Mad3p, a pseudosubstrate inhibitor of APCCdc20 in the spindle assembly checkpoint. *Genes Dev.* **21**, 655-667.

Casaletto, J. B., Nutt, L. K., Wu, Q., Moore, J. D., Etkin, L. D., Jackson, P. K., Hunt, T. and Kornbluth, S. (2005). Inhibition of the anaphase-promoting complex by the Xnf7 ubiquitin ligase. *J. Cell Biol.* **169**, 61-71.

Cross, D. A. and Smythe, C. (1998). PD 98059 prevents establishment of the spindle assembly checkpoint and inhibits the G2-M transition in meiotic but not mitotic cell cycles in Xenopus. *Exp. Cell Res.* **241**, 12-22.

Dumont, J., Umbhauer, M., Rassinier, P., Hanauer, A. and Verlhac, M. H. (2005). p90Rsk is not involved in cytostatic factor arrest in mouse oocytes. *J. Cell Biol.* **169**, 227-231.

Enquist-Newman, M., Sullivan, M. and Morgan, D. O. (2008). Modulation of the mitotic regulatory network by APC-dependent destruction of the Cdh1 inhibitor Acm1. *Mol. Cell* **30**, 437-446.

Geley, S., Kramer, E., Gieffers, C., Gannon, J., Peters, J. M. and Hunt, T. (2001). Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. *J. Cell Biol.* **153**, 137-148.

Gotoh, Y. and Nishida, E. (1995). Activation mechanism and function of the MAP kinase cascade. *Mol. Reprod. Dev.* **42**, 486-492.

Grimison, B., Liu, J., Lewellyn, A. L. and Maller, J. L. (2006). Metaphase arrest by cyclin E-Cdk2 requires the spindle-checkpoint kinase Mps1. *Curr. Biol.* **16**, 1968-1973.

Gross, S. D., Schwab, M. S., Lewellyn, A. L. and Maller, J. L. (1999). Induction of metaphase arrest in cleaving Xenopus embryos by the protein kinase p90Rsk. *Science* **286**, 1365-1367.

Gross, S. D., Schwab, M. S., Taieb, F. E., Lewellyn, A. L., Qian, Y. W. and Maller, J. L. (2000). The critical role of the MAP kinase pathway in meiosis II in Xenopus oocytes is mediated by p90(Rsk). *Curr. Biol.* **10**, 430-438.

Guadagno, T. M. and Ferrell, J. E., Jr (1998). Requirement for MAPK activation for normal mitotic progression in Xenopus egg extracts. *Science* **282**, 1312-1315.

Haccard, O., Sarcevic, B., Lewellyn, A., Hartley, R., Roy, L., Izumi, T., Erikson, E. and Maller, J. L. (1993). Induction of metaphase arrest in cleaving Xenopus embryos by MAP kinase. *Science* **262**, 1262-1265.

Hansen, D. V., Tung, J. J. and Jackson, P. K. (2006). CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc. Natl. Acad. Sci. USA* **103**, 608-613.

Inoue, D., Ohe, M., Kanemori, Y., Nobui, T. and Sagata, N. (2007). A direct link of the Mos-MAPK pathway to Erp1/Emi2 in meiotic arrest of Xenopus laevis eggs. *Nature* **446**, 1100-1104.

Kimata, Y., Trickey, M., Izawa, D., Gannon, J., Yamamoto, M. and Yamano, H. (2008). A mutual inhibition between APC/C and its substrate Mes1 required for meiotic progression in fission yeast. *Dev. Cell* **14**, 446-454.

Kosako, H., Gotoh, Y. and Nishida, E. (1994a). Mitogen-activated protein kinase kinase is required for the mos-induced metaphase arrest. *J. Biol. Chem.* **269**, 28354-28358.

Kosako, H., Gotoh, Y. and Nishida, E. (1994b). Regulation and function of the MAP kinase cascade in Xenopus oocytes. *J. Cell Sci.* **Suppl. 18**, 115-119.

Liu, J. and Maller, J. L. (2005). Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. *Curr. Biol.* **15**, 1458-1468.

Liu, J., Grimison, B., Lewellyn, A. L. and Maller, J. L. (2006). The anaphase-promoting complex/cyclosome inhibitor Emi2 is essential for meiotic but not mitotic cell cycles. *J. Biol. Chem.* **281**, 34736-34741.

Liu, J., Grimison, B. and Maller, J. L. (2007). New insight into metaphase arrest by cytostatic factor: from establishment to release. *Oncogene* **26**, 1286-1289.

Lorca, T., Galas, S., Fesquet, D., Devault, A., Cavadore, J. C. and Doree, M. (1991). Degradation of the proto-oncogene product p39mos is not necessary for cyclin proteolysis and exit from meiotic metaphase: requirement for a Ca(2+)-calmodulin dependent event. *EMBO J.* **10**, 2087-2093.

Lorca, T., Cruzalegui, F. H., Fesquet, D., Cavadore, J. C., Mery, J., Means, A. and Doree, M. (1993). Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of Xenopus eggs. *Nature* **366**, 270-273.

Madgwick, S., Hansen, D. V., Levasseur, M., Jackson, P. K. and Jones, K. T. (2006). Mouse Emi2 is required to enter meiosis II by reestablishing cyclin B1 during interkinesis. *J. Cell Biol.* **174**, 791-801.

Masui, Y. and Markert, C. L. (1971). Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool.* **177**, 129-145.

Miller, J. J., Summers, M. K., Hansen, D. V., Nachury, M. V., Lehman, N. L., Loktev, A. and Jackson, P. K. (2006). Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. *Genes Dev.* **20**, 2410-2420.

Mochida, S. and Hunt, T. (2007). Calcineurin is required to release Xenopus egg extracts from meiotic M phase. *Nature* **449**, 336-340.

Mori, M., Hara, M., Tachibana, K. and Kishimoto, T. (2006). p90Rsk is required for G1 phase arrest in unfertilized starfish eggs. *Development* **133**, 1823-1830.

Nishiyama, T., Ohsumi, K. and Kishimoto, T. (2007a). Phosphorylation of Erp1 by p90rsk is required for cytostatic factor arrest in Xenopus laevis eggs. *Nature* **446**, 1096-1099.

Nishiyama, T., Yoshizaki, N., Kishimoto, T. and Ohsumi, K. (2007b). Transient activation of calcineurin is essential to initiate embryonic development in Xenopus laevis. *Nature* **449**, 341-345.

Ohe, M., Inoue, D., Kanemori, Y. and Sagata, N. (2007). Erp1/Emi2 is essential for the meiosis I to meiosis II transition in Xenopus oocytes. *Dev. Biol.* **303**, 157-164.

Ohsumi, K., Koyanagi, A., Yamamoto, T. M., Gotoh, T. and Kishimoto, T. (2004). Emi1-mediated M-phase arrest in Xenopus eggs is distinct from cytostatic factor arrest. *Proc. Natl. Acad. Sci. USA* **101**, 12531-12536.

Okumura, E., Fukuhara, T., Yoshida, H., Hanada Si, S., Kozutsumi, R., Mori, M., Tachibana, K. and Kishimoto, T. (2002). Akt inhibits Myt1 in the signalling pathway that leads to meiotic G2/M-phase transition. *Nat. Cell Biol.* **4**, 111-116.

Phillips, K. P., Petrunewich, M. A., Collins, J. L., Booth, R. A., Liu, X. J. and Baltz, J. M. (2002). Inhibition of MEK or cdc2 kinase parthenogenetically activates mouse eggs and yields the same phenotypes as Mos(–/–) parthenogenotes. *Dev. Biol.* **247**, 210-223.

Posada, J., Yew, N., Ahn, N. G., Vande Woude, G. F. and Cooper, J. A. (1993). Mos stimulates MAP kinase in Xenopus oocytes and activates a MAP kinase kinase *in vitro*. *Mol. Cell. Biol.* **13**, 2546-2553.

Rauh, N. R., Schmidt, A., Bormann, J., Nigg, E. A. and Mayer, T. U. (2005). Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. *Nature* **437**, 1048-1052.

Reimann, J. D. and Jackson, P. K. (2002). Emi1 is required for cytostatic factor arrest in vertebrate eggs. *Nature* **416**, 850-854.

Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J. and Vande Woude, G. F. (1988). Function of c-mos proto-oncogene product in meiotic maturation in Xenopus oocytes. *Nature* **335**, 519-525.

Sagata, N., Watanabe, N., Vande Woude, G. F. and Ikawa, Y. (1989). The c-mos proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature* **342**, 512-518.

Schmidt, A., Duncan, P. I., Rauh, N. R., Sauer, G., Fry, A. M., Nigg, E. A. and Mayer, T. U. (2005). Xenopus polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev.* **19**, 502-513.

Schmidt, A., Rauh, N. R., Nigg, E. A. and Mayer, T. U. (2006). Cytostatic factor: an activity that puts the cell cycle on hold. *J. Cell Sci.* **119**, 1213-1218.

Schwab, M. S., Roberts, B. T., Gross, S. D., Tunquist, B. J., Taieb, F. E., Lewellyn, A. L. and Maller, J. L. (2001). Bub1 is activated by the protein kinase p90(Rsk) during Xenopus oocyte maturation. *Curr. Biol.* **11**, 141-150.

Sharp-Baker, H. and Chen, R. H. (2001). Spindle checkpoint protein Bub1 is required for kinetochore localization of Mad1, Mad2, Bub3, and CENP-E, independently of its kinase activity. *J. Cell Biol.* **153**, 1239-1250.

Shoji, S., Yoshida, N., Amanai, M., Ohgishi, M., Fukui, T., Fujimoto, S., Nakano, Y., Kajikawa, E. and Perry, A. C. (2006). Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. *EMBO J.* **25**, 834-845.

Sudakin, V., Chan, G. K. and Yen, T. J. (2001). Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *J. Cell Biol.* **154**, 925-936.

Tang, W., Wu, J. Q., Guo, Y., Hansen, D. V., Perry, J. A., Freel, C. D., Nutt, L., Jackson, P. K. and Kornbluth, S. (2008). Cdc2 and Mos Regulate Emi2 Stability to Promote the Meiosis I-Meiosis II Transition. *Mol. Biol. Cell* **19**, 3536-3543.

Tung, J. J., Hansen, D. V., Ban, K. H., Loktev, A. V., Summers, M. K., Adler, J. R., 3rd and Jackson, P. K. (2005). A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of Xenopus eggs. *Proc. Natl. Acad. Sci. USA* **102**, 4318-4323.

Tung, J. J., Padmanabhan, K., Hansen, D. V., Richter, J. D. and Jackson, P. K. (2007). Translational unmasking of Emi2 directs cytostatic factor arrest in meiosis II. *Cell Cycle* **6**, 725-731.

Tunquist, B. J. and Maller, J. L. (2003). Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.* **17**, 683-710.

Tunquist, B. J., Schwab, M. S., Chen, L. G. and Maller, J. L. (2002). The spindle checkpoint kinase bub1 and cyclin e/cdk2 both contribute to the establishment of meiotic metaphase arrest by cytostatic factor. *Curr. Biol.* **12**, 1027-1033.

Tunquist, B. J., Eyers, P. A., Chen, L. G., Lewellyn, A. L. and Maller, J. L. (2003). Spindle checkpoint proteins Mad1 and Mad2 are required for cytostatic factor-mediated metaphase arrest. *J. Cell Biol.* **163**, 1231-1242.

Wu, J. Q., Hansen, D. V., Guo, Y., Wang, M. Z., Tang, W., Freel, C. D., Tung, J. J., Jackson, P. K. and Kornbluth, S. (2007a). Control of Emi2 activity and stability through Mos-mediated recruitment of PP2A. *Proc. Natl. Acad. Sci. USA* **104**, 16564-16569.

Wu, Q., Guo, Y., Yamada, A., Perry, J. A., Wang, M. Z., Araki, M., Freel, C. D., Tung, J. J., Tang, W., Margolis, S. S. et al. (2007b). A role for Cdc2- and PP2A-mediated regulation of Emi2 in the maintenance of CSF arrest. *Curr. Biol.* **17**, 213-224.

Yamamoto, T. M., Iwabuchi, M., Ohsumi, K. and Kishimoto, T. (2005). APC/C-Cdc20-mediated degradation of cyclin B participates in CSF arrest in unfertilized Xenopus eggs. *Dev. Biol.* **279**, 345-355.

Yamano, H., Gannon, J., Mahbubani, H. and Hunt, T. (2004). Cell cycle-regulated recognition of the destruction box of cyclin B by the APC/C in Xenopus egg extracts. *Mol. Cell* **13**, 137-147.