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Minireview

Protein kinases in control of the centrosome cycle

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Abstract The centrosome is the major microtubule nucleating center of the animal cell and forms the two poles of the mitotic spindle upon which chromosomes are segregated. During the cell division cycle, the centrosome undergoes a series of major structural and functional transitions that are essential for both interphase centrosome function and mitotic spindle formation. The localization of an increasing number of protein kinases to the centrosome has revealed the importance of protein phosphorylation in controlling many of these transitions. Here, we focus on two protein kinases, the polo-like kinase 1 and the NIMA-related kinase 2, for which recent data indicate key roles during the centrosome cycle.

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1. Centrosome dynamics through the cell cycle

The mammalian centrosome is composed of two barrel-shaped centrioles, each formed by nine triplets of short microtubules (MTs), surrounded by a fibrous meshwork termed the pericentriolar material. Though small, the centrosome is a vital organelle in animal cells as it directs the nucleation and organization of microtubules. By consequence, the centrosome is essential during interphase for intracellular organelle transport, cell migration and the establishment of cell shape and polarity. Equally important is its role in mitosis when, by constituting the two spindle poles, the centrosome orchestrates the formation of the mitotic spindle.

Electron microscopy has given us a good view of the structural changes that take place at the centrosome through the cell cycle [1–3]. In brief, upon entry into G1, the two centrioles separate slightly in a process of disorientation. The semi-conservative duplication of centrioles then begins around the time of S phase entry as indicated by the appearance of two pro-centrioles. These elongate throughout S and G2 reaching full size by the next mitosis. In late G2, a process of centrosome maturation takes place that is characterized by the recruitment of extra pericentriolar material leading to an overall increase in centrosome size. As cells enter mitosis, centrosome separation towards either pole leads to the establishment of a bipolar mitotic spindle. Following cytokinesis, each daughter cell inherits one centrosome.

Protein phosphorylation is one of the key mechanisms controlling centrosome function during the cell cycle. Indeed, it was demonstrated more than a decade ago that there is a significant increase in the level of phosphorylated epitopes detectable at the centrosome during mitosis [4]. As illustrated in Table 1, several structurally distinct protein kinases have been found to localize at the centrosome, either transiently or throughout the cell cycle. These include members of the cyclin-dependent kinase (Cdk) family which play a cardinal role not only in cell cycle progression but also in regulation of the centrosome. In particular, Cdk2 has recently been shown to be required for centrosome duplication in embryonic and somatic cells ([5,6], Meraldi P., Lukas J., Fry A.M., Bartek J. and Nigg E.A., Nature Cell Biol., in press). Furthermore, Cdk1 ($p34^{cdc2}$) plays a role at early mitosis in both recruitment of proteins to the centrosome [7] and modification of MT dynamics [8]. Apart from the Cdks, other families of protein kinases are also implicated in controlling the centrosome cycle such as the aurora-related kinases that are required for centrosome separation and mitotic spindle assembly [9–12]. In this short review, we highlight the contribution of two additional families of protein kinases, the polo-like kinases and the NIMA-related kinases, to the centrosome regulation through the cell cycle.

2. Polo kinases and spindle formation

The *Drosophila* gene *polo* is the founding member of a serine-threonine kinase family, the polo-like kinases (Plks), found universally from fungi to vertebrates [13,14]. Their activation profile together with functional studies in different organisms point to multiple roles for Plks during mitosis. These include the activation of Cdk1 through phosphorylating Cdc25, the control of the metaphase to anaphase transition through regulating APC activity and a requirement for cytokinesis. In addition, at the onset of mitosis, several Plks display a transient association with the centrosomes or the spindle pole body (SPB), the major MT organizing center in yeast [13,15–17]. Moreover, disruption of Plk function almost invariantly leads to some form of mitotic spindle defect. Hence, Plks appear to have an important function in the centrosome cycle that is essential for the correct establishment of the bipolar mitotic spindle.

While *polo* mutations lead to a diverse array of mitotic spindle abnormalities in *Drosophila* embryos, the frequent appearance of monopolar spindles suggests that part of this defect emanates from the inability to separate centrosomes [18,19]. The same result is seen in fission yeast where loss of Plo1, the *Schizosaccharomyces pombe* Plk, also induces monopolar spindle formation with unseparated spindle poles [20]. Plk activation in different organisms coincides with the time

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Table 1
Protein kinases associated with the centrosome

Protein	Function
Cyclin-dependent kinases:	
Cdk1 ($p34^{cdc2}$) [35,36]	Centrosome maturation, phosphorylation of the kinesin-like motor protein Eg5 and regulation of MT nucleation and dynamics.
Polo-like kinases[a]:	
Plk1, Polo, Plx1 [15–17]	Centrosome maturation and establishment of the bipolar spindle.
Aurora-related kinases:	
Aurora-related kinase 1[b] [9–12] (IAK, Aik1, Eg2, AIR-1)	Centrosome separation and spindle formation.
Aik3 [37]	Mitotic function?
NIMA-related kinases:	
Nek2[29]	Centrosome separation, phosphorylation of C-Nap1.
NIMA?[c] [28]	Recruitment of Cdk1/cyclin B to the spindle pole body.
Other kinases:	
cAMP-dependent kinase II [38,39]	Regulation of MT dynamics?
Ca^{2+}/calmodulin kinases II [40]	MT stability?
Casein kinase I-α [41]	
Casein kinase II [42]	
Fyn [43]	Signaling in T lymphocytes.
Phosphoinositide 3-kinase [44]	
LK6 [45]	
PKC-τ [46]	
Phosphatases[d]:	
Protein phosphatase 4 [47]	MT organization.
Protein phosphatase 1-α [48]	Regulation of MT dynamics.

[a]Yeast Plks (Cdc5p, Plo1p) have also been localized to the SPB [13].
[b]A second subclass of aurora-related kinases appears to have no function at the centrosome and instead is required for cytokinesis.
[c]NIMA is a low abundance protein, its endogenous localization has yet to be determined.
[d]Protein phosphatases have also been reported to be associated with the centrosome.

of centrosome separation and the identification of some candidate Plk substrates lends further support to an active role in this process. Putative substrates include β-tubulin, two MAPs (85 kD and 220 kD/Asp) and a kinesin-like motor protein (pavarotti) that has been independently shown to be required for spindle formation [13,21]. Centrosome separation, however, is a complex mechanism that probably depends on not only activation of motor proteins, but also recruitment of additional protein complexes to the emerging spindle poles. In this light, it is important to note that disruption of Plk function, as well as preventing separation, also leads to a failure in centrosome maturation. In fly embryos, *polo* mutants fail to recruit the CP190 protein to the centrosome [18] and, in a similar manner, microinjection of human Plk1 antibodies into immortalized cells leads to a mitotic block with monopolar spindles and small immature centrosomes that fail to recruit either γ-tubulin or MPM-2 phosphoepitopes [22]. Clearly, we are still at an early stage in dissecting the role of Plks at the centrosome in early mitosis but data would tend to point to a dual role in stimulating both recruitment and activation of proteins required for centrosome separation and bipolar spindle formation.

As mentioned above, one major substrate of Plk is the Cdc25C phosphatase which is activated by Plk phosphorylation [23]. This in turn leads to activation of Cdk1 which further activates Cdc25C and, as a result of this feedback loop, the onset of mitosis occurs. It is possible that this activation cascade and positive feedback loop is enhanced by the concentration of the relevant molecules at the centrosome. This emphasizes the principle of the centrosome as a 'meeting place' to focus kinases and substrates [24]. Although this review aims to illustrate how protein kinases regulate centrosomes, centrosomes may also be important in regulating kinases.

3. NIMA-related kinase 2 (Nek2) in an early step to centrosome separation

The mitotic separation of centrosomes and the establishment of the bipolar spindle are intimately linked processes. Protein complexes comprising molecular motors bind and cross-link MTs in a manner that jointly leads to centrosome separation and spindle assembly. Recent studies with Nek2, a member of the NIMA kinase family, have indicated the possibility of an earlier step in the process of centrosome separation that precedes the action of MTs and protein motors.

Nek2 is a mammalian serine/threonine kinase that is structurally related to the mitotic regulator NIMA of the filamentous fungus *Aspergillus nidulans* [25]. *nimA* was first identified as a temperature-sensitive mutant that became blocked in G2 when raised to the restrictive temperature and cloning of the gene identified it as a serine/threonine kinase with a peak activity at the G2/M transition. Overexpression of NIMA in *Aspergillus* promotes a premature condensation of chromosomes and, when expressed from high level promoters, the premature appearance of mitotic spindles [26]. More recent data show that NIMA directly cooperates with Cdk1 to promote mitotic progression [27] and, intriguingly, is required for the localization of Cdk1/cyclin B to the nucleus and spindle pole body [28]. However, as no NIMA substrates have yet been identified, its molecular action remains obscure.

Human Nek2 is the most closely related vertebrate kinase to NIMA and its protein abundance and kinase activity are also cell cycle-regulated, but with peak levels in S/G2 and a low activity in a mitotic arrest. Overexpression of Nek2 in mammalian cells has no obvious effect on chromatin condensation. However, it causes a striking alteration in the structure of the centrosome. Specifically, active Nek2 induces a pronounced splitting of centrosomes, characterized by the sepa-

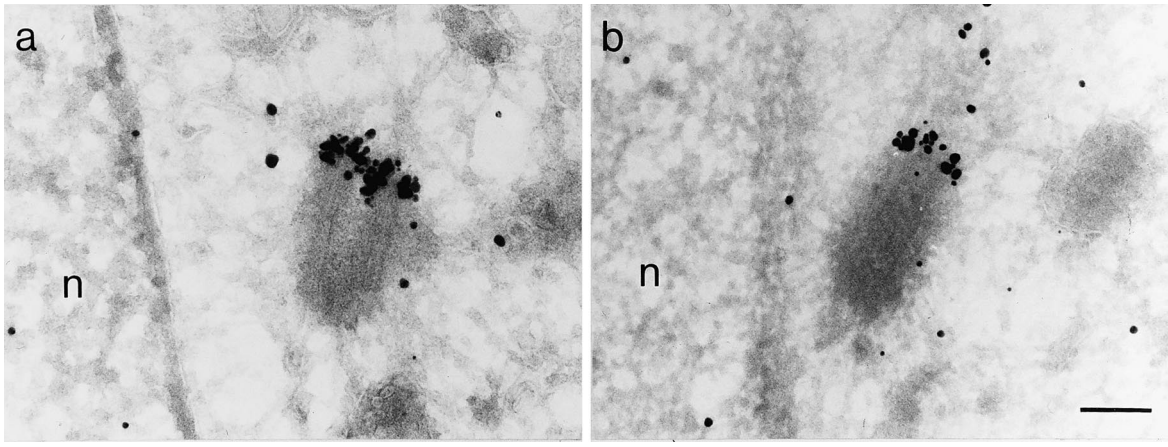

Fig. 1. Localization of C-Nap1. Immunoelectron microscopy on ultrathin cryosections of U2OS osteosarcoma cells with anti-C-Nap1 (R63) antibodies and silver-enhanced Nanogold reveals the strict localization of C-Nap1 to the proximal end of centrioles (a and b). n, nucleus. Bar, 250 nm.

ration of the two centrioles often by a distance of up to half a cell length. This splitting is not associated with any other aspect of spindle formation nor recruitment of proteins such as Eg5 to the centrosome [29]. It also appears to occur from any point of the cell cycle (P.M and E.A.N. unpublished results). In contrast, a catalytically inactive mutant of Nek2 does not induce splitting, indicating that this phenotype is dependent on Nek2 kinase activity. Localization studies and biochemical fractionation confirmed the importance of this result by demonstrating that Nek2 is a core component of the centrosome throughout the cell cycle [29,30]. Hence, we proposed that Nek2-dependent phosphorylation of centrosomal substrates may stimulate a loss of cohesion between the duplicated centrosomes in late G2. This in turn may represent a prerequisite step for subsequent motor protein-driven centrosome separation. Overexpression of Nek2 at inappropriate times in the cell cycle would promote an illegitimate splitting without spindle formation.

In an attempt to search for targets of the Nek2 kinase which may be involved in centrosome-centrosome cohesion, we recently isolated a candidate substrate through a yeast two-hybrid interaction screen. The isolated clone represented the carboxy-terminal domain of a 281 kDa novel coiled-coil protein that was subsequently found to be a core component of the centrosome [30]. Hence, we named this protein C-Nap1, for centrosomal Nek2-associated protein 1. Database searches revealed the presence of a potential homologue in mouse as well as weak similarities to many coiled-coil proteins. C-Nap1 was also independently isolated using human autoimmune sera reactive against centrosomal antigens [31].

Intriguingly, immunoelectron microscopy performed following either pre-embedding [30] or ultrathin cryosectioning (Fig. 1) shows that C-Nap1 is specifically associated with the proximal end of the two centrioles. There is little penetration inside the centriole barrel and no specific staining elsewhere in the pericentriolar material. Equivalent studies with Nek2 indicate a remarkably similar pattern of staining, demonstrating a strict co-localization of Nek2 and C-Nap1 at the proximal end of centrioles. Importantly, whereas C-Nap1 antibody staining of interphase centrosomes is consistently strong, mitotic spindle poles are, in comparison, very poorly labeled. Indeed, loss of C-Nap1 staining is first detected at early prophase when centrosomes begin to separate and continues until after telophase when C-Nap1 again becomes clearly detectable on centrosomes of early G1 post-mitotic cells. Finally, Nek2 is able to phosphorylate the carboxy-terminal domain of C-Nap1 both in vitro and upon co-expression of the two proteins in tissue culture cells, suggesting that C-Nap1 may be a bona fide substrate of the Nek2 kinase.

Based on these observations, our current working model implicates C-Nap1 in a bridge structure that links the two centrosomes throughout interphase (Fig. 2). Electron dense material connecting the proximal ends of centrioles has been observed on purified centrosomes [32] and C-Nap1 localization suggests that it is in a strategic position to anchor the bridge to the centriole. At the G2/M transition, phosphorylation by Nek2 might regulate C-Nap1 interaction or stability leading to dissolution of the 'bridge' prior to the second step of motor protein-driven centrosome separation. Clearly, being active in G2 and a putative regulator of C-Nap1, Nek2 is an ideal candidate kinase to trigger this event. However, it should be pointed out that serum stimulation and drug treatment can also induce transient splitting in interphase cells at a time when Nek2 is not supposed to be active [33,34]. Indeed, we found recently that overexpression of certain other active kinases can also trigger centrosome splitting, albeit to different

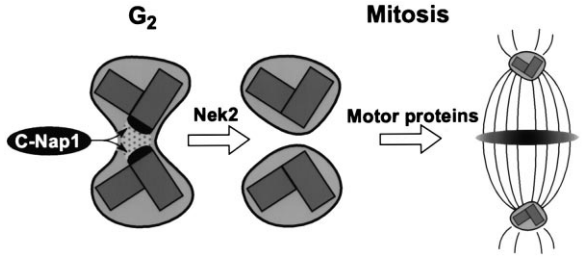

Fig. 2. Working model for the role of Nek2 and C-Nap1 in an early step of centrosome separation. Based on the results of Nek2 overexpression and C-Nap1 localization, we propose that active Nek2, by phosphorylating C-Nap1, causes the removal of a bridge structure between the two pairs of centrioles during the G2/M transition. In a second step, activation of MT-associated motor proteins (e.g. Eg5) leads to separation of the two centrosomes and formation of the bipolar mitotic spindle.

extents (P.M. and E.A.N, unpublished results). Whether these act directly on C-Nap1, Nek2 or other targets remains to be determined.

4. Conclusion

Phosphorylation has a multitude of effects on the properties of centrosomes during the cell cycle. We are only beginning to understand how individual protein kinases and phosphatases regulate centrosome function. Yet, the centrosome cycle clearly constitutes an important subject for future study, as aberrant regulation of this process may lead to the formation of abnormal spindles and hence cause missegregation of chromosomes.

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