Mini-Review

Polo Kinase: The Choreographer of the Mitotic Stage?

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THE regulation of protein function through phosphorylation is fundamental in controlling cell cycle progression. To date, most attention has focused on the cyclin-dependent protein kinases (cdks)[1] (for review see reference 21). However, whereas the $p34^{cdc2}$-cyclin B complex appears to regulate the mitotic "state" and in this way changes the overall organization of the cell, members of another conserved serine/threonine kinase family appears to be able to control the dynamics of cellular architecture. These are the polo-like kinases (plks) which orchestrate several mitotic events including the formation of the bipolar spindle, and at least in some organisms, the process of cytokinesis. It appears that in some of its roles the plk cooperates with $p34^{cdc2}$ and indeed recent work (15) has suggested that one plk can help maintain the mitotic state by phosphorylating the cdc25 phosphatase that activates $p34^{cdc2}$.

The founding member of this kinase family is encoded by the *Drosophila* gene *polo* (20, 25). Homologous genes encoding polo-like kinases have been identified from various eukaryotes: *CDC5* of *S. cerevisiae* (14); *plo1* of *S. pombe* (22); the *Plx1* gene of *Xenopus* (reference 15; Tavares, A., H. Ohkura, C. Smythe, and D.M. Glover, unpublished results); and the *Plk* genes of mouse and human (3, 7, 9, 12, 16). All these proteins exhibit a high degree of identity not only in the NH_2-terminal catalytic domain, but also in an equally long COOH-terminal domain where three consensus motifs characterize this kinase family (Fig. 1). The extension of identity beyond the catalytic domain strongly suggests that they are true homologues, although as yet there have been no reports that show functional exchangeability between species. Perhaps a more important question is whether the polo-like kinases of different organisms have equivalent biological functions. This would appear to be the case, at least one common role being observed in fission yeast, *Drosophila* and human cells where they regulate centrosome separation in mitosis. As with the cdks, mammals also appear to have evolved a group of plk family members that function in G1. These cousins are represented by the mouse kinases Snk and Fnk (24, 4), and the human kinase Prk (19) that are induced in response to mitogens. Finally, another kinase, Sak (6), which appears to be involved in cell cycle regulation, was described as being polo-like because of some homology in the catalytic domain, but lacks the three COOH-terminal consensus subdomains, and thus it is not clear that it is a family member. In this short article, we will concentrate upon reviewing the functions of the plks that function during mitosis.

Pas de Deux: The Centrosome Cycle

The *polo*^{1} mutation of *Drosophila* was first identified as a maternal effect mutant giving rise to embryos that show abnormal mitotic networks of microtubules, and in which centrosomal components fail to organize into discrete structures (25). The mutation also affects centrosome behavior in larval neuroblasts, which show a wide range of mitotic defects including the formation of monopolar spindles and spindles with broad poles (25, 20). Multipolar spindles are seen in male meiosis, and these mutant males show meiotic nondisjunction and reduced fertility. The pleiotropy of the mutant phenotype could reflect diverse roles for the protein, but may also in part reflect the hypomorphic nature of this particular allele of *polo* (Fig. 2).

Loss of polo-like kinase function also leads to the formation of monopolar spindles in organisms as diverse as fission yeast and man. One of the two major phenotypes resulting from gene disruption of the *plo1* gene in fission yeast is a failure of the spindle pole bodies to form a bipolar spindle (reference 22; Fig. 2). Lane and Nigg (17) have now observed that microinjection of antibodies to human Plk1 into cultured human cells blocks the cells in mitosis with monopolar spindles that are nucleated by centrosomes that appear to have duplicated but have not separated. These findings are remarkable given the entirely different structure of the spindle pole bodies of yeasts and the centrosomes of animal cells. They suggest that the polo kinase is part of a fundamental signal to generate a bipolar spindle that has been conserved in spite of the evolutionary divergence of the structure of the spindle poles.

It seems that not only is the process of centrosome separation affected by the injection of anti-Plk1 antibodies into human cells, but also that the centrosomes fail to undergo the increase in size normally seen immediately before mitosis (17). They remain small and contain reduced amounts of immunoreactivity to antibodies against γ-tubulin and to MPM-2, a monoclonal antibody that reacts with a large number of proteins phosphorylated specifically at mitosis. This has led Lane and Nigg (17) to suggest that human

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1. *Abbreviations used in this paper*: cdk, cyclin-dependent protein kinase; plk, polo-like kinase.

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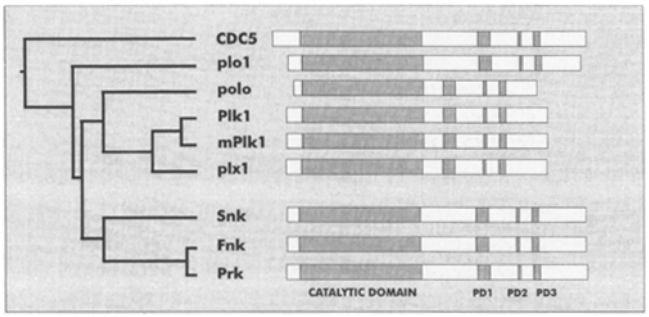

Figure 1. Phylogenetic analysis of the polo-like kinases inferred from the amino acid sequence alignments. The primary structure of the proteins is also represented, with the four regions of higher homology in dark boxes. These are the catalytic domains and three sub-domains in the COOH-terminal (PD1, PD2, and PD3 — **P**olo **D**omain) that constitute a characteristic of the members of this kinase family. Snk, Fnk, and Prk seem to have a G1/S associated function while the other kinases seem to be active during the M-phase.

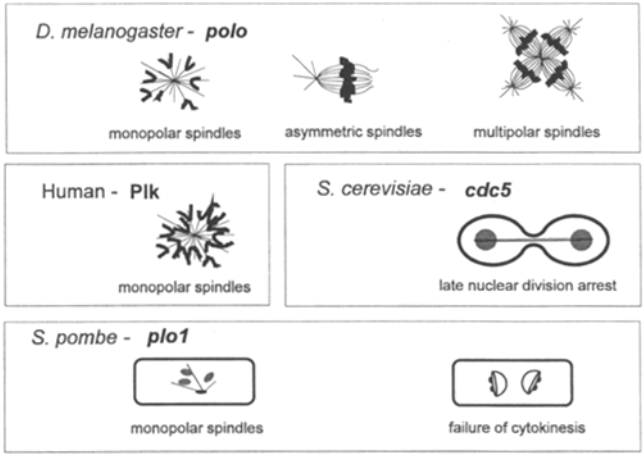

Figure 2. Mutant phenotypes of polo-like kinases in different organisms. In humans monopolar spindles are observed when anti-Plk1 antibodies are injected into cultured cells.

Plk1 plays a role in centrosome maturation, a concept that receives some support from the inability of centrosomes to recruit the CP190 antigen in *Drosophila polo*^{1}–derived embryos (25).

Immunostaining experiments in mammalian cells indicate that Plk1 is associated with the polar region of mitotic spindle from prophase to metaphase (8, 18). It is not clear whether there are any plk substrates associated with the centrosome per se, or indeed what these might be. Recent work with the *Drosophila* polo kinase indicates that it is likely to have a number of microtubule associated substrates (26). These studies identified a number of proteins that were differentially phosphorylated in extracts of wild-type embryos or embryos derived from *polo* females either by endogenous kinases, or by exogenously added polo kinase. One of these substrates appears to be β-tubulin, and other substrates are enriched in microtubule preparations, and are thus the type of molecule that might have been expected to be phosphorylated by the enzyme.

Coda: The Late Stages of Mitosis and Cytokinesis

It is clear from both fission yeast and budding yeast mutants, that loss of function of the respective genes *plo1*$^{+}$and *CDC5* results in late mitotic phenotypes. In addition to being required to generate a bipolar spindle, *plo1*$^{+}$ is also required for the process of septation (reference 22; Fig. 2). The *plo1*$^{+}$ gene is required for both actin ring formation and deposition of septal material, and because most septation mutants are defective in only one of these processes, the *plo1*$^{+}$ gene is likely to function high in the septation cascade.

The overexpression of *plo1*$^{+}$ in wild-type cells has the opposite effect in leading to the formation of multiple septa without nuclear division. Remarkably, septation can be induced by the overexpression of *plo1*$^{+}$ in G1-arrested cells; in cells arrested in G2; or in cells lacking the cyclin B homologue cdc13 that undergo repeated S-phases in the absence of mitosis. Thus, *plo1*$^{+}$ overexpression seems to overcome the normal dependency of cytokinesis upon the prior initiation of mitosis. The finding that *plo1*$^{+}$ function is both essential and sufficient for actin ring and septum formation has led to the proposal that *plo1*$^{+}$ is the key rate limiting inducer of this whole pathway (22). In short, plo1 kinase behaves as a "septum promoting factor" in *S. pombe.*

In contrast to the *Drosophila* and *S. pombe* mutants, *cdc5* mutants of *S. cerevisiae* retain the ability to form a bipolar spindle, but arrest nuclear division at a late stage when chromosomes have separated and the spindle elongated (2, 11) (Fig. 2). The gene product might still be required for some aspects of microtubule behavior, however, since following the release of a temperature-sensitive *cdc5* strain from a block at the nonpermissive temperature, cells become temporarily insensitive to the microtubule depolymerizing drug MBC (28).

It is not clear whether the plks of animal cells have an equivalent function in cytokinesis. *polo* mutants of *Drosophila* show polyploid cells in larval brains, together with multipolar spindles in male meiosis. It is, however, difficult to tell whether these phenotypes are consequences of defects in cytokinesis or of earlier aberrant centrosome behavior (25). No effects upon cytokinesis were seen when anti-HsPlk1 antibodies were injected into human cells already in metaphase (17). However, it is always possible that the requirement for the polo-like kinase in cytokinesis occurs earlier in the mitotic cycle and is obscured by the dramatic effects on centrosome behavior. The movement of mammalian polo-like kinase from the polar regions of the spindle microtubules during prophase and metaphase to the spindle mid-zone during anaphase, and to the midbody during telophase suggests a role in cytokinesis as a distinct possibility (8, 18). This pattern of subcellular localization in late mitosis is consistent with the reported association of Plk1 with the kinesin-like protein MKLP-1 (18), thought to mediate movement of overlapping pole–pole microtubules, and would appropriately position the kinase to modify substrates in the vicinity of the contractile ring at the onset of cytokinesis. However, at the present time direct evidence for a role of the enzyme in the cytokinesis of animal cells is lacking.

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Pas de Caractère: Polo-like Kinase Activates cdc25

The potential intimacy of the interaction between $p34^{cdc2}$ and polo-like kinases was recently demonstrated by the surprising findings of Kumagai and Dunphy (15) who set out to purify a kinase activity from *Xenopus* egg extracts that would activate the cdc25 phosphatase. This phosphatase, which activates $p34^{cdc2}$, has been known for some time to be positively regulated by its own state of phosphorylation. The cdc25-activating kinase that they purified turned out to be a *Xenopus* polo-like kinase, Plx1. Once phosphorylated by Plx1, the $p34^{cdc2}$ specific phosphatase activity of cdc25 is stimulated in vitro. It has already been shown that once it has been activated, $p34^{cdc2}$ can itself phosphorylate cdc25 forming part of a positive feedback loop that amplifies $p34^{cdc2}$ activation. It was now found that Plx1 and $p34^{cdc2}$ phosphorylate cdc25 on a set of sites corresponding to positions of phosphorylation by an M-phase extract (15). In addition there seem to be a number of additional sites that are phosphorylated by the egg extract, suggesting further enzymes may be involved. At present it is unclear whether plk might provide the activity that first activates $p34^{cdc2}$, or whether it plays a role in maintaining it in an active state, thus regulating progression through mitosis.

Cdc25 phosphorylated by Plx1 could be recognized by the monoclonal antibody MPM-2. Components of the mitotic spindle and of the anaphase-promoting complex become MPM-2 antigens during mitosis (see e.g., 27, 13). If polo-like kinases are major "MPM-2 kinases," this would be consistent with a general role in regulating the function of mitotic structures. It would also explain the failure to accumulate MPM-2 antigens at the centrosome in mammalian cells following the injection of antibodies to the human Plk1 (17).

Pas d'Action: Timing of Polo Kinase Activity

Activity measurements have not been made for the polo-like kinases of either fission yeast or budding yeast. One can only assume that their activation might occur during periods indicated by the timing of their cell cycle phenotype. If one compares the timing of spindle pole body separation and actin ring formation as the first indicators of bipolar spindle formation and cytokinesis, one is struck by the differences in the division cycles of these two yeasts. In the *S. pombe* cell cycle, these two events take place roughly at the same time, at a very early stage of mitosis, and consequently, there would be no need of a mechanism to control the independent timing of these two events. On the other hand in *S. cerevisiae* SPB separation takes place in S phase, whereas *cdc5* mutants arrest after spindle elongation at the time of actin ring formation (1, 2, 23). Thus, events which take place roughly simultaneously in *S. pombe*, are temporally separated in the *S. cerevisiae* cell cycle. While no SPB separation defects have been observed in *cdc5* mutants, a role in this process cannot yet be categorically ruled out. However, it is possible that *S. cerevisiae* might have abandoned one function of its polo-like kinase, or that another related kinase might have become specialized for this role, although a preliminary search of the yeast genome database suggests that this latter possibility is unlikely.

Timing of activity has only been examined in cultured mammalian cells and in the syncytial *Drosophila* embryo. In *Drosophila* embryos, $p34^{cdc2}$ kinase activity peaks in the beginning of mitosis, whereas polo kinase activity is maximal at late anaphase-telophase (5). In cultured mammalian cells, on the other hand, the activity of plk1 follows closely the profile of $p34^{cdc2}$ activity (8, 10, 18). How can one account for these apparent differences in activity profiles? At first sight, the timing of polo kinase activity in *Drosophila* syncytial embryos would appear inconsistent with a role in spindle formation. However, the mitotic cycle of the *Drosophila* embryo is unique: it is the shortest cell cycle known in all organisms, with no gap phases or cytokinesis; and lacking the checkpoint to prevent mitosis if DNA replication is incomplete. Interestingly, centrosome separation begins during telophase of the previous cycle, the time of maximal polo kinase activity. In mammalian cells centrosome separation takes place at prophase coinciding with the activity peak of both Plk1 and $p34^{cdc2}$ kinases. Consequently, the observations may not be as disparate as they appear at first sight.

Finale et Apothéose

The mitotic polo-like kinases are conserved enzymes that play a leading role in mitotic progression with at least three separate functions. First, they are essential to establish bipolarity of the spindle in organisms as diverse as fission yeast, flies, and mammals. Second, in the yeasts they are essential for the cytokinesis pathway, and indeed they can drive the whole of the pathway for actin ring formation and septation in *S. pombe*. Although there may be a requirement for the plks in the cytokinesis of animal cells, this has yet to be proven. A third property is the ability to activate cdc25 and so contribute to maintaining $p34^{cdc2}$ in an active state. This is a property that plk shares with $p34^{cdc2}$ itself, and would be an effective way of maintaining the mitotic state. It remains to be seen to what extent and how the two enzymes cooperate in other aspects of mitotic progression. At present very little is known about the mechanisms that regulate the activation of the plks. Active polo kinase obtained from *Drosophila* embryo extracts is inactivated following phosphatase treatment (26). Moreover, polo is underphosphorylated in *polo*^{1} mutants. The mammalian plks also appear to be regulated by their phosphorylation state (10). However, the kinases responsible for this activation are unknown. The identification of genes from *Drosophila*, fission yeast, and budding yeast that show interactions with the respective *polo, plo1,* and *CDC5* genes should prove to be an effective way to elaborate both the mechanisms that regulate the activation of the polo-like kinases, and to identify their substrates.

We apologize to authors whose work we have not cited due to space constraints.

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