

## Polo-like kinases and centrosome regulation

Wei Dai<sup>\*.1</sup>, Qi Wang<sup>1</sup> and Frank Traganos<sup>1</sup>

<sup>1</sup>*Brander Cancer Institute, Department of Medicine, New York Medical College, Valhalla, New York, NY 10595, USA*

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### Polo and Polo like kinases

Protein kinases play a pivotal role in the regulation of the centrosome cycle. Extensive research in the past decade or so has led to the identification of several families of protein kinases that are important to the regulation of centrosome functions. These kinase families include cyclin-dependent kinases (Cdks) (Winey, 1999; Okuda *et al.*, 2000), Aurora kinases (Descamps and Prigent, 2001; Hannak *et al.*, 2001), *Mps1* (monopolar spindle) gene product (Hinchcliffe and Sluder, 2001; Fisk and Winey, 2001), NIMA family of kinases (Mayor *et al.*, 1999; Fry *et al.*, 2000), and the Polo family of protein kinases (Glover *et al.*, 1996; Wianny *et al.*, 1998; Glover *et al.*, 1996). Protein kinases of the Polo family, conserved through evolution, have been described in major eukaryotic models such as *Saccharomyces cerevisiae* (Kitada *et al.*, 1993), *Schizosaccharomyces pombe* (Ohkura *et al.*, 1995), *Caenorhabditis elegans* (Ouyang *et al.*, 1999; Chase *et al.*, 2000), *Drosophila melanogaster* (Sunkel and Glover, 1988; Llamazares *et al.*, 1991), *Xenopus laevis* (Kumagai and Dunphy, 1996; Duncan *et al.*, 2001), mouse (Donohue *et al.*, 1995; Lake and Jelinek, 1993; Simmons *et al.*, 1992), and human (Golsteyn *et al.*, 1994; Hamanaka *et al.*, 1995; Li *et al.*, 1996). The founding member of this family, Polo, was originally identified in *Drosophila* and was shown to be a serine–threonine kinase that is required for mitosis (Llamazares *et al.*, 1991). Subcellular localization of Polo protein and its kinase activity is cell cycle-dependent. It is predominantly cytoplasmic during interphase and is associated with condensed chromosome during mitosis (Llamazares *et al.*, 1991) at which time its kinase activity is also at peak (Fenton and Glover, 1993). In addition to the conserved kinase domain, Polo family members all share a unique amino acid sequence termed Polo box in the carboxy-terminal half of these proteins (Li *et al.*, 1996; Nigg, 1998). Mutations in the

Polo box that do not affect the kinase activity are capable of abolishing its biological activity *in vivo* (Lee *et al.*, 1998), indicating the importance of this conserved motif.

CDC5 and Plo1 are structural as well as the functional homologues of Polo in budding yeast and fission yeast, respectively (Nigg, 1998). Both genes are essential, and loss of their function leads to mitotic arrest (Kitada *et al.*, 1993; Ohkura *et al.*, 1995). Vertebrate cells contain three Polo-like kinases (Plk1, Plk2 and Plk3) that exhibit marked sequence homology to Polo (Table 1). The *C. elegans* genome also contains three Polo structural homologues (Ouyang *et al.*, 1999; Chase *et al.*, 2000). Interestingly, to date no additional gene products structurally homologous to Polo have been identified in *Drosophila*. Given the lower evolutionary hierarchy of *C. elegans* compared to *Drosophila*, it is reasonable to predict that the fruit fly may contain additional Polo homologues. There is circumstantial evidence supporting this notion. Fruit flies homozygous for the original *polo*<sup>1</sup> mutant allele are capable of developing to adulthood (Sunkel and Glover, 1988). Interpretations are that the mutation is weakly hypomorphic and that polo deficiency is partially rescued by the wild-type protein from an heterozygous mother (Donaldson *et al.*, 2001b). An alternative explanation could be that there exists yet to be identified polo member(s) that may complement, to certain degree, *polo*<sup>1</sup> mutation.

In mammalian cells, Plk1 (alternatively named Plk (Hamanaka *et al.*, 1995)) is the best studied Polo member to date (Donaldson *et al.*, 2001a). Extensive investigations have uncovered a variety of cell cycle events that are regulated by Plk1, including the onset of mitosis (Toyoshima-Morimoto *et al.*, 2001; Roshak *et al.*, 2000), DNA-damage checkpoint activation (Smits *et al.*, 2000), regulation of the anaphase promoting complex (Golan *et al.*, 2002; Kotani *et al.*, 1998), phosphorylation of the proteasome (Feng *et al.*, 2001), as well as centrosome duplication and maturation (Golsteyn *et al.*, 1995).

Mammalian Plk3 (alternatively named Prk (Li *et al.*, 1996) and Fnk (Donohue *et al.*, 1995)), was originally shown to be an immediate early gene product. The amount of Plk3 mRNA (but not Plk1 mRNA) is rapidly and transiently increased in response to mitogenic stimulation; in cycling cells, Plk3 kinase activity, but not its protein level, fluctuates significantly, and its kinase activity appears to peak during late S and G2 phase (Li *et al.*, 1996). Both Plk3 and

\*Correspondence: W Dai; E-mail: wei\_dai@nymc.edu

**Table 1** Polo family of kinases

Organisms	Name	SPBs or centrosome function
<i>Saccharomyces cerevisiae</i>	CDC5	Yes
<i>Schizosaccharomyces pombe</i>	Plo1	Yes
<i>Caenorhabditis elegans</i>	Plc1, Plc2, Plc3	Not determined
<i>Drosophila melanogaster</i>	Polo	Yes
<i>Xenopus laevis</i>	Plx1, Plx2, Plx3	Not determined
Mouse**	Plk1, Plk2*, Plk3	Yes
Human**	Plk1, Plk2*, Plk3	Yes

\*Centrosome function of these gene products has not been directly studied. \*\*Mammalian Plk1, Plk2, and Plk3 were alternatively named Plk, Snk, and Fnk/Prk, respectively

Plk1 are capable of rescuing CDC5 temperature sensitive (*ts*) mutants at restrictive temperature, indicating functional conservation of these kinases throughout evolution (Ouyang *et al.*, 1997; Lee and Erikson, 1997). At present, it remains unclear which mammalian Polo-like kinase represents the true ortholog of Cdc5. In addition to its role in regulation of mitosis, Plk3 is activated during DNA damage checkpoint activation (Xie *et al.*, 2001b) and severe oxidative stress (Xie *et al.*, 2001a).

Plk2 (alternatively named Snk (Simmons *et al.*, 1992)) was originally identified as the product of an immediate early gene, and it is the least well characterized of the mammalian Polo homologs. Recent studies have shown that Plk2 and Plk3 each associate with CIB, a calmodulin-related protein (Kauselmann *et al.*, 1999). Both Plk2 and Plk3 have been implicated in long-term synaptic plasticity and thus may have important post-mitotic functions.

### Cdc5, Plo1 and spindle pole bodies

In yeast, the functional counterparts to centrosomes are the spindle pole bodies (SPBs). CDC5, MOB1 and MPS1 are some of the essential genes in *S cerevisiae* that are involved in regulating SPBs. Mob1p is known to physically interact with Mps1p (Luca and Winey, 1998), the latter being a protein kinase essential for duplication of SPBs (Schutz *et al.*, 1997) and centrosomes (Fisk and Winey, 2001) as well as for spindle checkpoint activation (Weiss and Winey, 1996). In fact, Mob1p is a phosphorylation target of Mps1p *in vitro* (Luca and Winey, 1998). Mob1p undergoes dynamic subcellular relocation during mitosis. It primarily localizes to SPBs during mid-anaphase and then moves to a ring at the budding neck prior to cytokinesis; however, in the absence of CDC5, this translocation process is blocked (Luca *et al.*, 2001), suggesting dependence of Mob1p on upstream phosphorylation events during subcellular relocation. CDC5 also functionally interacts with several other genes such as DBF2 and CDC15 whose products are localized to SPBs (Menssen *et al.*, 2001). Dbf2p is localized to SPBs for much of the cell cycle and relocates to the bud neck during late mitosis, suggesting that it participates in

cytokinesis (Frenz *et al.*, 2000). Cdc15p localizes to SPBs in a unique pattern. Cdc15p is associated with SPB of the mother cell much of the cell cycle until late mitosis when it is also associated with SPB of the daughter cell. It appears that the binding of Cdc15p to the daughter spindle pole is essential for triggering cytokinesis (Menssen *et al.*, 2001). Thus, Cdc5p may regulate translocation of Cdc15p to and from SPBs through phosphorylation. The most direct evidence indicating a role for CDC5 in regulating SPBs comes from the study of meiosis in the budding yeast (Schild and Byers, 1980). A *ts* mutant of CDC5 fails to complete meiosis I due to arrest at a stage after SPB duplication and separation at the restrictive temperature. In these mutant cells, SPBs lack the normal spindle microtubules that are characteristic of meiosis I in wild-type cells.

In fission yeast, *Plo1* is required for the assembly and function of the mitotic spindle. Both loss of *Plo1* function and over-expression of this gene results in formation of cells in which condensed chromosomes are associated with monopolar spindles (Ohkura *et al.*, 1995), indicating a failure in bipolar spindle formation. *Plo1p* localizes to SPBs and mitotic spindles and its subcellular localization is cell cycle-dependent (Mulvihill *et al.*, 1999). *Plo1p* is not associated with SPBs during interphase. However, it becomes strongly associated with SPBs during early stages of mitosis until anaphase. Association of *Plo1p* with SPBs appears to require the activity of mitosis-promoting factor. In certain yeast mutants, *Plo1p* becomes inappropriately associated with SPBs in the cell cycle. For example, in *Stf1.1* mutants *Plo1p* interacts with SPBs during interphase bypassing the requirement for Cdc25, an activator for the mitosis-promoting factor; conversely, when septation occurs due to deregulated *Spg1* pathway, *Plo1p* is not associated with SPBs during mitosis (Mulvihill *et al.*, 1999).

Although no definitive SPB target(s) of Cdc5p or *Plo1p* has been identified, the observed changes in their subcellular localization and existing genetic studies are consistent with a role of these kinases in SPB function. It is likely that Cdc5p and *Plo1p* serve to coordinate spindle pole functions with other cellular events throughout the cell cycle, especially during mitosis. The identification of physiological substrates of these Polo-like kinases in SPBs would greatly facilitate the elucidation of their mode of action in cell division in the lower eukaryotes.

### Polo and centrosomes

*Drosophila gene* Polo is required for cytokinesis as well as for organization of spindle poles. Fruit flies homozygous for the original *polo*<sup>1</sup> allele exhibit certain abnormalities in spindle poles during development (Sunkel and Glover, 1988). These defects include multiple branched spindles in syncytial polo-derived embryos, spindles with broad poles and fewer circular mitotic figures in larval neuroblasts. In strongly

hypomorphic mutants (*polo<sup>9/10</sup>*), a majority of cells are arrested in a metaphase-like stage (Donaldson *et al.*, 2001b). The arrested cells lack asters at each spindle pole although they all possess bipolar spindles with robust arrays of microtubules. This may be partly due to the involvement of Polo in recruiting centrosomal components. In *polo<sup>9/10</sup>* mutants, CP190 and  $\gamma$ -tubulin, two centrosome-specific antigens, do not concentrate on centrosomes; instead they scatter throughout the mitotic spindles (Donaldson *et al.*, 2001b). The absence (or greatly diminished levels) of these proteins in centrosomes is not due to reduced levels of these proteins in the cells (Donaldson *et al.*, 2001b). Thus, Polo kinase activity is required for localization of these proteins to the microtubule organization center. In contrast, centrosomin and abnormal spindle protein (Asp) are present at spindle poles in these mutant cells (Donaldson *et al.*, 2001b), indicating an independence of their subcellular localization on Polo.

Genetic studies have identified several mutants that lie in the same pathway as *Polo* (Gonzalez *et al.*, 1998; do Carmo *et al.*, 2001). Both *Polo* and *Asp* mutants share similar phenotypes and there exists a synergism between these mutants (Gonzalez *et al.*, 1998), suggesting a common role in spindle pole function. *Asp* mutant cells contain bipolar spindles but with disorganized broad poles, and the distribution of  $\gamma$ -tubulin in these cells is also abnormal. *Asp* is a microtubule-associated protein that appears to mediate the interaction of centrosomes with spindle microtubules. Biochemical analyses show that Polo interacts with and phosphorylates *Asp* *in vitro*, resulting in an MPM-2 epitope (do Carmo *et al.*, 2001). Furthermore, extracts of *polo<sup>1</sup>*-deprived embryos are incapable of supporting microtubule aster nucleation by salt-stripped centrosomes; however, addition of phosphorylated *Asp* or kinase-active Polo protein can complement this defect (do Carmo *et al.*, 2001), indicating a common role of Polo and *Asp* in promoting the mitotic organizing activity of centrosomes. Given that subcellular localization of *Asp* to centrosomes is independent of Polo (Donaldson *et al.*, 2001b) it is reasonable to suggest that phosphorylation of *Asp* by Polo mainly affects the nucleating activity of this spindle protein.

Similar to the situation in yeast, Polo is also required for spindle pole function during meiosis because chromosome nondisjunction, as well as failure to undergo cytokinesis, is observed during meiosis in *polo<sup>1</sup>* mutants (Riparbelli *et al.*, 2000). A close examination reveals that there are several major defects that are related to the centrosome cycle or centrosomal function. Although no appreciable defects in spindle poles during meiosis I are observed, the spindles of meiosis II are abnormal. The growth of sperm aster microtubules is also significantly compromised. In addition, sperm centrosome duplication is uncoupled from female meiosis in *polo<sup>1</sup>* mutants.

The stability of Polo also contributes to centrosomal function (de Carcer *et al.*, 2001). Heat shock protein, Hsp90, is required for proper centrosomal function in *Drosophila* as well as in vertebrate cells (Lange *et al.*,

2000). Polo was identified as one of those proteins that are stabilized by this chaperone (de Carcer *et al.*, 2001). Hsp90 and Polo are found to be part of a protein complex. Inhibition of Hsp90 leads to the disappearance of Polo protein and thus inactivation of Polo kinase activity. It also renders protein extracts prepared from these cells incapable of complementing salt-stripped centrosomal preparations to nucleate microtubules. Consistently, supplementation of the kinase active recombinant Polo to the *in vitro* nucleation assays restores the ability of those centrosomes to nucleate microtubules. Hence, Polo appears to affect not only the organization (assembling) of centrosomes by recruiting key components but also their nucleating ability.

### Mammalian Plks and centrosomes

#### *Plk1*

Several mammalian Plks are involved in regulating centrosomal function although the molecular basis of their roles remains unclear. Plk1 was first implicated in the centrosome cycle because of its subcellular localization (Golsteyn *et al.*, 1995). Subsequent expression of a recombinant green fluorescence protein confirmed the centrosomal localization of Plk1 (Arnaud *et al.*, 1998). However, while Plk1 is concentrated at the centrosomal region during interphase, as the cell cycle progresses, Plk1 associates with mitotic spindle poles, and eventually, from metaphase on, relocates to the midzone from which the midbody eventually derives as the cell goes through telophase (Golsteyn *et al.*, 1995).

Inhibition of the activity of Plk1 by antibody injection results in cells with monoastrial microtubules nucleated from duplicated but unseparated centrosomes in both transformed and non-transformed cells (Lane and Nigg, 1996), suggesting that Plk1 may be involved in regulating centrosome maturation not duplication. This notion is supported by the observations that Plk1 antibody injection does not block DNA synthesis (Lane and Nigg, 1996) and that DNA replication and centrosome duplication are coordinated and regulated by Cdk2–cyclin E (Winey, 1999). Although the mechanism controlling centrosome maturation remains to be elucidated HsEg5, a plus end-directed kinesin-related motor protein, appears to be an important mediator for centrosome separation and bipolar spindle formation (Blangy *et al.*, 1995, 1997). HsEg5 is phosphorylated by Cdk1 and the phosphorylated form specifically associates with centrosomes and mitotic spindles (Blangy *et al.*, 1995).

Plk1 is capable of activating Cdk1 kinase activity by phosphorylation of cyclin B at the onset of mitosis resulting in translocation of this protein to the nucleus, forming an active Cdk1/cyclin B complex (Toyoshima-Morimoto *et al.*, 2001). Thus, Plk1's role in the centrosomal cycle may be through direct regulation of centrosomal components and through activation of other protein kinases, which in turn regulate the activity of centrosomal targets.

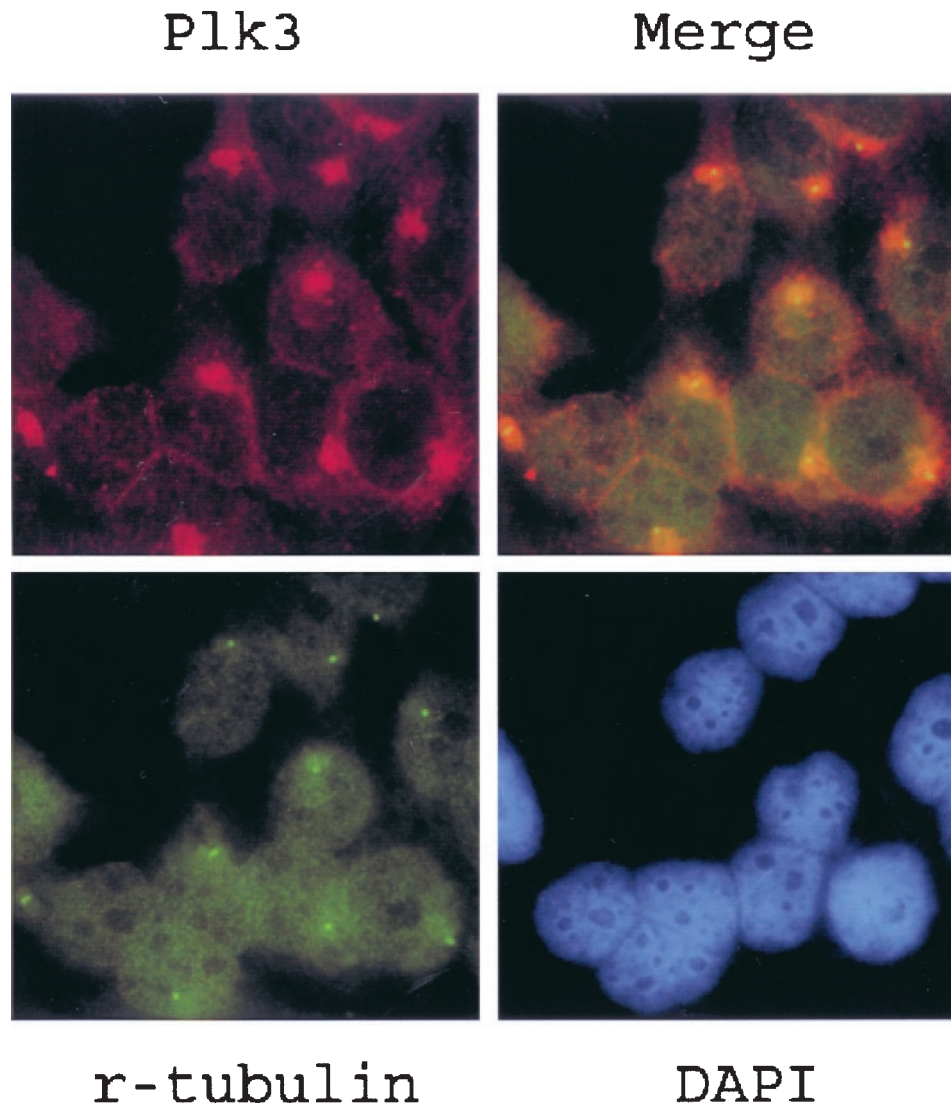
### Plk3

Using a Plk3-specific monoclonal antibody, we have observed that during interphase Plk3 primarily localizes, as a concentrated dot, to the microtubule organization center as shown by co-staining with  $\gamma$ -tubulin (Figure 1). As the cell cycle progresses through S, G2, and M phases, Plk3 appears to comigrate with duplicated centrosomes; in all stages of the cell cycle Plk3 is associated with centrosomes or mitotic spindle poles (Wang *et al.*, 2002). In addition, the localization of Plk3 to centrosomal region is microtubule-dependent because depolymerization of microtubules by cold or nocodazole treatment disrupts its centrosomal localization (Wang *et al.*, 2002). It remains unclear why both Plk1 and Plk3 are localized to centrosomal regions during interphase. Given that the kinase activity of Plk1, as well as its protein level, peak at

mitosis it is likely that Plk3, but not Plk1, may play a major role in controlling centrosomal function during interphase. This is consistent with the observation that levels of Plk3 do not fluctuate greatly during the cell cycle and that its kinase activity peaks around late S and G2 phase (Ouyang *et al.*, 1997). Thus, Plks may fulfill different functions in regulating microtubule dynamics during the cell cycle just as Cdks play different roles in regulating various events associated with cell cycle progression.

### Plks, the Golgi complex, and microtubule organization center

Our recent studies indicate that Plk3 appears to be associated with the Golgi apparatus as well during the cell cycle in many cell types (Figure 2). This is not



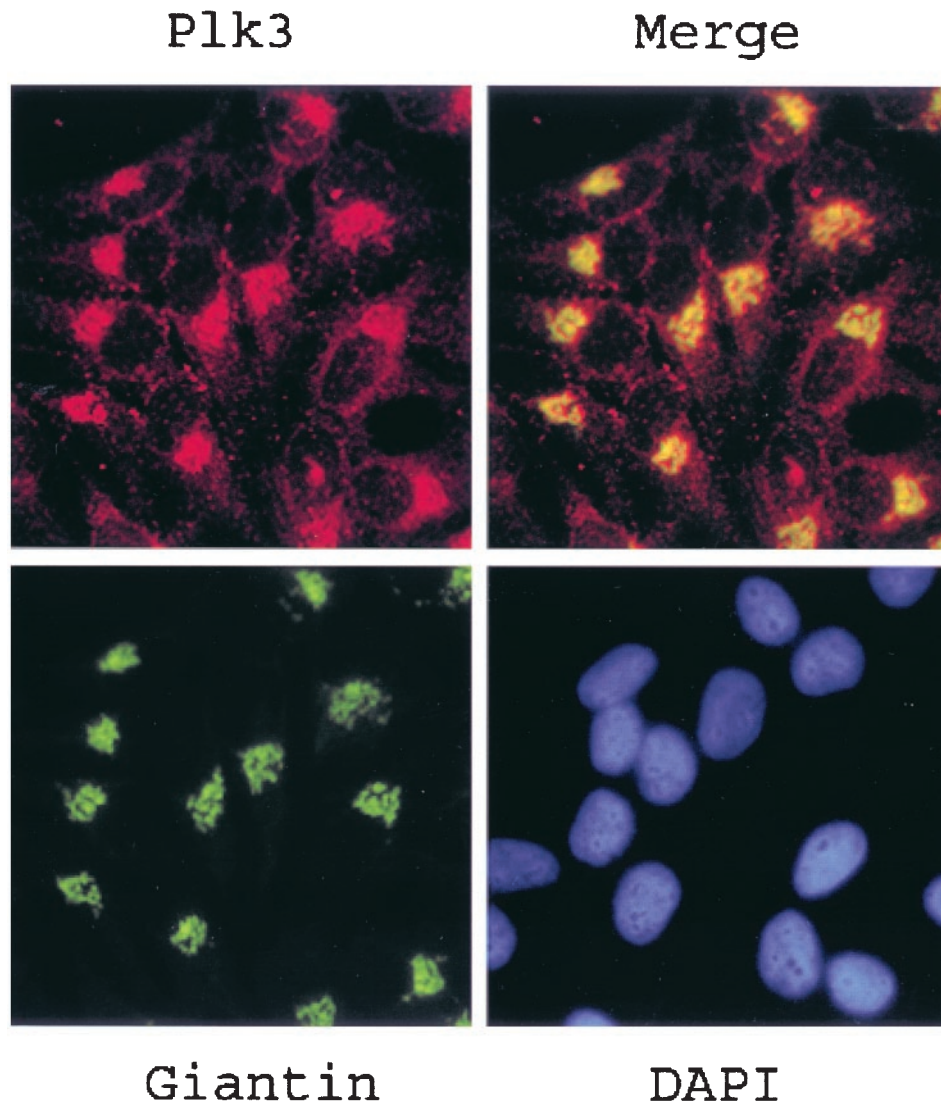
**Figure 1** Colocalization of Plk3 with  $\gamma$ -tubulin. Lung carcinoma A549 cells were double-stained with antibodies to Plk3 (red) and  $\gamma$ -tubulin (green), a centrosome-specific marker. Cellular DNA was stained with DAPI. Fluorescence microscopy was performed on a Nikon microscope and images were captured using a digital camera (Optronics) using Optronics MagFire and Image-Pro Plus softwares

surprising because the Golgi complex is closely associated with centrosomes. The striking co-localization of Plk3 with giantin, a Golgi-specific membrane protein, indicates a function of this Polo-family kinase in regulating the dynamics of the Golgi complex. Given that Golgi cisternae undergo extensive reorganization during mitosis and that the contents of the Golgi apparatus also need to be equally partitioned during cell division (Seemann *et al.*, 2002), Plks may play a key role in fragmentation, dispersal and reassembling of the Golgi apparatus during mitosis of animal cells.

Besides their close proximity, a functional relation exists between the Golgi apparatus and centrosomes. Drug-induced disruption of microtubules results in disruption of the Golgi apparatus; the microtubule-dependent motor proteins cytoplasmic dynein and kinesin bind to the Golgi membrane and are implicated in vesicular transport within the complex (Thyberg and

Moskalewski, 1999). On the other hand, the Golgi complex also has a microtubule-organizing capability. The Golgi complex can directly stimulate microtubule nucleation both *in vivo* and *in vitro*; the nucleation of Golgi-based microtubules in interphase cells involves  $\gamma$ -tubulin (Chabin-Brion *et al.*, 2001).

Plk3 and other Plks may have adapted during evolution to coordinate disassemble and reassemble of the Golgi complex during cell division through protein phosphorylation. In fact, Plk1 interacts with and phosphorylates GRASP65 (Golgi reassembly stacking protein of 65 kDa) (Lin *et al.*, 2000) which appears to be required for mitosis-specific fragmentation of the Golgi apparatus (Sutterlin *et al.*, 2001). Clearly, centrosomes and the Golgi apparatus share some common regulatory molecules during cell division. Plks appear to be one major group of protein kinases that control the dynamics of these cellular organelles.



**Figure 2** Colocalization of Plk3 with a Golgi-specific antigen. Interphase A549 cells were double-stained with antibodies to Plk3 (red) and giantin (green), a Golgi-specific marker. Cellular DNA was stained with DAPI

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