REVIEW

Polo-like kinases: a team that plays throughout mitosis

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When the first mutant allele of the *Drosophila* gene *polo* was first characterized over 10 years ago, attention focused on the defects that centrosome behavior exhibited at various stages of development (Sunkel and Glover 1988). The subsequent realization that the serine-threonine kinase it encodes is highly conserved from yeasts to humans has provoked a flurry of investigation into the function of the enzyme. A role for the polo-like kinases (plks) in regulating centrosome behavior has been borne out in several organisms, and the enzymes have attracted further attention recently with the realization that they regulate multiple stages of mitotic progression. In this article we review the current status of our understanding of the functions of plks from the time of commitment to M phase in the activation of Cdc25, through the activation of the anaphase promoting complex (APC), to the regulation of late mitotic events essential for cytokinesis. We discuss how to reconcile the sometimes apparently disparate observations made upon plk function in different organisms.

The team members

The plks are recognizable as a team because in addition to a highly conserved amino-terminal catalytic domain, their carboxyl termini contain three conserved regions, the polo boxes (for review, see Glover et al. 1996; Lane and Nigg 1997). Whereas in the yeasts and *Drosophila* only a single plk gene has been identified to date (Llamazares et al. 1991; Kitada et al. 1993; Ohkura et al. 1995), the higher vertebrates have multiple plk genes of which *Plk1* is most similar to *Drosophila polo*. Two other plks, Snk and Fnk, have been described in mouse, as well as their human counterparts, hSnk and Prk [Clay et al. 1993; Lake and Jelinek 1993; Hamanaka et al. 1994; Holtrich et al. 1994; Golsteyn et al. 1994; Li et al. 1996; B. Ouyang and W. Dai (GenBank accession no. AF059617)]. We suggest that it may be appropriate to adopt a new nomenclature for these enzymes, and we will refer to Snk as Plk2, and to Fnk/Prk as Plk3, but will for the time being continue to use this nomenclature side by side with the original terms. A fourth mouse enzyme, Sak, which also appears to have a cell-cycle function was described as being as polo-related kinase by Fode and colleagues (1994). At the moment, however, we reserve judgement as to whether it is a bona fide family member, because although it shows high homology with plks in the catalytic domain, it lacks the three characteristic polo boxes in the carboxyl terminus.

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Positioning the players: the timing and localization of plk is consistent with multiple M-phase functions

All the plks so far examined share the common property of associating with the spindle poles early in mitosis. This is remarkable given the disparate architecture of these microtubule organizing centers (MTOCs) in different organisms, and indeed between different cell types in the same organism (Fig. 1). The *Saccharomyces cerevisiae* Cdc5p and *Schizosaccharomyces pombe* Plo1 kinases are found in association with the spindle pole bodies (SPBs), although their precise location with respect to SPB ultrastructure has not been determined (Shirayama et al. 1998; D. Mulvihill, H. Ohkura, D. Glover, and I. Hagan, in prep.). Immunolocalization studies reveal that the fission yeast enzyme moves onto the SPB and the forming spindle at mitotic entry, but is lost from the spindle about half way through anaphase when SPB association becomes dramatically weaker. The metazoan counterparts are found at the centrosomes from prophase until anaphase (Golsteyn et al. 1995; Lee et al. 1995; Adams et al. 1998; Logarinho and Sunkel 1998; Qian et al. 1998). In addition, a punctate distribution of the enzyme over chromatin from prophase until anaphase in positions that correspond to the centromeres has been described in *Drosophila* and mouse (Logarinho and Sunkel 1998; Wianny et al. 1998). A change in the localization of the animal cell plks occurs at the onset of anaphase, when the plks are no longer found in the centromeres but accumulate in the central spindle where they remain clearly visible in the midbody at telophase after the centrosomal staining is lost (Figs. 2 and 3). The animal cell centrosome is usually a corpuscular structure comprised of microtubule nucleating pericentriolar material organized around a pair of centrioles. However, there are no centrioles in the mammalian oocyte, and

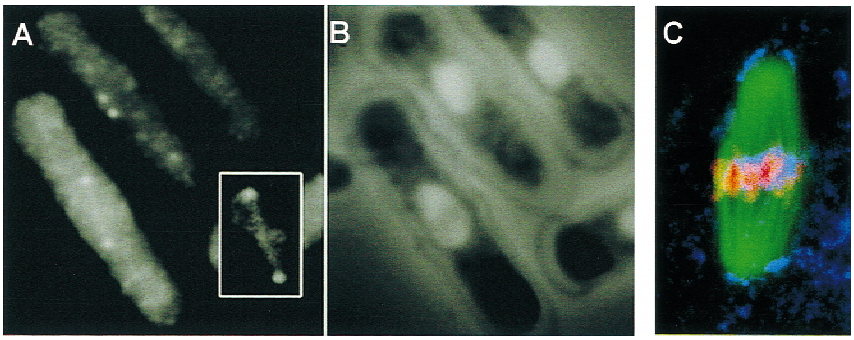

Figure 1. Plks localize to spindle poles of radically different architecture. Fission yeast cells immunostained to reveal Plo1 (*A*) and DNA (*B*). The enzyme associates with the SPBs of the middle cell at the onset of mitosis, and remains there until partway through anaphase. The spindle staining of the later mitotic cell can be seen more clearly in the inset (taken from D. Mulvihill, H. Ohkura, D. Glover, and I. Hagan, in prep.). (*C*) Metaphase II spindle of a mouse oocyte. (Red) DNA; (green) microtubules; (blue) Plk1. Plk can be seen associated with the centromeres of chromosomes, and at punctate foci along the spindle poles, which lack centrioles and so are unusually broad (taken from Wianny et al. 1998).

consequently the meiotic spindles have very broad poles (Maro et al. 1985). Nevertheless, despite these differences in spindle architecture, mouse plk1 maintains its ability to localize to the broad poles of the meiotic spindle where it is associated with a string of multiple punctate foci (Wianny et al. 1998). This localization of Plk to specific components of the spindle apparatus at different stages of mitosis or meiosis is likely to reflect temporally and spatially distinct functions of the enzyme.

The timing of activity of plks throughout the cell division cycle also gives general support to the notion that plk function is required at several points during mitotic progression. In syncytial *Drosophila* embryos, for example, the peak of polo kinase activity at late anaphase–telophase is quite distinct from the peak of cyclin-dependent kinase 1 (cdk1; also known as $p34^{cdc2}$ and MPF) activity on the entry into mitosis (Fenton and Glover 1993). This would be consistent with a late mitotic function for the enzyme. At the same time, a role in centrosome separation is not excluded because of the peculiarities of the syncytial nuclear division cycles. These accelerated cycles first occur at intervals of ~10 min, and to accomplish the cycle within this short time frame, centrosome separation begins during the telophase of the previous cycle, the time at which polo is maximally active.

The profiles of plk activity in *S. cerevisiae* and *S. pombe* are pointers to the differences in mitotic regulation between these two organisms. Charles and coworkers (1998) assayed the profile of Cdc5p kinase activity across the division cycle in budding yeast cells released from a G_1 arrest. They found a single peak of activity during the cycle that rises to its maximum after the histone H1 kinase activity associated with the mitotic B-type cyclin Clb2p, and ahead of maximal anaphase-promoting complex activity (see also below). Unexpectedly, studies by Mulvihill and coworkers (D. Mulvihill, H. Ohkura, D. Glover, and I. Hagan, in prep.) show two well-separated peaks of Plo1 kinase activity during each division cycle. The first peak is after Plo1 has been loaded onto the poles and is coincident with spindle formation and actin ring formation, processes that are implicated from the mutant phenotype to be regulated by the enzyme (Ohkura et al. 1995). The second peak occurs late in mitosis and is coincident with septum formation, consistent with the third postulated role of Plo1. Viewed in this context, it is certainly possible that the single peak of activity in the syncytial *Drosophila* embryos could represent overlapping enzymatic functions that

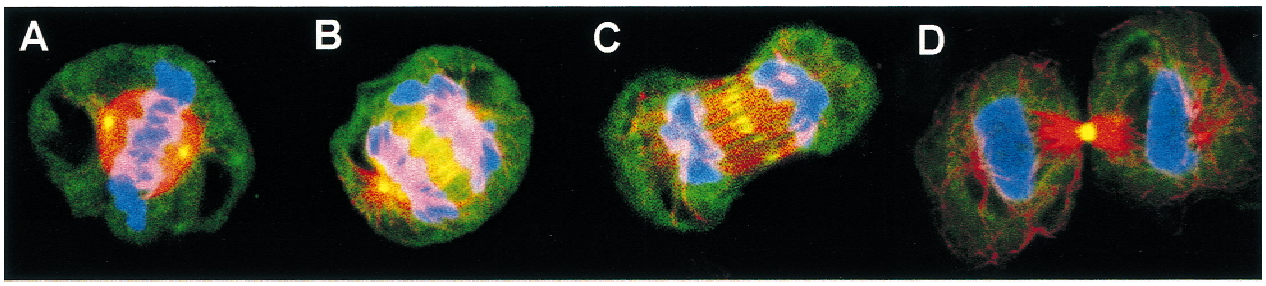

Figure 2. Localization of Plk1 in dividing HeLa cells. Cells are stained to reveal DNA (blue); microtubules (red); and Plk1 (green). Overlap of microtubule and Pk1 staining appears yellow. Plk1 is associated with the punctate centrosomes at metaphase (*A*) and early anaphase (*B*). The enzyme becomes associated with the mid-zone region of the spindle in early- and late-anaphase (*C*). As cytokinesis takes place, Plk1 is found at the mid-body at the center of the cleavage furrow (*D*). (Previously unpublished micrographs of Á. Tavares, University of Dundee, UK).

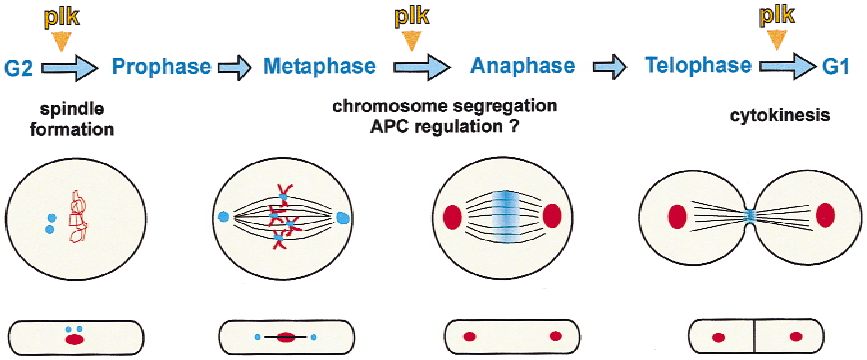

Figure 3. The subcellular localization of fission yeast Plo1 and animal cell Plk in mitosis is in accord with the multiple functions of this family of mitotic kinases. DNA (orange); polo-like kinase (blue).

coincide in timing in these rapid nuclear division cycles, and which may be separated temporally in dividing cells.

The broad peak of Plk1 activity in dividing mammalian cells may also be best explained if the enzyme has temporally overlapping functions. The activation of Plk1 in cultured mammalian cells appears to follow closely the onset of cdk1 activity (Golsteyn et al. 1995; Hamanaka et al. 1995; Lee et al. 1995). However, the enzyme then appears to remain active until beyond the point at which cdk1 activity has begun to decay (Lee et al. 1995; Kotani et al. 1998).

The first chukka[1]: entry into M phase

Plks and the Cdk1 activation loop

The activity of Cdk1–cyclin B is required throughout the cell to bring about the changes in cellular architecture during mitosis. In most eukaryotes, the activity of Cdk1 reflects the balance between the action of inhibitory kinases of the Wee1/Mik1 type and their opposing phosphatases, homologs of the product of the fission yeast gene *cdc25* (Nurse 1990). Once the balance is tipped in favor of the Cdc25 phosphatase, then a positive feedback loop is established whereby Cdk1 can contribute towards maintaining Cdc25 in a hyperphosphorylated and active state to drive an irreversible commitment to mitosis. The discovery by Kumagai and Dunphy (1996) that Plx associates with, phosphorylates, and can thereby activate the *Xenopus* cdc25C gene product in vitro raised the intriguing question of whether plk could be the 'trigger' kinase that initiates the onset of the G_2–M transition. This exciting discovery led to a series of recent studies showing that Plx1 does participate in the Cdk1–cyclin B amplification loop in meiotic maturation in *Xenopus* oocytes (Abrieu et al. 1998; Karaiskou et al. 1998: Qian et al. 1998). Qian and colleagues (1998) showed that Plx1 was normally activated concurrently with Cdk1 during meiosis, and that when it was microinjected into oocytes, it could accelerate the rate of activation of Cdc25 and Cdk1–cyclin B. When Plx1 is immunodepleted or neutralized with antibodies, the activation of Cdc25 and cdk1–cyclin B is suppressed (Abrieu et al. 1998; Qian et al. 1998). The reversal of the antibody-mediated inhibition in vitro by addition of Cdc25 suggests that Plx is upstream of Cdc25. Thus it seems that Plx1 is capable of activating Cdc25 and being part of the positive-feedback loop that amplifies Cdk1-cyclin B activity both in vivo and in a cell-free system (Fig. 4). On the other hand, Plx1 does not seem to be required for the activation of cdk1-cyclin A, which in turn fails to activate Plx1 (Abrieu et al. 1998).

However, if Plx1 is truly a part of the feedback activation loop, its activation, like the activation of the majority of Cdc25 (Kovelman and Russell 1996), must lie downstream of the activation of the activity that first triggers Cdk1. Several pieces of evidence suggest this is the case. First of all, in the absence of Plx1 activity, the phosphorylation and activation of Cdc25 are not prevented but only delayed indicating that other kinases are capable of fully phosphorylating Cdc25 (Qian et al. 1998). Moreover, Plx1 itself requires phosphorylation to be activated upon entry into M phase, thus pointing towards an alternative trigger (Karaiskou et al. 1998; Qian et al. 1998). In addition, Karaiskou et al. (1998) also observe that the activation of Plx1 seems to occur slightly later than the activation of Cdk1–cyclin B, and it is totally prevented by the Cdk-specific inhibitor p21CIP. This suggests that at least in this system, Plx1 kinase

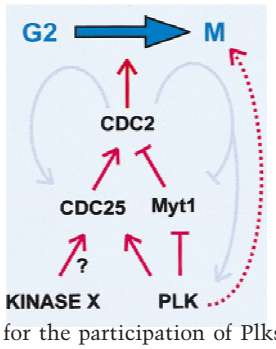

Figure 4. Model for the participation of Plks in the Cdk amplification loop. In most eukaryotes, Cdk1 (CDC2) is activated by the Cdc25 phosphatase, and inhibited by the Wee1/Mik1/Myt1 group of kinases. In *Xenopus* Plk (Plx) is shown as an activator of Cdc25, and a repressor of Myt1. Once Cdk1 has been activated then it triggers a positive feedback loop (light blue arrows) that can promote the further activation of Cdc25, possibly mediated through Plo kinase. Whether another kinase *X* acts as the initial trigger for this loop by activating either Cdc25 or Cdk1 (CDC2) is discussed in the text.

[1]The game of polo is divided into three to six periods, or chukkas. Players may use the intervals between chukkas to change ponies (Hobson 1993).

activation depends on cdk1 activity and that its initial activation is mediated by a small proportion of cdk1–cyclin B that has escaped inhibition by inhibitory kinases. The same may be true in other systems, as Mundt and coworkers (1997) observed that overproduction of Plk1 in HeLa cells did not lead to obvious advancement into mitosis as would expected for a mitotic trigger. But why should the positive feedback loop be triggered in this particular way and at this specific time? Is it perhaps more reasonable to think of the amplification loop as a means of activating both plk1 and cdk1 mitotic kinases, in which case there is still a need to search further for the triggering event.

What is the relationship between M-phase entry and centrosome/SPB separation?

In contrast to these newly emerging biochemical studies, genetic analyses upon both *Drosophila* and fission yeast have rather emphasized a role for plk activity in centrosome assembly and separation in the formation of the bipolar spindle (Fig. 5). One notable feature of the *polo*^{1} allele of *Drosophila* was a failure of the CP190 centrosomal antigen, to assemble into centrosomes in mutant syncytial embryos that display highly disorganized spindle microtubules (Sunkel and Glover 1988). Spindle defects were also seen in mitotic cells in the larval central nervous system, including characteristic monopolar spindles in which it appears that centrosomes have failed to separate (Llamazares et al. 1991). Similarly, in fission yeast either disruption of the *plo1* gene or its overexpression resulted in the formation of monopolar spindles as a consequence of the failure of the SPB to complete either its duplication or separation (Ohkura et al. 1995). Likewise, studies of the loss of plk function in higher eukaryotes have also indicated a requirement for the correct of the spindle poles early in mitosis. Microinjection of anti-plk1 antibodies into HeLa cells, or anti-Plx1 antibodies into individual blastomeres of the *Xenopus* embryo also resulted in the formation of monopolar spindles (Lane and Nigg 1996; Qian et al. 1998).

The relationship between the process of separation of the spindle pole MTOC and entry into M phase is not clear. Whereas the injection of anti-Plk1 antibodies led to mitotic arrest with a monopolar spindle formed around a smaller than usual centrosome in the immortalized HeLa cell line, nonimmortalized human cells were found to arrest in G_2 (Lane and Nigg 1996). This suggests that a requirement for plk function during entry into mitosis has in some way been overcome by the oncogenic events that led to the establishment of the HeLa cell line. Although the underlying basis for this finding is not understood, one possibility might be that the tumor cells have lost a checkpoint present in normal cells that prevents full commitment to mitosis if centrosomes are not properly matured.

Recent experiments in fission yeast also point strongly towards the importance of local events at the SPB in regulating commitment to mitosis as well as spindle formation and suggest that the Plo1 kinase may play a critical role in these processes. The absolute requirement for Cdc25 activity to mediate mitotic entry can be completely by passed by *stf1*, a semidominant mutant of the spindle pole component Cut12 (Bridge et al. 1998). An attractive explanation is that semidominant *cut12* alleles (like *stf1.1*) could influence commitment to mitosis through the inappropriate activation of the Cdc2 amplification mechanism at the SPB in the absence of Cdc25. Could an interaction between the semidominant Cut12 protein of the *stf1.1* mutant and Plo1 kinase provide an inappropriate trigger for the activation of p34^{cdc2}? Because *stf1.1* cells can drive mitosis in the complete absence of Cdc25 (Hudson et al. 1991), the *stf1* mutation must be counteracting the activity of Wee1, perhaps by inhibiting the enzyme directly. In this respect, it is note-

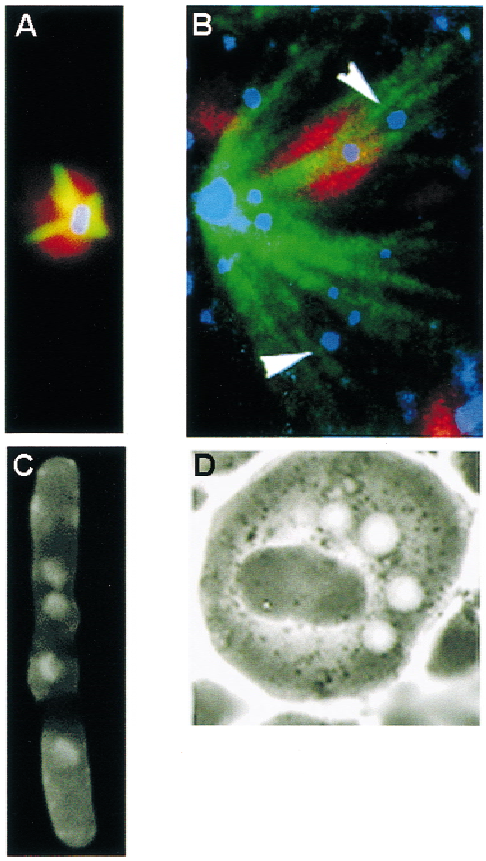

Figure 5. Common aspects of the mutant phenotypes of *S. pombe plo1* and *D. melanogaster polo*^{1} mutants. Monoastral spindles in an *S. pombe plo1* cell (*A*), and a *D. melanogaster polo*^{1} neuroblast (*B*). (Red) DNA; (green) spindle microtubules; (blue) SPB or centrosomal-associated antigens. The centrosomal associated antigen shown in blue in the *Drosophila* cell is often not found at the spindle pole, and in this micrograph can be seen associated with the spindle microtubules (arrowheads). An *S. pombe plo1* cell (*C*) and a *D. melanogaster* spermatid (*D*) in which the previous two rounds of cytokinesis has failed. Both cells are tetranucleate. In the spermatid, the nuclei are the four clear spherical structures, the dark structure being the mitochodrial aggregate. *A* and *C* were provided by Hiro Ohkura (University of Edinburgh, UK), and *B* by Richard Adams (University of Edinburgh, UK). *D* is taken from Carmena et al. (1998).

worthy that hyperphosphorylation of the *Xenopus* $p34^{cdc2}$ inhibitory kinase Myt1 is reduced strongly in cycling extracts in which Plx1 is inactivated (Abrieu et al. 1998).

The association of plks and $p34^{cdc2}$ with the spindle pole upon commitment to mitosis is a common feature in all systems characterized to date, suggesting that elements of the controls emerging from the analyses of fission yeast may be universally conserved. For the moment, however, it must remain an open question whether the monopolar spindles seen in animal or yeast cells following perturbation of plk function are caused by an inability to mature the spindle pole sufficiently for mitosis, or a weak commitment to mitosis because of insufficient cdc2 activation by a defective amplification loop. Alternatively polo kinases may be required to regulate the functions of other proteins essential for spindle formation such as the mitotic motor proteins. Indeed the consequences of losing polo function do resemble the consequences of loss of motors such as KLP61F of *Drosophila* (Heck et al. 1993) and Cut7 of fission yeast (Hagan and Yanagida 1992).

The second chukka: do plks play through the metaphase–anaphase transition?

A function for centromeric plk?

The centrosomal localization of plks would seem to correlate with a function in establishing the bipolar spindle on mitotic entry. What of the enzyme localized at centromeres? The loss of plk from the centromeres at the onset of anaphase suggests that the enzyme might function in regulating centromere behavior at this crucial transition in M phase. Defects in chromosome segregation in male meiosis were in fact reported in the first description of the *polo*[1] allele. When the segregation of marked chromosomes was followed, most nondisjunction appeared to take place in the second meiotic division suggestive of defects in the separation of sister chromatids (Sunkel and Glover 1988).

Meiotic defects were also reported in the early studies of a temperature-sensitive *cdc5* mutant. In mitosis, the mutant arrests with a bipolar spindle at the late stages of nuclear division (Byers and Goetsch 1974). In meiosis, it exhibits abnormal behavior of the SPBs in the first division, and a failure of spindles to elongate in meiosis II (Schild and Byers 1980). However rather unexpected results were reported by Sharon and Simchen (1990a,b) who examined chromosome segregation when mononucleate cells arrested with two SPBs in meiosis I were shifted to the permissive temperature. Interestingly, some chromosomes were found to segregate reductionally and others equationally in a manner reflecting a property of specific centromeres. Thus it seems that both in *Drosophila* and budding yeast, plk function is required to mediate the appropriate pattern of chromosome segregation at anaphase in the meiotic divisions. These observations could be consistent with a proposed role for the plks in regulating the APC, which directs the degradation of chromosomal proteins postulated to maintain the cohesion of sister chromatids (Ciosk et al. 1998).

Do polo-like kinases regulate the APC?

In budding yeast and mammalian cells, the timing of late-mitotic plk activity has been shown by direct comparison to precede the activity of the APC (Charles et al. 1998; Kotani et al. 1998). The APC functions as a cell-cycle-regulated ubiquitin-protein ligase (E3) responsible for the degradation of mitotic cyclins, as well as the anaphase inhibitors Pds1p of budding yeast and Cut2 of fission yeast, the cohesin Scc1p, and Ase1p, a protein associated with the spindle mid-zone. Little is known of the mechanisms that regulate the timing of APC activation, although members of the Fizzy–Cdc20 protein family are reported to bind to and activate the complex. This family of proteins are highly conserved. In *Drosophila* the genes *fizzy* and *fizzy-related* are both required for the APC-dependent degradation of cyclins. In budding yeast, Cdc20p is required for the destruction of Pds1p, whereas a related protein Hct1p/Cdh1p is involved in destruction of mitotic cyclins and Ase1p. Thus there appear to be at least two major pathways of APC activity; one required for sister-chromatid separation and the other for cyclin B degradation (for review, see Townsley and Ruderman 1998; Wolf and Jackson 1998).

Two groups have demonstrated recently that *cdc5* mutants of *S. cerevisiae* are defective in their ability to degrade the mitotic cyclin Clb2p without affecting the degradation of Pds1p or the Clb5p cyclin (Charles et al. 1998; Shirayama et al. 1998). These observations raise the question of whether the inability to resolve the mitotic spindle in *cdc5* mutants, is because of loss of APC activity responsible for mitotic cyclin degradation. Several experiments substantiate this notion. First of all, overexpression of the wild-type, but not 'kinase-dead' mutant, enzyme decreases the levels of Clb2p and increases APC activity (Charles et al. 1998). Secondly, overexpression of an amino-terminally truncated form of Cdc5 (that increases the stability of the enzyme) is lethal, but the lethality can be suppressed in mutants with reduced APC activity.

A novel mutant allele of CDC5 has been described recently that appears to be defective in mitotic exit following DNA damage (Toczyski et al. 1997). Normally budding yeast cells become extensively delayed in mitosis following DNA damage, but eventually adapt to this checkpoint arrest and exit mitosis. Charles and coworkers (1998) find that this mutant form of Cdc5p was less effective at stimulating APC activity even though the kinase is catalytically active. This suggests that the mutation may affect substrate recognition by kinase, and consequently adaptation to the checkpoint delay could be caused by a defect in the ability to activate Clb2p proteolysis.

Suggestions that polo-like kinases can activate the APC have also come from recent studies with animal-cell-derived systems. Descombes and Nigg (1998) have shown that the *Xenopus* homolog Plx1 is required for the

destruction of APC targets that drive exit from M phase. Working with *Xenopus* egg extracts that are arrested in the second meiotic metaphase as a result of the activity known as cytostatic factor (CSF), they find that the addition of a catalytically inactive mutant form of Plx1 blocks the destruction of cyclin B and the inactivation of Cdk1 that is triggered normally by addition of Ca^{2+}. If exogenous APC-dependent substrates, such as the fission yeast Cut2, were added to the extracts, their degradation was also prevented in the presence of the inactive mutant protein. Finally, M-phase exit would not take place in this system following immunodepletion of Plx1, but could be restored by the addition of catalytically active enzyme. Thus it seems that Plx activity is a component of the pathway that overcomes CSF arrest. In *Xenopus*, CSF activity is known to require a MAP kinase cascade under the control of c-Mos (Sagata 1997). c-Mos itself is normally proteolytically degraded during Ca^{2+} mediated activation, but is stable in extracts to which the catalytically inactive Plx1 has been added. Descombe and Nigg (1998) postulate that Plx1 is most likely to lie downstream of the c-Mos/MAP kinase cascade in this pathway of events. It could either inactivate a hypothetical inhibitor of the APC present in CSF arrest, or act directly to activate the APC in concert with members of the *fizzy*–Cdc20p family of proteins.

The latter hypothesis finds support in the recent work of Kotani and coworkers (1998), who found that mouse Plk1 would coimmunoprecipitate with several substrate polypeptides, of which two proved to be the APC components Cdc16p and Cdc27p. They also showed that if activated by Cdk1, bacterially expressed plk would phosphorylate the bacterially expressed APC proteins Cdc16p, Cdc27p, and Tsg4p directly, and phosphorylate and activate purified mammalian APC. Although the biochemical study of Kotani et al. (1998) is extremely convincing, the generality of their findings remains to be established. It is not clear whether $p34^{cdc2}$ can universally activate plk as $p34^{cdc2}$ consensus phosphorylation sites are not conserved on the plks of different species. Moreover, neither Cdk1 nor MAP kinase was found capable of activating Plk by other workers (Hamanaka et al. 1995; Lee et al. 1995).

Although some aspects of the *cdc5* and *polo* mutant phenotypes in *S. cerevisiae* and *D. melanogaster* are consistent with a function in APC regulation, the effects of perturbing plk function in some other organisms appear at first sight less supportive of such a role. It is possible that different organisms have evolved alternative ways of coordinating the essential late mitotic activity of the plks with other mitotic functions that may or may not include a direct involvement in APC activation. There is, for example, no indication of metaphase arrest in *S. pombe plo1* disruptants. If such cells have sufficient residual enzyme to evade the block to the formation of a bipolar spindle, then they arrest after exit from mitosis prior to cytokinesis (Ohkura et al. 1995). Of course, it is possible that there is sufficient residual Plo1 function in these cells to activate the APC. A similar argument may be advanced in the case of $polo^{1}$ mutants of *Drosophila*, in which during spermatogenesis cyclin B degradation appears to take place normally in mutant cells that show defects both in chromosome segregation and cytokinesis during meiosis (Carmena et al. 1998).

It is also possible that the earlier arrest seen in *cdc5* mutants may be a peculiarity of *S. cerevisiae*. In the budding yeast, SPB duplication takes place during S phase, and a short intranuclear spindle begins to form at a point corresponding to the beginning of G_2, rather than at the onset of M phase. Is it also significant that, so far, Cdc5p appears to be unique in the plk family in that it contains 'destruction boxes' (Charles et al. 1998). Cdc5p is ultimately itself destroyed by APC-activated proteolysis, and this is prevented in *hct1/cdh1* mutants. Its proteolysis, together with Cdc20p, may assist in switching off cyclin and Pds1p hydrolysis, respectively, as cells enter G_1 phase. The stability of plks does appear to vary between species. Fang and coworkers (1998) report that in *Xenopus* extracts Plk1 is an APC substrate. In *Drosophila*, however, the maternally provided Polo kinase is sufficiently enduring to provide function sufficient for development to late larval stages, which would be inconsistent with its destruction at cell division (Sunkel and Glover 1988). However, it is always possible that local degradation of the enzyme at specific sites within the cell could regulate polo activity within each cell cycle.

The third chukka: late M-phase roles of the plks

$plo1^{+}$ *is required for actin ring and septum formation*

The original observations of the mutant phenotypes of *plo1* disruptants of *S. pombe* revealed not only a failure of spindle formation, but also failure to form the actin ring and septum, prerequisites for cytokinesis (Fig. 5). The function of the enzyme in these late mitotic events was further illustrated by the dramatic ability of $plo1^{+}$ overexpression to drive the formation of multiple septa in cells arrested at any stage of the cell cycle (Ohkura et al. 1995). Thus overexpression of Plo1 kinase overcomes the normal dependency of septation upon the completion of mitosis.

It should therefore perhaps come as no surprise to learn that the two peaks of Plo1 kinase activity seen by D. Mulvihill, H. Ohkura, D. Glover, and I. Hagan (in prep.) correspond to the timing of actin ring formation and septation, respectively. The initiation of cytokinesis occurs upon commitment to mitosis when a ring of a protein Dmf1, is deposited underneath the plasma membrane at the cell equator. Dmf1 is rapidly joined by filamentous actin and a number of other proteins (Sohrmann et al. 1996). The later stage of septation is controlled by a regulatory network headed by a G protein, Spg1 (Schmidt et al. 1996). Spg1 is found associated with the SPB throughout the cell cycle and is activated upon commitment to mitosis (Sohrmann et al. 1996). The ability of Plo1 to induce septation in interphase may be because of premature maturation of the SPB, which could inappropriately activate the SPB-bound Spg1 network. Alternatively the timing of the second peak of kinase

activity at the end of mitosis may argue for a direct role for Plo1 in activating the Spg1 pathway in a normal mitosis. Support for either of these models comes from a genetic screen that set out to identify mutants that are only able to grow in the presence of elevated levels of Plo1 (H. Ohkura, F. Cullen, I. Hagan, and D. Glover, in prep.). This screen has identified a large number of new mutant alleles of genes encoding different components of the Spg1 and ring formation pathways.

The late nuclear division mutants of S. cerevisae

In the budding yeast, *CDC5* is one of several genes that have a similar mutant phenotype of arrest at the stage of late nuclear division. Interestingly, several of these genes are the budding yeast homologs of other members of the fission yeast Spg1 network. They include, for example, the *S. cerevisiae* genes *TEM1* and *CDC15*, counterparts of the *S. pombe* genes *spg1* and *cdc7*. It is undetermined whether the products of these genes, like Cdc5p, show the same association with the SPB.

The cytological consequences of overexpressing Cdc5p protein kinase in budding yeast have never been reported. However, Lee and Erikson (1997) found that when they expressed mammalian Plk1 in budding yeast cells, it would drive the formation of multiple septa within the bud neck. It could therefore be that Cdc5p might also play an analogous role in septation to Plo1 in fission yeast cells, but that in the budding yeast an earlier role in APC regulation obscures this function.

Although neither Plo1 nor Cdc5p have been localized yet to the forming septum in fission yeast or budding yeast, respectively, it is tantalizing that when the mammalian Plk1 is expressed in budding yeast cells, this enzyme is seen to localize not only to the spindle poles, but also the cytokinetic bud neck filaments (Lee et al. 1998). Interestingly Lee and colleagues (1998) also report that the localization of Plk1 in neck filaments is prevented by mutations in polo box 1. These same mutations also lead to inability to complement the *cdc5* mutation, and yet kinase activity in vitro is not affected. It is therefore tempting to conclude that the plk function is disrupted because of mislocalization of the kinase. It will be of great interest to know whether these same sequences direct the localization of plks in their native organisms.

A requirement for plks for cytokinesis in animal cells

The plks of animal cells localize to the central spindle region in late anaphase and the midbody at telophase (Golsteyn et al. 1995; Lee et al. 1995), sites that might imply a role for the enzyme in cytokinesis in animal cells. Until recently, however, evidence for such a role was lacking. Lane and Nigg (1996) were unable to observe any cytokinesis defects following the injection of anti-Plk antibodies into cultured cells. On the other hand, Mundt and coworkers (1997) did see the formation of multinucleate cells following the overexpression of the enzyme suggesting that the precise level of enzyme activity may be important for the correct execution of cytokinesis.

The first clues to the possible functional significance of the localization of plks to the central region of the spindle in late mitosis came from the observation that Plk1 could associate with a kinesin-like protein known as both CHO1 and MKLP1 and could phosphorylate this protein in vitro (Lee et al. 1995). Subsequently it was found that *Drosophila* polo kinase associates with the homologous motor protein, encoded by the gene *pavarotti* (*par*) (Adams et al. 1998). It appears significant that mutations in *pav* prevent cytokinesis and show the formation of a defective spindle mid-zone region in late anaphase. Direct evidence for plk function in cytokinesis can be seen from defects at several stages of spermatogenesis in a number of hypomorphic mutant alleles of *Drosophila polo* (Carmena et al. 1998) (Fig. 5). Once again, the earliest abnormalities observed were a failure to form the correct mid-zone and midbody structures at late anaphase–telophase, accompanied by a failure to assemble components of the contractile ring correctly. In fact, Polo and Pav proteins appear to be mutually dependent for their correct localization. Polo kinase fails to localize to spindle poles or the spindle mid-zone in *pav* mutants (Adams et al. 1998), and Pav–KLP accumulates at the spindle poles in meiosis in *polo* males and often fails to become associated with the spindle mid-zone. This suggests models in which the failure of the two proteins to localize correctly in either mutant could be caused by a direct consequence of the disruption to spindle morphology, or because Polo kinase requires Pav–KLP for its movement to the correct site on the spindle. In either case, it is possible that the motor properties of Pav–KLP might be changed as a consequence of phosphorylation by Polo kinase.

Could the plks be a component of the signaling system that initiates cytokinesis? Rappaport has provided compelling evidence that in Echinoderm embryos asters can dictate the position of the cleavage furrow (Rappaport 1961), whereas in cultured animal cells it is suggested that a signal can originate from the mid-zone of the spindle (Cao and Wang 1996). These two general hypotheses could be reconciled if the signaling molecule(s), for which Polo-like kinase could be one prime candidate, were initially localized at the poles, and subsequently at the central spindle anticipating the position of the cleavage furrow, and if the extent of this relocalization were to vary between different cell types. The work of Giansanti and her colleagues (1998) suggests that the structure of the contractile ring and the central spindle at late anaphase are mutually dependent. Thus it seems likely that the plks could have several substrates in the cleavage furrow, both associated with the central spindle and the contractile ring.

The importance of these studies is that they present a unifying role for the plks in regulating the early events of cytokinesis. The fission yeast Plo1, like its animal cell counterparts, localizes to the spindle poles at the onset of mitosis, but structures analogous to the central spindle and cleavage furrow are not found in fission yeast cytokinesis. However, other proteins required for septation, such as the protein kinase encoded by *cdc7*, are also

found at the SPBs. This establishes the SPBs as a potential source for signaling molecules that regulate the onset of cytokinesis in the fission yeast. Animal cells may show a variation in this theme by which the Plks become redistributed from the spindle poles and centromeres to the central region of the spindle at the later stages of division. This could be an evolutionary adaptation that has paralleled the increase in size and complexity of the metazoan mitotic spindle in comparison with its yeast counterpart.

Extra time: do plks have roles outside of M phase?

The budding yeast gene *CDC28* for cyclin-dependent kinase is well documented to have roles not only in mitosis, but also throughout the cell cycle. Could this be true for *CDC5*? There is indeed evidence for an additional role for *CDC5*, in the initiation of DNA replication. In fact, *CDC5* was first cloned as a multicopy suppressor of certain mutant alleles of *DBF4* (Kitada et al. 1993), a gene encoding a protein that targets the Cdc7p protein kinase to the prereplicative origin recognition complex (ORC) for the initiation of DNA replication (related to the fission yeast cdc7 protein kinase essential for septation). A possible role for Cdc5p in DNA replication was later put forward by Hardy and Pautz (1996), who showed that both Cdc5p and Cdc7p kinases could interact with Dbf4. Moreover *cdc5* mutants showed a plasmid-maintenance defect and a genetic interaction with a mutant gene encoding a component of the ORC, *orc2*. As the transition from the postreplicative to the prereplicative initiation complex occurs late in M phase, it is possible that another late mitotic function of Cdc5p kinase is to regulate this transition. This could be achieved by phosphorylating proteins of the ORC, or more directly by modifying the Dbf4p protein so that it can subsequently recruit Cdc7p to origins.

Whereas the yeasts can achieve cell-cycle progression utilizing a single cyclin-dependent kinase, mammals have evolved multiple forms of both Cdks and their activating Cdc25 phosphatases. At least three plks have been described in mammalian cells, the specific functions of which are still obscure. The existence of these multiple forms mirrors the presence of multiple forms of Cdks and Cdc25s in mammals, and by analogy suggests that the plks could play related but nonoverlapping roles at different stages in the progression through the cell cycle.

Studies of the function of the different mammalian enzymes in budding yeast serve only to confirm that these proteins are family members. Lee and Erikson (1997) showed that Plk1 would complement a *cdc5* temperature-sensitive mutation, and more recent work has indicated that human Plk3 (Prk) also has this ability (Ouyang et al. 1997). Thus any differing function of the Plk family members in mammalian cells appears to have no significance in complementation tests with lower eukaryotes.

However, there is evidence for a requirement for some plks in the G_0 stage of the mammalian cell cycle. The genes for mouse Plk2 (Snk) and Plk3 (Fnk) are both immediate-early response genes, whose expression is induced by the addition of growth factors to serum-deprived G_0 cells but then declines before the end of G_1 (Simmons et al. 1992; Donohue et al. 1995). Some mouse Plk3 (Fnk) protein is also present in quiescent cells prior to mitogenic stimulation that results in a transient phosphorylation and activation of the protein (Chase et al. 1998). The induction of Plk2 and Plk3 transcripts during G_1 contrasts with the transcription of Plk1 after the G_1/S transition (Hamanaka et al. 1995). Transient ectopic expression of Plk1 can induce quiescent cells to enter S phase (Hamanaka et al. 1994), but because the enzyme normally has negligible activity in G_1 (Hamanaka et al. 1994; Golsteyn et al. 1994) it is possible that it is acting nonphysiologically in these experiments in substituting for either Plk2 or Plk3.

Human Plk3 (Prk) shows a relatively low kinase activity during mitosis, G_1 and G_1/S phases and peaks during late S/G_2 stages of the cell cycle (Ouyang et al. 1997). This timing correlates with the completion of DNA synthesis and the activation of p34cdc2 kinase. As with Plk1, Plk3 (Prk) can phosphorylate Cdc25 in vitro, suggesting it too may play a role in regulating the onset of M phase. In support of this idea, Ouyang and colleagues also report that the enzyme can potentiate the progesterone-induced meiotic maturation of *Xenopus* oocytes meiotic maturation, but it is probably prudent to be cautious in interpreting the consequences of this heterologous expression. Likewise, mouse Plk3 (Fnk) protein levels are reported to increase as cells progress from G_1 to mitosis, whereupon the enzyme becomes phosphorylated. This modification correlates with increases in kinase activity. Later in mitosis, Plk3 (Fnk) is dephosphorylated, and by the time cells enter G_1, it is all present as the dephosphorylated form (Chase et al. 1998). Moreover, this mitotic activation of Plk3 (Fnk) requires the function of Cdk1. Thus it seems that Plk3 (Fnk) may have two different functions: for cells to re-enter the cell cycle in response to mitogenic factors and during mitosis.

What about future polo games?

There is much still to learn about how the activity of the plks is regulated. We already know that regulation can occur through transcription, through the biosynthesis and stability of the protein, by post-translational mechanisms—especially phosphorylation, and through the changing pattern of the intracellular location of the enzyme throughout the cell cycle. It is still a particular puzzle as to where exactly plks lie in the network of mitotic phosphorylation events: What are the kinases that activate the plks? What are the substrates of the enzymes? The increase of Plk activity at mitosis correlates with phosphorylation, and phosphatase treatment reduces the activity of Plk1, Polo, and Plx1 substantially (Lee et al. 1995; Tavares et al. 1996; Mundt et al. 1997; Qian et al. 1998). However, there remain uncertainties as

Table 1. *plk substrates*

	Putative substrate	Comments	Reference
G_2–M	Cdc25	activated by phosphorylation	Kumagai and Dunphy (1996)
	Myt1	a putative substrate from *in vivo* studies	Abrieu et al. (1998)
Spindle function	Asp	a microtubule-associated protein	A. Tavares (unpubl.)
	β-tubulin		Tavares et al. (1996)
	KLP61F	KLP required for centrosome separation	A. Tavares (unpubl.)
	MKLP1	KLP found in spindle mid-zone	Lee et al. (1995)
	Pav–KLP	KLP required for spindle mid-zone structure	A. Tavares (unpubl.)
APC	Cdc27	APC component	Kotani et al. (1998)
	Cdc16	APC component	Kotani et al. (1998)
Cytokinesis	MKLP1	KLP found in spindle mid-zone	Lee et al. (1995)
	Pav–KLP	KLP required for spindle mid-zone structure	A. Tavares (unpubl.)

to how this fits into the cdk1 regulatory cascade, as we discussed, and it is likely that there are further activating kinases to be identified. The function of the conserved carboxy-terminal domain of the protein also remains a mystery. The polo boxes may have a function in directing the correct subcellular localization of the enzyme as suggested by Lee et al. (1998), or association with currently unidentified regulatory subunits. Other regulatory functions may also be attributed to this regions of the protein, as small truncations of the carboxyl terminus result in an substantial increase in the activity of the kinase (Lee and Erikson 1997; Mundt et al. 1997).

The Sak protein kinase is a closely related enzyme, but lacks the conserved polo boxes found in other plks. What exactly is its relationship to the plks? It does appear to have a cell-cycle function, and is only expressed in cells with proliferative capacity. Its mRNA levels increase from late G_1 to M phase; antisense RNA or overexpression of Sak suppresses cell growth; and, overexpression of Sak protein increases the incidence of multinucleated cells, suggesting a possible role in cytokinesis (Fode et al. 1994, 1996). This is perhaps just one of many mitotic kinases about which we currently know very little.

The list of proteins phosphorylated by the plks is steadily growing (Table 1). They include components of the Cdk1 activation loop, APC components, and several microtubule-associated proteins some of which were identified by Tavares et al. (1996) by comparing phosphorylated proteins in wild-type and *polo* mutant embryo extracts. Genetic studies may help in searching for substrates and/or interacting proteins. A recently published study shows a strong synergistic interaction between mutations in the genes *polo* and *abnormal spindle* (*asp*) (Gonzalez et al. 1998). *asp* encodes a 220-kD microtubule-associated protein essential for the correct behavior of the spindle poles and M-phase microtubules (Saunders et al. 1997), and is indeed an excellent Polo kinase substrate in vitro becoming an MPM-2 epitope upon phosphorylation (Á. Tavares and C. Avides, unpubl.). It will be interesting to determine whether phosphorylation by Polo kinase modifies Asp protein function. Another gene that may play an important role in regulating Polo function is encoded by the gene *scant* (White-Cooper et al. 1996). Females transheterozygous for mutations in *polo* and *scant* produce embryos showing abnormal behavior of the spindle poles. We await the molecular identification of the *scant* gene product with anticipation.

Finally, an understanding of these aspects of mitosis will be important in the treatment of human cancer. A growing number of reports indicates that tumor cells display abnormal centrosome behavior (e.g., Lingle et al. 1998) sometimes associated with loss of *p53* function (e.g., Fukasawa et al. 1996). Although the conventional view has been that the mitotic abnormalities observed for over a century in human tumors is rather a downstream consequence of other oncogenic events, this is not entirely clear. Mitotic abnormalities could in the first instance contribute to the generation of aneuploidy that is so important for tumor development. Some support for this comes from the finding of oncogenic lesions in genes that regulate mitotic checkpoints controlling either the onset of anaphase or cytokinesis (Cahill et al. 1998). Levels of Plk1 have been shown to be elevated in tumor cells (Holtrich et al. 1994; Wolf et al. 1997; Yuan et al. 1997), and one report has shown the enzyme to be able to cause oncogenic focus formation in NIH-3T3 cells, the transformed cells being capable of forming tumors in nude mice (Smith et al. 1997). Individuals with defective DNA damage checkpoint responses such as found in ATM or Li–Fraumeni syndrome have an elevated incidence of cancer. It is perhaps highly significant in this regard that *cdc5* was identified in screens for yeast genes that fail to down-regulate the checkpoint response on prolonged exposure to the insult (Toczyski et al. 1997). Thus the plks may not only play critical roles in the process of oncogenesis, but also they may well represent future therapeutic targets.

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