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Extra View

Matrimony ties Polo down

Can this kinase get free?

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Cell cycle progression in female meiotic systems is characterized by the presence of two or more pre-programmed arrests. One such arrest is invariable throughout all species—a lengthy G_2 arrest that separates the end of pachytene (by which time homologous chromosomes have condensed, paired and undergone recombination[1]) from nuclear envelope breakdown (NEB). The termination of G_2 arrest (as defined by NEB) and the subsequent entry of the oocyte into prometaphase is regulated by an intracellular signaling cascade whose ultimate feature is the activation of the cyclin B-Cdk1 complex by the Cdc25 phosphatase family. In oocytes, activation of Cdc25 is often mediated by Polo-like kinases (Plks).[2-6] Recent work by Xiang et al. demonstrated that in Drosophila female meiosis, Polo's role in promoting NEB is regulated by a meiosis-specific stoichiometric inhibitor called Matrimony (Mtrm), which binds to the C-terminal Polo-box domain (PBD) of Polo.[7] In addition to a PBD-binding site, Mtrm contains putative Plk and cyclin B-Cdk1 phosphorylation consensus motifs. These motifs suggest a unique mechanism of Polo inhibition by Mtrm and a possible auto-amplification loop by which cyclin B-Cdk1-mediated destruction or dissociation of Mtrm from Polo allows for rapid and irreversible G_2 exit and entry into prometaphase.

Although many important aspects regarding the temporal control of the meiotic G_2/M transition remain to be elucidated, we know a great deal about its ultimate effector cyclin B-Cdk1.[8-10] In eukaryotes participating in "open" mitosis or meiosis, activated cyclin B-Cdk1 functions to phosphorylate nuclear components, including the lamina, causing dissolution of the nuclear envelope—the hallmark of mitotic and meiotic entry.[11] Proper initiation of this inaugural event depends on multiple layers of tight control over numerous cell cycle regulators, including cyclin B-Cdk1 itself.[12]

Beyond binding of Cdk1 to its positive regulatory subunit, cyclin B, the phosphorylation of several key residues on Cdk1 act together to either promote or inhibit the activity of the complex. On one hand, phosphorylation of a threonine residue (Thr161 in metazoan Cdk1) in the activation loop (T-loop) of cyclin B-Cdk1 by Cdk-activating kinase serves to activate the kinase domain of Cdk1. Conversely, inhibitory phosphorylation at two other sites (Thr14 and Tyr15 in metazoan Cdk1) by kinases of the Myt1 and Wee1 families maintains the newly formed cyclin B-Cdk1 complexes in an inactive state. Removal of this inhibition is achieved by dephosphorylation of these residues by the Cdc25 family of phosphatases, which results in rapid activation of cyclin B-Cdk1 and thus mitotic/meiotic entry.[13,14]

Multiple lines of evidence demonstrate that Cdc25-mediated activation of cyclin B-Cdk1 is essential for initiating NEB in female meiosis.[15] For example, female *Cdc25b*-deficient mice are sterile due to permanent oocyte arrest at prophase of meiosis I with intact nuclear envelopes, while microinjection of *Cdc25b* mRNA into these oocytes catalyzes NEB within one hour.[16] Kinase assays using extracts derived from *Cdc25b*-deficient oocytes reveal low cyclin B-Cdk1 activity. Additionally, Xiang et al. have demonstrated that Drosophila oocytes homozygous for a loss-of-function allele of *twine*, the meiotic homolog of Cdc25 in Drosophila, display a significantly delayed onset of NEB.[7] Thus, Cdc25 is essential to meiotic cyclin B-Cdk1 activation, and therefore, in the termination of G_2 arrest in oocytes.

Mechanisms that regulate Cdc25 activation in oocytes are less well characterized. Recently, Plks have been implicated as positive effectors in the circuit controlling meiotic entry.[17] For example, the *C. elegans* Plk homolog, PLK-1, has been shown to be required for proper timing of NEB. Chase et al. demonstrated that silencing of PLK-1 expression in *C. elegans* oocytes by RNA-mediated interference (RNAi) significantly delays NEB, resulting in a nucleus that persists until just after fertilization. This delay in NEB can be phenocopied in *ncc-1* RNAi oocytes (NCC-1 is the *C. elegans* homolog of Cdk1), suggesting that PLK-1 and NCC-1 may function in the same pathway.[4] Work on Xenopus oocytes also indicates that Plx1 (the Xenopus Plk homolog) functions as a "trigger" kinase for Cdc25 activation. Specifically, Qian et al. have demonstrated that immunodepletion of Plx1 completely inhibits both Cdc25 and cyclin B-Cdk1 activation, and furthermore, inhibiting cyclin B-Cdk1 does not affect Cdc25 phosphorylation or the activity of Plx1.[6] Work on porcine oocytes, which exploits a characteristically long G_2 arrest relative to rodents, also clearly demonstrated that Plk1 is activated prior to cyclin B-Cdk1 activation.[3,18] Thus, in multiple systems, Plks play a critical role in controlling meiotic re-start.

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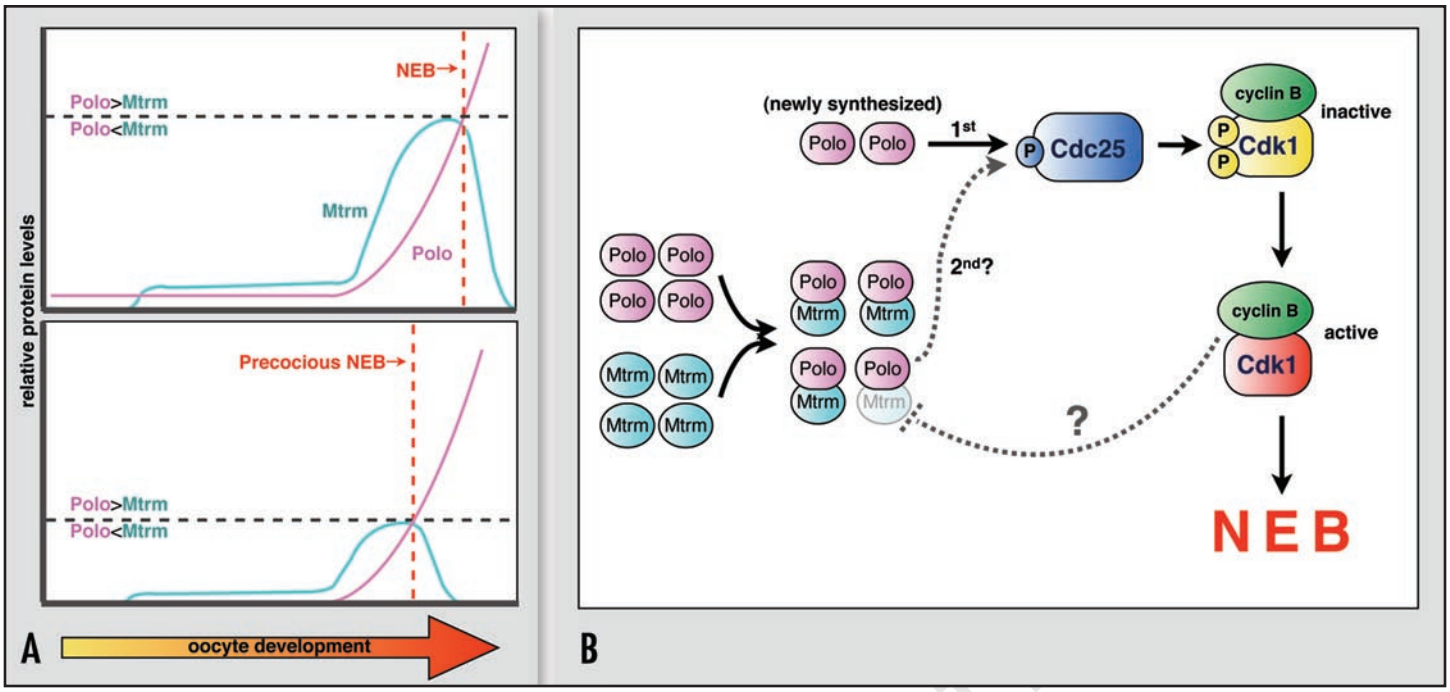

Figure 1. Mtrm-induced inhibition of Polo controls proper timing of NEB. (A) A model (adapted from Fig. 9 of Xiang et al.) demonstrating stoichiometric inhibition of Polo by Mtrm prior to NEB onset in female meiosis. In the top panel, which depicts a wildtype oocyte, an excess of Mtrm regulates Polo activity until Polo protein levels exceed the available amount of Mtrm. Polo then initiates the chain of events leading to the initiation of NEB at the appropriate time in oocyte development. The lower panel depicts precocious NEB in the absence of a sufficient amount of Mtrm created by heterozygosity for a loss-of-function allele of *mtrm*. (B) A model for cyclin B-Cdk1 auto-amplification via dissociation or destruction of Mtrm from the Mtrm-Polo complex. This allows for unbound Polo to further activate Cdc25 and trigger rapid onset of NEB.

Mtrm Controls Polo in Drosophila Oocytes

Achieving a "switch-like" transition from prophase to prometaphase requires fine-tuning mechanisms that act to ensure that NEB onset occurs both rapidly and at the appropriate time. How then can Plks, which are known control a large number of meiotic events,[17] be temporally and specifically regulated as to allow for a precise oocyte re-start? Given that Plks act upon a large number of substrates,[19] one can imagine that a simple and gradual increase in Plk activity would not permit precise and rapid re-entry into the meiotic cell division cycle. Instead, the oocyte needs a tightly controlled mechanism to regulate Plk, either by the transcriptional or translational repression of stimulatory proteins that activate Plk or by the presence of inhibitory proteins that preclude Plk activity until the appropriate time. Xiang et al. have provided evidence that the temporal control of Polo activity in Drosophila oogenesis is mediated by a small protein called Matrimony (Mtrm) that serves as a stoichiometric inhibitor of Polo.[7] Mtrm appears to block Polo activity during the initial stages of Polo synthesis, allowing Polo to exert its effect only when the level of Polo protein exceeds the amount of Mtrm (see Fig. 1).

The first indication of a physical interaction between Mtrm and Polo came from a large scale yeast two-hybrid screen and was subsequently confirmed by co-immunoprecipitations.[7,20] Additional data obtained by multidimensional proteomic identification technology (MudPIT) indicated a 1:1 stoichiometric relationship between Mtrm and Polo. Utilizing three independent affinity purifications from ovarian extracts expressing C-terminally tagged Mtrm protein, Polo was the only interacting protein recovered at levels similar to those of Mtrm.[7]

Not only do Mtrm and Polo physically interact, but genetic analysis has demonstrated a functional relationship between the two proteins. Drosophila females heterozygous or homozygous for a null allele of *mtrm* display dosage-dependent precocious NEB. Moreover, the precocious NEB observed in *mtrm/+* oocytes can be suppressed by simultaneously reducing the dose of *polo*$^{+}$, supporting the idea that the functionality of this relationship hinges upon the ability of Mtrm to inhibit Polo in a stoichiometric fashion.[7] Indeed, decreasing the expression of Mtrm or increasing the expression of Polo causes precocious NEB, while decreasing the dose of both proteins maintains the stoichiometry and thus maintains proper timing of NEB. Therefore, the inhibition of Polo activity by Mtrm allows for proper timing of NEB in Drosophila oocytes.

As illustrated in Figure 1A, the expression patterns of both Mtrm and Polo are fully consistent with a model in which the stoichiometric inhibition of Polo controls the timing of NEB. The accumulation of Mtrm in the oocyte cytoplasm begins well before Polo is first observed. Subsequent Polo protein expression is thought to be harnessed by binding to Mtrm until the stage at which NEB normally occurs in Drosophila oocyte development.[7] Xiang et al. propose that in this stage, Polo protein levels begin to exceed that of Mtrm, and the newly synthesized Polo is, thus, unhindered and available for use by the cell. Presumably, this un-bound Polo then activates Cdc25. As shown in the lower panel in Figure 1A, oocytes with reduced levels of Mtrm allow unbound Polo to accumulate at earlier stages of oogenesis, thus accelerating the onset of NEB. Moreover, as might be expected if the primary function of Mtrm is to regulate Polo to allow for proper timing of NEB, Mtrm is rapidly degraded at the onset of NEB (see below). Additional evidence

supporting the functionality of a stoichiometric relationship between Mtrm and Polo to regulate the timing of NEB comes from the observation that effects of reducing the levels of Mtrm can be ameliorated by simultaneously reducing the levels of Twine, the meiotic homolog of Cdc25 in Drosophila.[7]

Functional Characterization of the Mtrm-Polo Interaction

The findings presented above raise the question—how does Mtrm physically interact with Polo so as to regulate its function? Plks contain two functional domains, an N-terminal Ser/Thr kinase domain and a C-terminal Polo-box domain (PBD). In many cases, the PBD has been shown to preferentially bind phosphothreonine/serine residues located in the consensus motif (S-pS/pT-P/X) residing in target proteins.[21-24] Mtrm contains one such functional PBD-binding site, a STP with the central threonine at position 40. Utilizing MudPIT mass spectrometry, Mtrm T40 was found to be consistently phosphorylated in vivo. Mutation of T40 to nonphosphorylateable alanine ablates the interaction between Mtrm and Polo as evidenced by coimmunoprecipitations and genetic analysis, indicating that the PBD of Polo recognizes and binds to a version of Mtrm phosphorylated at T40.[7]

Recent proteomic and bioinformatic analysis of PBD-interacting proteins have revealed that PBD-binding sites frequently co-occur with Plk phosphorylation motifs.[25] Mtrm is no exception, containing a putative Plk phosphorylation consensus motif (D/E-X-S/T-Ø-X-D/E) with the phosphorylatable serine at position 137 in addition to the bona fide T40 PBD-binding site. MudPIT mass spectrometric analysis revealed that residue S137 is phosphorylated at reproducibly high levels[7]—consistent with the view that Mtrm not only binds to the C-terminal PBD to regulate Polo activity, but that Mtrm is also a substrate of Polo.

What kinase phosphorylates Mtrm at residue T40 and primes Mtrm for binding to the PBD? The observation that Mtrm and Polo interact in the budding yeast two-hybrid system strongly suggests that Mtrm T40 is phosphorylated in this assay since phosphorylation of T40 appears to be critical for Mtrm-Polo binding in Drosophila. Interestingly, when Mtrm protein is expressed alone in *S. cerevisiae*, Mtrm T40 is not successfully phosphorylated, indicating that a yeast kinase is not a likely culprit of the PBD-binding site phosphorylation event (our unpublished results). Instead, perhaps the simplest model to explain how Mtrm and Polo bind in the yeast two-hybrid system is that Polo, itself, phosphorylates Mtrm T40, priming its own PBD-binding site. Although T40 does not lie within a well-defined Polo consensus motif, several recent reports have suggested that Plks are promiscuous in recognizing their targets.[19,26,27] Moreover, studies by Neef et al. demonstrated that Plk1 is capable of priming its own PBD-binding site to facilitate its interaction between the anaphase-specific docking partners MKlp2 and PRC1-2 in HeLa cells.[26-28] Thus, the notion that Plks can serve as their own priming kinase is not unprecedented.

The possibility that Polo phosphorylates Mtrm residues T40 and/or S137 suggests that Polo is bound to Mtrm in a catalytically active configuration. In fact, Mtrm-Polo protein complexes analyzed by MudPIT mass spectroscopy supports this view. That is, Polo's T-loop region is phosphorylated on residue T182, which strongly correlates with a catalytically active kinase domain.[17] This observation raises the question: if Mtrm-bound Polo is able to phosphorylate Mtrm, then what prevents the kinase from acting upon other substrates such as Cdc25? Genetic and cell biological evidence presented in Xiang et al. support the view that Polo is functionally inactive while in complex with Mtrm in vivo, at least with regards to the ability of Polo to phosphorylate other substrates. One mechanism by which a catalytically active Polo kinase could appear inactive is via sequestration of Polo by Mtrm to some subcellular compartment, away from Polo's key meiotic substrates. However, Xiang et al. find Mtrm and Polo to be localized throughout the cell at the pertinent stages of Drosophila oogenesis.[7]

If not by sequestration, then how else might Mtrm inhibit Polo? Unlike other PBD-interacting proteins and/or substrates of Plks, Mtrm has the unique ability to bind to Polo with a relatively high affinity (likely in the micromolar range). This is not a typical characteristic of kinase-substrate interactions nor does it seem to be the case for other PBD-interacting proteins with Plks.[19,25] Thus, it is possible that Mtrm and Polo may share some third interaction domain that mediates high-affinity binding. Mtrm may also uniquely bind to Polo as to alter its configuration, interlocking the two proteins together and perhaps preventing Polo's N-terminal kinase binding cleft from acting upon other substrates. Future analysis of the domains of Mtrm that are necessary and sufficient for Polo inhibition are required to address this question.

Does the Destruction of Mtrm Free Polo?

Given that Mtrm and Polo form a stable complex, then mechanisms to disrupt their interaction likely exist and may play a role in freeing Mtrm-bound Polo once oocyte re-start is initiated. Intriguingly, Mtrm also contains a putative cyclin B-Cdk1 phosphorylation consensus motif (S/T-P-X-R/K). As previously mentioned, once the levels of Polo exceed the concentration of Mtrm, un-bound (newly synthesized) Polo can activate Cdc25 and thus activate cyclin B-Cdk1. This view, coupled with evidence that Mtrm is rapidly degraded at the onset of NEB,[7] suggests the possibility that activated cyclin B-Cdk1 facilitates an auto-amplication loop serving to release the remaining Mtrm-bound Polo via cyclin-B-Cdk1-dependent dissociation or destruction of Mtrm (see Fig. 1B). Further support for such a model is found in other species and is a common mechanism used to ensure rapid and complete cycle transitions. Xenopus oocyte studies found that detectable Plx1 activity occurs downstream of cyclin B-Cdk1 activation, indicating that Plx1 participates in the auto-amplification loop between Cdc25 and cyclin B-Cdk1.[2] Similarly, in mouse oocytes, inhibition of Plk1 activity occurs following treatment with two specific cyclin B-Cdk1 inhibitors.[5] Because Polo T182 has been shown to be phosphorylated in the Mtrm-Polo complex,[7] destruction or dissociation of Mtrm could lead to an immediate increase in Polo activity, since Plk kinase domains phosphorylated within their T-loop region do not appear to be inhibited via intra-molecular binding of the PBD.[24]

Overall, the details of the interaction between Mtrm and Polo, particularly regarding the roles of the putative Plk and cyclin B-Cdk1 phosphorylation consensus motifs, remain unclear, but the work presented by Xiang et al. clearly demonstrates the tremendous value in elucidating such mechanisms. Indeed, Plk overexpression has been implicated in a broad range of human cancers,[29] and unique Plk features such as the PBD will undoubtedly serve as ideal targets

for selective Plk inhibition. At least in terms of Drosophila female meiosis, it seems that nature has already taken advantage of this opportunity.

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