Emi1 and Erp1:
Who Can Stop These Eggs?

Vertebrate eggs prevent parthenogenetic development by producing cytostatic factor (CSF), which blocks exit from metaphase of meiosis II until fertilization. CSF was never purified but recently suspected to inhibit the anaphase-promoting complex (APC), an ubiquitin ligase required for entry into anaphase. In a recent paper in *Genes & Development*, Schmidt et al. describe the Xenopus APC inhibitor Erp1, which seems to be the best candidate yet for the downstream effector of CSF activity.

Cytostatic factor (CSF) has been studied extensively in *Xenopus laevis* oocytes, which yield cell-free extracts that faithfully reproduce cell cycle events in vitro. Hormonal induction causes immature oocytes, which are arrested in meiotic prophase, to undergo maturation: they perform the first meiotic division and then, without an intervening S phase, proceed to metaphase of the second division. Fertilization triggers a surge in free cytoplasmic Ca^{2+}, which causes entry into anaphase followed by the onset of embryonic (i.e., mitotic) cleavage divisions. The classic injection experiments by Masui and Markert identified two different activities in the cytoplasm of mature oocytes: maturation-promoting factor (MPF) induced resumption of meiosis and, unlike MPF, never reappeared during the embryonic cell cycles. MPF was eventually purified and identified as the universal cell cycle regulator cyclin-dependent kinase 1 (Cdk1) bound to cyclin B. CSF was never purified, probably because several pathways rather...
than a single activity contribute to CSF function. Two main strategies have since been used to analyze CSF. The first one was to unravel, step by step, the pathway leading from the resumption of meiosis to the establishment of the metaphase II arrest. A second, more recent, approach was to examine regulators of APC activity for a role in CSF arrest and release (reviewed in Tunquist and Maller, 2003).

Analysis of the germ cell-specific protein kinase Mos provided the first molecule required to generate CSF activity. Similar to CSF, Mos appears during maturation and disappears shortly after fertilization. Mos activates a MAP kinase cascade whose sole downstream target is the kinase p90Rsk. In Xenopus oocytes, this pathway prevents complete destruction of Cdk1-cyclin B between meiosis I and II by increasing cyclin synthesis and counteracting APC-dependent cyclin degradation. Cdk1-cyclin B activity is required to suppress DNA replication after meiosis I and to drive oocytes into meiosis II. Once oocytes are in metaphase II, p90Rsk is not required anymore for the maintenance of the CSF arrest. However, the Mos-dependent pathway contributes more to the CSF arrest than "just" promoting entry into meiosis I-to-II transition.

The Ca^{2+} wave at fertilization causes entry into anaphase through activation of the APC, which requires association of the complex with the Cdc20 activator. Ubiquitination by APC-Cdc20 then triggers cyclin degradation and sister chromatid separation. Because APC-Cdc20 is essential for release from the CSF arrest, inhibition of APC-Cdc20 was thought to be CSF’s ultimate function. The spindle assembly checkpoint (SAC) has been identified as a potent inhibitor of APC-Cdc20. In somatic cells, the SAC is activated by kinetochore lacking spindle microtubules and blocks anaphase in response to spindle damage. In Xenopus oocytes, SAC components are not activated by kinetochore (because the nucleocyttoplasmic ratio is too low) but by p90Rsk in the cytoplasm. While SAC components are required to generate CSF activity in Xenopus oocytes, they seem to be dispensable for CSF in mouse oocytes. The Mos/MAPK pathway might inhibit APC-Cdc20 through additional mechanisms.

Recently, the APC inhibitor Emi1 has emerged as a CSF candidate. Cyclin accumulation in prophase of the mitotic cell cycle requires inhibition of APC-Cdc20 by Emi1, which is thought to block Cdc20’s ability to interact with APC substrates. In prometaphase, Emi1 is degraded to allow activation of the APC. Phosphorylation of a DSGxxS motif in Emi1 creates a “phosphodegron,” a docking site for the SCF-βTrCP ubiquitin ligase. Exogenous Emi1 causes cell cycle arrest in cleaving embryos and blocks cyclin degradation in Ca^{2+}-treated CSF extracts, demonstrating that while Emi1 can block both the embryonic and the meiotic cell cycle. The idea of Emi1 as a CSF component is supported by the detection of Emi1 in mature mouse oocytes and the finding that phosphorylation by p90Rsk strengthens Emi1’s interaction with Cdc20 (Paronetto et al., 2004). Whether Emi1 is essential for the CSF arrest in Xenopus eggs, however, is controversial. One group reported that Emi1 is more abundant than Cdc20 in CSF extracts and remains stable until the first mitotic division upon Ca^{2+} release (Reimann and Jackson, 2002). Another group failed to detect endogenous Emi1 under similar conditions and found rapid degradation of exogenous Emi1 in meiotic extracts, which is more consistent with Emi1’s behavior in mitosis (Ohsumi et al., 2004).

One solution to these inconsistencies may arise from the discovery of Thomas Mayer and colleagues that vertebrates contain an additional conserved APC inhibitor, the Emi1-related protein Erp1 (Schmidt et al., 2005). This protein is a prime candidate for the most downstream CSF component, at least in Xenopus oocytes: Erp1 appears during maturation, is essential for blocking APC-Cdc20 in metaphase II, and is abruptly degraded in response to Ca^{2+}. Erp1 was identified as a protein binding to polo-like kinase (Plk1) in an effort to unravel the mechanism by which Plk1 activates APC-Cdc20 upon fertilization. Inactivation of Plk1 was known to prevent cyclin degradation in Ca^{2+}-treated CSF extracts (Descombes and Nigg, 1998). Plk1 can phosphorylate APC subunits in vitro, but a clear in vivo function for these sites failed to emerge, nurturing the idea that Plk1 might inactivate an APC inhibitor. Indeed, Erp1’s degradation requires phosphorylation of a βTrCP degron by Plk1 (Schmidt et al., 2005). Interestingly, Plk1 also phosphorylates the degron of Emi1, suggesting that Plk1 has a general role in liberating the APC from its inhibitors (Hansen et al., 2004; Moshe et al., 2004).

Plk1 appears early during maturation, when it is required for the upregulation of Cdk1-cyclin B activity. This raises the question of how Ca^{2+} actually triggers the degradation of Erp1. Plk1 and its kin all contain a C-terminal polo box domain that binds to phosphopeptides containing S-pS/pT (Elia et al., 2003). Plk1 therefore phosphorylates substrates that have previously been phosphorylated by a “guiding kinase.” It is tempting to speculate that Ca^{2+}/camodulin-dependent kinase II (CaMKII) is the kinase that guides Plk1 to Erp1. CaMKII is activated by Ca^{2+} released upon fertilization, while a constitutively active version triggers activation of APC-Cdc20 even in the absence of Ca^{2+} (Lorca et al., 1993). Indeed, Erp1 contains potential CaMKII phosphorylation sites (RxxS/T) that can generate binding sites for the polo box domain.

Another question is how Erp1 and Emi1 inhibit APC-Cdc20’s ligase activity. Emi1 was reported to bind to Cdc20, while no interaction was detected for Erp1; whether Erp1 can bind to the APC was not tested. Erp1 and Emi1 inhibit the APC through a C-terminal, cysteine-rich region, which resembles an IBR (in between RING fingers) domain. This domain is thought to bind ubiquitin-conjugating enzymes (UBCs) in collaboration with the RING finger, which is a hallmark of ubiquitin ligases including the APC. Whether Erp1 and Emi1 inhibit the APC by affecting its interaction with the UBC remains to be tested.

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