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Regulation of Aurora-A kinase on the mitotic spindle

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Abstract The error-free segregation of duplicated chromosomes during cell division is essential for the maintenance of an intact genome. This process is brought about by a highly dynamic bipolar array of microtubules, the mitotic spindle. The formation and function of the mitotic spindle during M-phase of the cell cycle is regulated by protein phosphorylation, involving multiple protein kinases and phosphatases. Prominent among the enzymes implicated in spindle assembly is the serine/threonine-specific protein kinase Aurora-A. In several common human tumors, Aurora-A is overexpressed, and deregulation of this kinase was shown to result in mitotic defects and aneuploidy. Moreover, recent genetic evidence directly links the human Aurora-A gene to cancer susceptibility. Several of the physiological substrates of Aurora-A presumably await identification, but recent studies are beginning to shed light on the regulation of this critical mitotic kinase. Here, we review these findings with particular emphasis on the role of TPX2, a prominent spindle component implicated in a Ran-GTP-mediated spindle assembly pathway.

The Aurora family of mitotic kinases

In mammals, the Aurora family of serine/threonine protein kinases consists of three members, termed Aurora-A, -B and -C (Nigg 2001). Whereas Aurora-C is predominantly expressed in testis, suggesting an important role in male meiosis (Kimura et al. 1999), Aurora-A and -B are ubiquitously expressed, with peak activities during late G2- and M-phase of the somatic cell cycle (Bischoff et al. 1998). Aurora-B is a so-called chromosome passenger protein that displays a characteristic

redistribution from kinetochores on (pro-)metaphase chromosomes to the central spindle in anaphase and telophase cells (Adams et al. 2001). Aurora-B has been implicated in the establishment of correct (bipolar) microtubule–kinetochore attachments and in cytokinesis (Shannon and Salmon 2002). Aurora-A, the focus of this short review, has been localized to both centrosomes and spindle microtubules, and studies in different species point to a critical role in centrosome maturation and bipolar spindle assembly (Bischoff and Plowman 1999; Dutertre et al. 2002; Blagden and Glover 2003). Interestingly, the human Aurora-A gene localizes to a chromosomal region (20q13.2) that is amplified in a variety of human cancers (Sen et al. 1997). Concomitantly, Aurora-A is overexpressed in the corresponding tumors, and recent studies identified the Aurora-A gene as a low-penetrance tumor-susceptibility gene in both mice and humans (Ewart-Toland et al. 2003). Excess Aurora-A activity was shown to confer tumorigenic properties to cells (Bischoff et al. 1998; Zhou et al. 1998) and it is plausible that this reflects the ability of overexpressed Aurora-A to produce failure of cytokinesis, leading to centrosome amplification and polyploidization (Meraldi et al. 2002; Anand et al. 2003).

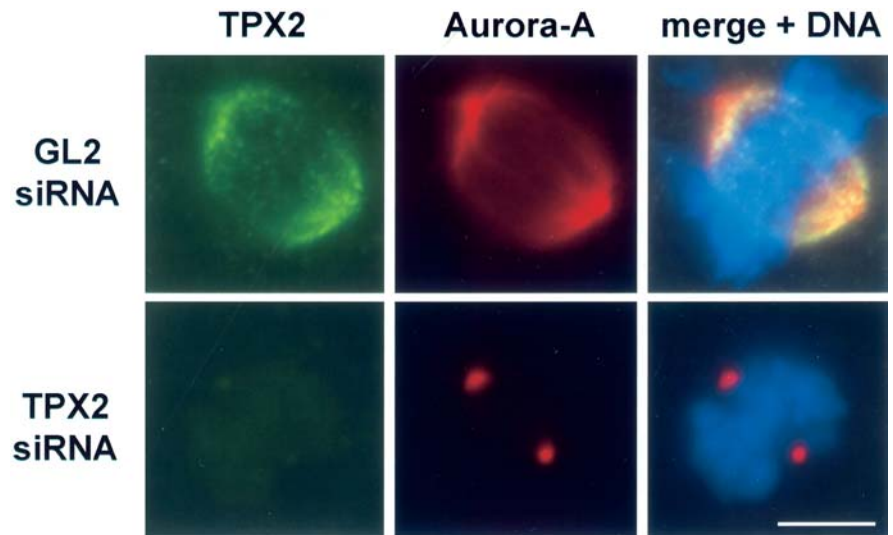
TPX2 controls both Aurora-A localization and activity

A number of recent findings have considerably advanced our understanding of the regulation of Aurora-A. The first insight came when a search for proteins interacting with Aurora-A revealed TPX2 as a prominent interaction partner of this kinase in mitotic human cells (Kufer et al. 2002). This was an intriguing finding, because TPX2 is not only a prominent component of the mitotic spindle (Wittmann et al. 2000), but also a key player in a spindle assembly process that is regulated by the small GTPase Ran (Gruss et al. 2001). According to a provocative model, established primarily in *Xenopus* egg extracts, the GTP form of Ran releases TPX2 from an inhibitory complex with the nuclear import factors importin- α/β

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Fig. 1 TPX2 localizes Aurora-A to the spindle. HeLa S3 cells were treated for 36 h with an siRNA duplex specific for human TPX2 (*TPX2*) or a control duplex (*GL2*) and then fixed and stained with the antibodies indicated. *Right-hand panels* show merged images, including DNA staining by 4',6-diamidino-2-phenylindole. *Bar* represents 10 μ m. This figure was reproduced from *The Journal of Cell Biology*, 2002, 158(4), 617–623 by copyright permission of The Rockefeller University Press



(Gruss et al. 2001). Because the GTP-exchange factor for Ran (RCC1) is associated with condensed chromosomes, while the GTPase for Ran (RanGAP) is primarily a soluble protein, a Ran-GTP gradient is established, which favors microtubule assembly in the vicinity of chromosomes (Dasso 2002; Kalab et al. 2002). This mechanism is expected to be particularly important in large cells (e.g. *Xenopus* eggs) where chromatin occupies only a small part of the cellular volume, but recent data indicate that Ran-GTP contributes to control spindle assembly also in human tissue culture cells (Gruss et al. 2002; Keryer et al. 2003).

In addition to identifying TPX2 as a physiological interaction partner of Aurora-A kinase, Kufer and coworkers further showed, by using siRNA (small, interfering RNA), that TPX2 is required for targeting Aurora-A to mitotic spindle microtubules, but not for targeting to centrosomes (Fig. 1) (Kufer et al. 2002). Two laboratories have independently confirmed the interaction between TPX2 and Aurora-A, and, moreover, found that TPX2 acts as an activator of the *Xenopus* Aurora-A kinase (also known as Eg2) (Eyers et al. 2003; Tsai et al. 2003). In a well-designed series of experiments Zheng and collaborators showed that TPX2 is released by Ran-GTP from the nuclear import receptor importin- α/β and then stimulates the activation of Aurora-A (Tsai et al. 2003). Independently, the Maller laboratory fractionated *Xenopus* egg extracts to search for activities that would be able to activate Aurora-A. Remarkably, this unbiased chromatographic separation approach also led to the identification of TPX2 (Eyers et al. 2003). Both the Zheng and Maller groups also provided evidence bearing on the mechanism by which TPX2 binding could lead to activation of Aurora-A. The activation of this kinase had previously been shown to depend on (auto)phosphorylation of a conserved threonine in the T-loop (human Thr288) (Walter et al. 2000; Littlepage et al. 2002). Moreover, this phosphorylation was shown to be counteracted by a phosphatase, most likely protein phosphatase 1 (PP1) (Walter et al. 2000; Katayama et al. 2001).

On the basis of this information, Zheng and Maller and their respective colleagues showed that the activation of Aurora-A by TPX2 reflects an antagonistic effect of TPX2 on the action of PP1, which then results in increased T-loop phosphorylation and activation of Aurora-A (Eyers et al. 2003; Tsai et al. 2003). In functional assays, it was further shown that spindle formation in egg extracts could be severely impaired by addition of a mutant Aurora-A kinase (Eg2^{T295A}) in which the T-loop phosphorylation site was replaced by a non-phosphorylatable residue, indicating that the TPX2-dependent stimulation of Aurora-A phosphorylation is required for spindle assembly (Tsai et al. 2003). These two studies thus concur to identify TPX2 as an important activator of Aurora-A in *Xenopus* eggs and early embryos, extending the original identification of the interaction between these proteins in human somatic cells (Kufer et al. 2002). In addition, they raised an interesting issue concerning the exact role of microtubules in the activation of Aurora-A by TPX2. Whereas data from the Zheng laboratory suggested that microtubules are required for this activation, the Maller group observed substantial activation without addition of microtubules. Recent studies with human Aurora-A and TPX2 indicate that TPX2 can activate Aurora-A directly, but that microtubules further enhance this activation (Fig. 2) (Bayliss et al. 2003; Kufer, unpublished data).

A crystal structure illuminates the mechanism of Aurora-A activation by TPX2

If, as suggested by the studies discussed above, TPX2 activates Aurora-A kinase by counteracting PP1, how does this occur mechanistically? A thorough structural study by Conti and coworkers recently answered this question (Bayliss et al. 2003). As previous data had demonstrated that the N-terminal half of TPX2 is

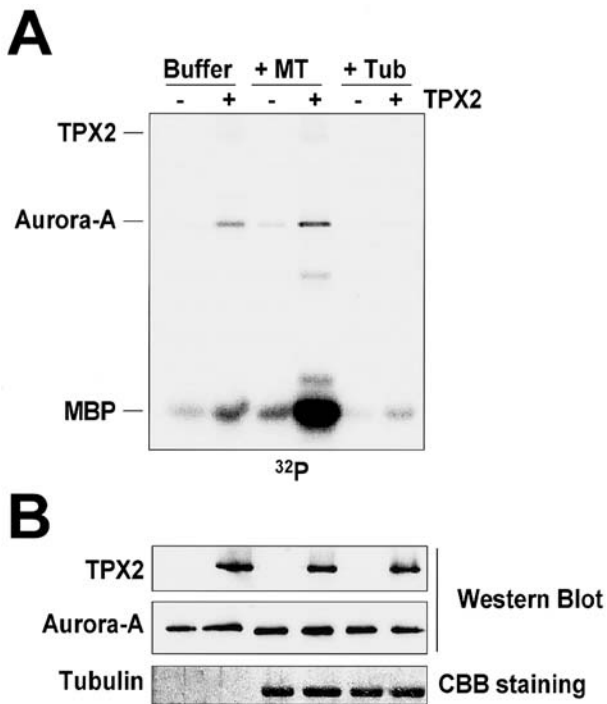


Fig. 2A, B Activation of Aurora-A by TPX2 and microtubules. In vitro kinase assays were performed with recombinant Aurora-A (produced in Sf9 insect cells) in the presence or absence of human TPX2 (produced in *Escherichia coli*). Where indicated, samples were incubated with taxol-stabilized microtubules (+MT) for 10 min at room temperature before starting the reaction by addition of [γ -³²P]ATP. Buffer only or tubulin dimers (+Tub) were used for controls. Kinase reactions were performed for 30 min at 30°C, using myelin basic protein (MBP) as a substrate. Phosphate incorporation was visualized by autoradiography (A). As a control for the presence of components and equal loading, Western blotting with the indicated antibodies and Coomassie Brilliant Blue (CBB) staining was performed (B)

sufficient to both bind and activate Aurora-A (Kufer et al. 2002; Eyers et al. 2003), attention was focused on the first N-terminal 43 amino acids, the region in TPX2 that shows the highest sequence conservation among species. After establishing that this fragment of TPX2 was indeed sufficient for binding and activating human Aurora-A, Conti and coworkers solved the crystallographic structure of the catalytic domain of Aurora-A in the presence or absence of this TPX2-derived activation domain. In the absence of the TPX2 fragment, the activation segment (T-loop) of Aurora-A was found to be in a flexible conformation, with the phosphorylated Thr288 exposed to the solvent and hence susceptible to dephosphorylation by PP1. In contrast, in the presence of the TPX2 peptide the activation segment was constrained in a different conformation, so that the phosphorylated Thr288 was oriented inwardly, and in this buried position was protected from dephosphorylation. These structural data very nicely explain the biochemical observation that binding of TPX2 protects Aurora-A from dephosphorylation by PP1. It follows that binding of TPX2 and (auto)phosphorylation of Thr288 (Thr295 in *Xenopus*) act synergis-

tically to lock Aurora-A in an active conformation. While this mechanism bears some resemblance to the localized movement of the activation segment that occurs in the intramolecular activation of the cAMP-dependent kinase (PKA), it is fundamentally different from the activation of Cyclin-dependent kinases (Cdks). In the latter case, the binding of the cyclin regulatory subunit induces an extensive conformational change affecting a large part of the kinase domain.

Interestingly, homology modeling suggests that Aurora-B exhibits a similar hydrophobic groove as Aurora-A. Considering that Aurora-B is also activated by an interacting protein, termed INCENP (Bishop and Schumacher 2002; Honda et al. 2003), it will be interesting to see whether the activation of Aurora-B by INCENP resembles the described mode of activation of Aurora-A by TPX2, in spite of the fact that TPX2 and INCENP bear no obvious sequence similarity.

Other contenders for Aurora-A activation

Localization data suggest that TPX2 acts primarily on Aurora-A that is associated with spindle microtubules (Kufer et al. 2002). In contrast, the regulation of the centrosome-associated pool of Aurora-A is likely to involve additional proteins. Two candidates for such a function have recently been reported. In *Drosophila*, the centrosome component centrosomin (CNN) was reported to be required for targeting Aurora-A to the centrosome and vice versa (Terada et al. 2003). Centrosomin was shown to interact with γ -tubulin ring complexes (γ -TuRCs) through its N-terminus and to *Drosophila* Aurora-A via its C-terminus (Terada et al. 2003). To determine whether this mechanism is conserved in other organisms it would obviously be important to characterize functional homologs of *Drosophila* centrosomin in other species. In the meantime, another structurally unrelated protein was described to activate Aurora-A at the centrosome in human cells (Hirota et al. 2003). This protein, known as Ajuba, contains LIM domains (Goyal et al. 1999). Previous studies had implicated Ajuba in cell adhesion and revealed its ability to shuttle between cell-cell contacts and the nucleus (Kanungo et al. 2000; Marie et al. 2003). Thus the reported role of Ajuba in the control of spindle assembly and chromosome segregation is surprising. However, it is possible that Ajuba might have a function in both cell signaling and spindle formation. Precedents for proteins with multiple functions, including roles at the cell periphery, signaling to the nucleus, and spindle function, can be found among components of the Wnt signalling pathway (Dikovskaya et al. 2001). More difficult to reconcile with an essential function of Ajuba in mitosis is the finding that keratinocytes from Ajuba^{-/-} knock-out mice are viable (Marie et al. 2003). Thus, the precise role of Ajuba in the regulation of Aurora-A and mitotic entry requires further study.

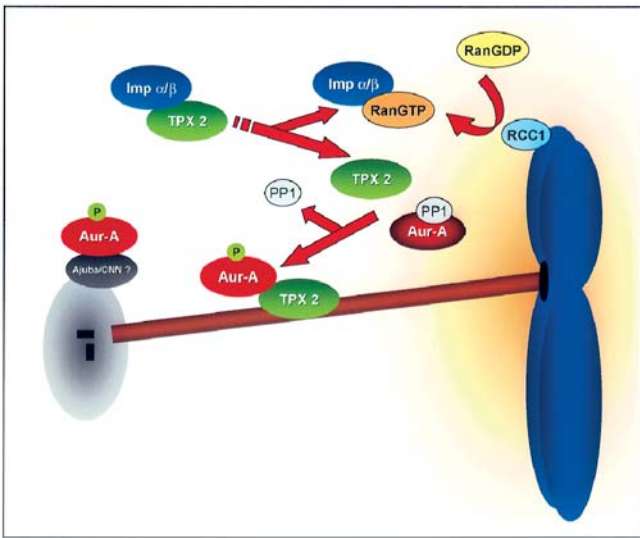


Fig. 3 A model linking Ran-GTP to Aurora-A activation by TPX2 on the spindle apparatus. The orange halo represents a gradient of Ran-GTP that could arise through asymmetric localization of the Ran-regulatory proteins RCC1 (=RanGEF) and RanGAP. Such a gradient has been described in *Xenopus* egg extracts (Dasso 2002; Kalab et al. 2002) but whether it occurs in somatic cells remains to be determined. For further explanation see text

Conclusions and perspectives

With TPX2, one important regulator of Aurora-A at the mitotic spindle has been identified. The proposed roles of centrosomin and Ajuba in regulating Aurora-A at the centrosome are also intriguing but await future detailed investigation. The identification of TPX2 is particularly interesting, for several reasons: first, TPX2 establishes a link between Ran-GTP and the activation of a key mitotic kinase on the spindle apparatus (Fig. 3) (Eyers et al. 2003; Tsai et al. 2003). If, as suggested by recent evidence (Dasso 2002, Kalab et al. 2002), the local concentration of Ran-GTP is controlled by chromatin-associated RCC1, the action of Ran-GTP could spatially coordinate spindle assembly with the positioning of chromatin. Second, although the interaction between Aurora-A and TPX2 was discovered only recently (Kufer et al. 2002), the mode of interaction between these two proteins is already understood in considerable molecular and mechanistic detail (Bayliss et al. 2003). Nevertheless, a number of important questions remain. In particular, it remains to be clarified how exactly microtubules enhance TPX2-mediated activation of Aurora-A. Do microtubules act directly on Aurora-A or do they act through binding to TPX2? The former scenario is not excluded, as Aurora-A has been reported to bind to microtubules in vitro (Roghi et al. 1998). Another point to bear in mind is that TPX2 targets not only Aurora-A to the mitotic spindle, but also the kinesin-like protein 2 (Klp2), from which its name originates (targeting protein for XKlp2), and perhaps yet other components (Wittmann et al. 1998, 2000). Thus, TPX2 could conceivably form an adapter for loading

various proteins onto the mitotic spindle. This in turn might contribute to bring Aurora-A in close proximity to its potential substrates. These include TPX2, but the physiological significance of TPX2 phosphorylation by Aurora-A (Kufer et al. 2002; Eyers et al. 2003; Tsai et al. 2003) and perhaps other mitotic kinases remains to be unraveled. Finally, the crystal structure of the Aurora-A/TPX2 complex provides a further impetus for the characterization of small-molecule inhibitors of Aurora kinases. The first such inhibitors have recently been described (Ditchfield et al. 2003; Hauf et al. 2003) and they may prove invaluable not only in basic research but also in therapeutic applications aimed at the treatment of cancer and other hyperproliferative diseases.

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References

- Adams RR, Carmena M, Earnshaw WC (2001) Chromosomal passengers and the (aurora) ABCs of mitosis. *Trends Cell Biol* 11:49–54
- Anand S, Penrhyn-Lowe S, Venkitaraman AR (2003) AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. *Cancer Cell* 3:51–62
- Bayliss R, Sardon T, Vernos I, Conti E (2003) Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol Cell* 12:851–862
- Bischoff JR, Plowman GD (1999) The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis. *Trends Cell Biol* 9:454–459
- Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, Schryver B, Flanagan P, Clairvoyant F, Ginther C, Chan CS, Novotny M, Slamon DJ, Plowman GD (1998) A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J* 17:3052–3065
- Bishop JD, Schumacher JM (2002) Phosphorylation of the carboxyl terminus of inner centromere protein (INCENP) by the Aurora B kinase stimulates Aurora B kinase activity. *J Biol Chem* 277:27577–27580
- Blagden SP, Glover DM (2003) Polar expeditions — provisioning the centrosome for mitosis. *Nat Cell Biol* 5:505–511
- Dasso M (2002) The Ran GTPase: theme and variations. *Curr Biol* 12:R502–R508
- Dikovskaya D, Zumburn J, Penman GA, Nathke IS (2001) The adenomatous polyposis coli protein: in the limelight out at the edge. *Trends Cell Biol* 11:378–384
- Ditchfield C, Johnson VL, Tighe A, Ellston R, Haworth C, Johnson T, Mortlock A, Keen N, Taylor SS (2003) Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J Cell Biol* 161:267–280
- Dutertre S, Descamps S, Prigent C (2002) On the role of aurora-A in centrosome function. *Oncogene* 21:6175–6183
- Ewart-Toland A, Briassouli P, De Koning JP, Mao JH, Yuan J, Chan F, MacCarthy-Morrogh L, Ponder BA, Nagase H, Burn J, Ball S, Almeida M, Linardopoulos S, Balmain A (2003) Identification of Stk6/STK15 as a candidate low-penetrance tumor-susceptibility gene in mouse and human. *Nat Genet* 34:403–412
- Eyers PA, Erikson E, Chen LG, Maller JL (2003) A novel mechanism for activation of the protein kinase aurora a. *Curr Biol* 13:691–697
- Goyal RK, Lin P, Kanungo J, Payne AS, Muslin AJ, Longmore GD (1999) Ajuba, a novel LIM protein, interacts with Grb2,

- augments mitogen-activated protein kinase activity in fibroblasts, and promotes meiotic maturation of *Xenopus* oocytes in a Grb2- and Ras-dependent manner. *Mol Cell Biol* 19:4379–4389
- Gruss OJ, Carazo-Salas RE, Schatz CA, Guarguaglini G, Kast J, Wilm M, Le Bot N, Vernos I, Karsenti E, Mattaj IW (2001) Ran induces spindle assembly by reversing the inhibitory effect of importin alpha on TPX2 activity. *Cell* 104:83–93
- Gruss OJ, Wittmann M, Yokoyama H, Pepperkok R, Kufer T, Sillje H, Karsenti E, Mattaj IW, Vernos I (2002) Chromosome-induced microtubule assembly mediated by TPX2 is required for spindle formation in HeLa cells. *Nat Cell Biol* 4:871–879
- Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, Heckel A, Van Meel J, Rieder CL, Peters JM (2003) The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J Cell Biol* 161:281–294
- Hirota T, Kunitoku N, Sasayama T, Marumoto T, Zhang D, Nitta M, Hatakeyama K, Saya H (2003) Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. *Cell* 114:585–598
- Honda R, Korner R, Nigg EA (2003) Exploring the functional interactions between Aurora B, INCENP, and Survivin in mitosis. *Mol Biol Cell* 14:3325–3341
- Kalab P, Weis K, Heald R (2002) Visualization of a Ran-GTP gradient in interphase and mitotic *Xenopus* egg extracts. *Science* 295:2452–2456
- Kanungo J, Pratt SJ, Marie H, Longmore GD (2000) Ajuba, a cytosolic LIM protein, shuttles into the nucleus and affects embryonal cell proliferation and fate decisions. *Mol Biol Cell* 11:3299–3313
- Katayama H, Zhou H, Li Q, Tatsuka M, Sen S (2001) Interaction and feedback regulation between STK15/BTAK/Aurora-A kinase and protein phosphatase 1 through mitotic cell division cycle. *J Biol Chem* 276:46219–46224
- Keryer G, Di Fiore B, Celati C, Lechtreck KF, Mogensen M, Delouee A, Lavia P, Bornens M, Tassin AM (2003) Part of Ran is associated with AKAP450 at the centrosome: involvement in microtubule-organizing activity. *Mol Biol Cell* 14:4260–4271
- Kimura M, Matsuda Y, Yoshioka T, Okano Y (1999) Cell cycle-dependent expression and centrosome localization of a third human aurora/Ipl1-related protein kinase, AIK3. *J Biol Chem* 274:7334–7340
- Kufer TA, Sillje HH, Korner R, Gruss OJ, Meraldi P, Nigg EA (2002) Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J Cell Biol* 158:617–623
- Littlepage LE, Wu H, Andresson T, Deanehan JK, Amundadottir LT, Ruderman JV (2002) Identification of phosphorylated residues that affect the activity of the mitotic kinase Aurora-A. *Proc Natl Acad Sci U S A* 99:15440–15445
- Marie H, Pratt SJ, Betson M, Epple H, Kittler JT, Meek L, Moss SJ, Troyanovsky S, Attwell D, Longmore GD, Braga VM (2003) The LIM protein Ajuba is recruited to cadherin-dependent cell junctions through an association with alpha-catenin. *J Biol Chem* 278:1220–1228
- Meraldi P, Honda R, Nigg EA (2002) Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53^{-/-} cells. *EMBO J* 21:483–492
- Nigg EA (2001) Mitotic kinases as regulators of cell division and its checkpoints. *Nat Rev Mol Cell Biol* 2:21–32
- Roghi C, Giet R, Uzbekov R, Morin N, Chartrain I, Le Guellec R, Couturier A, Doree M, Philippe M, Prigent C (1998) The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J Cell Sci* 111:557–572
- Sen S, Zhou H, White RA (1997) A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. *Oncogene* 14:2195–2200
- Shannon KB, Salmon ED (2002) Chromosome dynamics: new light on Aurora B kinase function. *Curr Biol* 12:R458–R460
- Terada Y, Uetake Y, Kuriyama R (2003) Interaction of Aurora-A and centrosomin at the microtubule-nucleating site in *Drosophila* and mammalian cells. *J Cell Biol* 162:757–763
- Tsai MY, Wiese C, Cao K, Martin O, Donovan P, Ruderman J, Prigent C, Zheng Y (2003) A Ran signalling pathway mediated by the mitotic kinase Aurora A in spindle assembly. *Nat Cell Biol* 5:242–248
- Walter AO, Seghezzi W, Korver W, Sheung J, Lees E (2000) The mitotic serine/threonine kinase Aurora2/AIK is regulated by phosphorylation and degradation. *Oncogene* 19:4906–4916
- Wittmann T, Boleti H, Antony C, Karsenti E, Vernos I (1998) Localization of the kinesin-like protein Xklp2 to spindle poles requires a leucine zipper, a microtubule-associated protein, and dynein. *J Cell Biol* 143:673–685
- Wittmann T, Wilm M, Karsenti E, Vernos I (2000) TPX2, a novel *Xenopus* MAP involved in spindle pole organization. *J Cell Biol* 149:1405–1418
- Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S (1998) Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 20:189–193