Cell Cycle-dependent Expression and Spindle Pole Localization of a Novel Human Protein Kinase, Aik, Related to Aurora of Drosophila and Yeast Ipl1*

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Mutations in Aurora of Drosophila and related Saccharomyces cerevisiae Ipl1 kinase are known to cause abnormal chromosome segregation. We have isolated a cDNA encoding a novel human protein kinase of 402 amino acids with a predicted molecular mass of 45.9 kDa, which shares high amino acid identities with the Aurora/Ipl1 protein kinase family; hence the cDNA is designated as aik (auroral/IPL1-related kinase). Amino acid sequence of C-terminal kinase domain of Aik shares 86, 86, 72, 59, and 49% identity with those of Xenopus XLP46APK and XLP46BPK, mouse STK-1, Aurora of Drosophila, and yeast Ipl1, respectively, whereas N-terminal domain of Aik shares high homology only with those of XLP46APK and XLP46BPK. Northern and Western blotting analyses revealed that Aik is expressed highly in testis and various proliferating cells including HeLa cells. In HeLa cells, the endogenous levels of aik mRNA and protein contents are tightly regulated during cell cycle progression. Both of these levels are low in G1/S, accumulate during G2/M, and reduce rapidly after mitosis. Its protein kinase activity is also enhanced at mitosis as inferred by exogenous casein phosphorylation. Immunofluorescence studies using a specific antibody have shown that Aik is localized to the spindle pole during mitosis, especially from prophase through anaphase. These results strongly suggest that Aik is a novel member of a protein kinase family possibly involved in a centrosome function(s) such as chromosome segregation or spindle formation.

Accurate segregation of chromosomes during mitosis is an important event in the progression of cell cycle. Chromosome segregation is a highly complex process requiring microtubule assembly and disassembly. Centrosome and pericentriolar materials play important roles in organizing the microtubules at interphase and mitotic phase. At the G1 phase of cell cycle, duplicated centrioles closely associate and function as a single unit, and then separate into two units at mitotic phase. Centrosome separation is initiated either before or after nuclear envelope breakdown, and then microtubules are arranged and the separated centrosome organizes a mitotic spindle. In accordance with chromosome movement toward the spindle pole, the spindle microtubules shorten. However, details of molecular mechanisms by which centrosomal cycle or microtubule nucleation is regulated have not been elucidated.

Multiple lines of evidence suggest that protein phosphorylation plays a pivotal role in controlling spindle assembly and its functioning. Several centrosomal components are known to be phosphorylated in Drosophila (1) and vertebrates (2–6). Phosphorylation of centrosomal proteins has been shown to control both the rate of the microtubule nucleation and its dynamic behavior (7). The reversible phosphorylation may control the function of microtubule-based motor proteins such as a kinesin-like protein (8). Several genetic studies on fungi and fruit flies have identified various protein kinases and phosphatases that play intriguing roles in chromosome segregation (9–11). In mammalian cells, centrosomal localization of several protein kinases has been reported on Cdc2 (12), Plk1 (13), Fyn (14), Src (15), and A kinase (16).

Among the protein kinases that have been implicated in the control of spindle function, the role of Cdc2/cyclin B complex is pivotal and has been well characterized (17). This kinase is activated during mitosis and inactivated immediately afterward. The Cdc2 kinase partly localized in the mitotic spindle is known to regulate the dynamics and length of microtubules (12). Ectopic expression of mutant cyclin B2 in HeLa cells causes cell division arrest with multiple mitotic spindle poles (17). In a recent study, a kinesin-like motor protein was identified as a substrate for Cdc2 kinase, which regulates the centrosomal localization (8).

Other kinases that control mitotic spindle formation have also been identified: budding yeast CDC5 (18), fission yeast Polo1 (19), Drosophila Polo (20), mammalian Polo-like kinase (Plk1) (13, 21–26), and Xenopus Plx1 (27). The polo1 and polo mutants show abnormal formation of monopolar spindle or unequally separated bipolar spindle in which one pole is unusually broad. Plk1 kinase is induced and activated at G2 phase and disappears at the end of mitosis. The subcellular localization of the Plk1 protein is in both centrosome and mitotic spindle at M phase. Plk1 is phosphorylated and activated by Cdc2 kinase (28). One of the substrates of Plk1 was identified as CHO1/MKLP-1 kinesin-like protein, and it was suggested that Plk1 is a regulator of the motor protein (26).

Ipl1 (29) and Aurora (11) gene products have conserved serine/threonine kinase domains, which are highly homologous to each other, and are suggested to be profoundly involved in normal chromosome segregation. Embryos derived from aurora mothers inappropriately display closely paired centrosomes at

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mitotic stages. Loss of function of aurora leads to a failure of the centrosomes to separate and form a bipolar spindle (11). Conditional ipl1<sup>ts</sup> mutants severely missegregate chromosomes at restrictive temperature (10). The substrate and the regulator of these kinases have not been identified, but type 1 protein phosphatase was shown to act in opposition to Ipl1 protein kinase in yeast (29). Other members of Aurora/Ipl1 family kinases have been isolated from Xenopus (XLP46APK, XLP46BPK) or mouse (30), but the functions of these proteins have not been well studied.

We have cloned a cDNA encoding a human novel serine/threonine kinase, Aik, which has a high homology with Aurora/Ipl1 family protein kinases. Accumulation and activation at M phase as well as spindle pole localization of Aik kinase suggest a possible role of the protein in centrosome function.

**EXPERIMENTAL PROCEDURES**

**Cloning and Sequencing of a Human Aurora/Ipl1-related Kinase cDNA—cDNAs from a human B cell plasmid library (CLONTECH) were screened randomly, and a novel kinase (Aurora/ipl1-related kinase, Aik) was identified. The obtained cDNA was a partial clone encoding only kinase domain, and a Agt11 human B cell cDNA library (Epstein-Barr virus-transformed) (CLONTECH) was screened using this (aik) cDNA fragment as a probe. Hybridization conditions were as described (31). Positive clones were isolated, and the insert DNA was subcloned into pBlueScript. The nucleotide sequences of both strands of the isolated clone were determined by the dideoxy chain termination method.**

**Cell Culture and Synchronization—HeLa cells (obtained from RIKEN Cell Bank) were cultured in MEM medium supplemented with 10% fetal calf serum. Megakaryoblastic leukemia cell line CMK, Epstein-Barr virus-transformed B cell line CGI, Raji, and HL60 were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, and neuroblastoma cell line NB69 (from RIKEN Cell Bank) was cultured with RPMI 1640 medium supplemented with 15% fetal calf serum. For S phase synchronization, HeLa cells were synchronized by double thymidine treatment (32). Cells were treated with 2.5 mM thymidine for 20–24 h, followed by a 10-h release in fresh medium and successive treatment with the drug for 16 h. For M phase synchronization, cells were synchronized by double thymidine treatment as above, followed by 400 ng/ml nocodazole treatment for 12 h. Cells to be released from the drug treatment were washed with PBS twice and refed with fresh medium. At appropriate time points, the cells were harvested for Northern or Western blotting.

**Northern Hybridization—Blotted filters with 2 µg of poly(A)<sup>+</sup> RNA lane from various human tissues were purchased from CLONTECH. Total RNAs from synchronized HeLa cells were isolated with ISOGEN according to the manufacturer’s procedure (Nippon Gene). Twenty-five µg of RNA was loaded on each lane and subjected to electrophoresis on denaturing agarose gel, followed by transfer to GeneScreen Plus membrane (DuPont NEN). Three blots were probed with <sup>32</sup>P-labeled random-primed DNA corresponding to the kinase domain of Aik cDNA (nucleotides 375–1884) or full-length human β-actin cDNA. Hybridization was performed as described (31). The blots were washed at 55 °C for 1 h in a buffer containing 2 × SSC and 0.5% SDS, and signals were visualized by autoradiography using Kodak XAR film.**

**Western Blotting—Polyclonal antiserum against Aik were generated against glutathione S-transferase-Aik fusion protein produced in Escherichia coli. The obtained immune sera were affinity-purified with Aik.**

**RESULTS**

**Cloning of a cDNA Encoding a Human Aurora/Ipl1-related Kinase, Aik—Homology search of randomly sequenced cDNAs revealed that one of these clones encoded a putative protein kinase. Since this clone did not contain initiation methionine, a Agt11 library was rescreened with the obtained clone. The nucleotide and deduced amino acid sequence of the cloned clone obtained have been deposited in DDBJ/EMBL/GenBank™ (accession number D84212). This cDNA encodes 402 amino acids with a predicted molecular mass of 45.9 kDa. The deduced amino acid sequence contains 11 conserved regions characteristic of protein kinase catalytic domain at the C terminus of the protein. Eleven conserved subdomains known in various protein kinases are indicated by Roman numerals (Fig. 1).**

A computer search of the protein sequence data base (GenBank™) revealed that the kinase domain of the isolated kinase shares 86, 86, 72, 59, and 49% identity with those of XLP46APK, XLP46BPK, STK-1 (30), Aurora (11), and Ipl1 (29), respectively. Therefore, we designated this novel kinase as an Aurora/Ipl1-related kinase, Aik. Multiple alignment of the kinase domains is shown in Fig. 2. Amino acids that are the same as Aik are indicated by asterisks. Since Aik shares little amino acid sequence identity with kinases other than these five, it is reasonable to assume that these kinases constitute a subfamily of serine/threonine kinases. Although the N-terminal domain of Aik shares little homology with Aurora and Ipl1, two closely related protein kinases from Xenopus laevis XLP46APK and XLP46BPK exhibited high homology with Aik throughout the entire sequence (approximately 62%). These results indicate that Aik is a novel member of the Aurora/Ipl1-related protein kinase family.

Aik Is Expressed in Testis and Some Proliferating Cell Lines—The tissue distribution of aik was examined by Northern hybridization. <sup>32</sup>P-Labeled random-primed cDNA corre-

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1 The abbreviations used are: PBS, phosphate-buffered saline; kb, kilobase pair(s); TBS, Tris-buffered saline plus Tween 20; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride.

2 An unpublished sequence of mouse Ayk1 (GenBank™ accession no. U80932) has recently been deposited that has high homology with the kinase.

3 A gene symbol of STK6 for Aik was obtained from the HUGO Nomenclature Committee of GDB.
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Fig. 1. Nucleotide and deduced amino acid sequences of human aik cDNA. The putative translation initiation methionine and the termination codons are underlined. The upstream in-frame stop-codon is boxed. The conceptual translation yields a 402-amino acid protein. Nucleotide and amino acid residue numbers are shown at the right of each line.

Cell Cycle-dependent Expression of aik—Despite intensive investigation of the phenotypes of ip1 and aurora mutants, their protein expression profiles and kinase activity have not been well characterized. HeLa cells have been used in many investigations as a suitable line to analyze the biological phenomena during cell cycle progression, because the procedure for cell cycle synchronization is established and mitotic indexes were examined in several studies (34). In addition, HeLa cells were found to express a high content of Aik protein as shown in Fig. 4. To examine the cell cycle dependence of aik expression, we performed Northern hybridization on HeLa cells synchronized by double thymidine block (Fig. 5A).

Fig. 2. Amino acid alignment of sequences of human Aik, Xenopus XLP46APK, mouse STK-1, Drosophila Aurora, and S. cerevisiae Ipl1. The protein sequences are presented in single-letter code. The identical amino acids with Aik are indicated as asterisks. Gaps were introduced into sequences to optimize alignments. Amino acid residue numbers are shown at the right of each line. Eleven conserved subdomains of protein kinases are indicated by Roman numerals.

Fig. 3. aik mRNA level in human tissues. Northern hybridization was performed with poly(A)^+ RNAs from human tissues. Blotted filters with 2 µg of poly(A)^+ RNA from various human tissues were hybridized with 32P-labeled aik cDNA or β-actin cDNA probes.

Fig. 5. Aik protein level was examined by Western blotting to see whether it is also dependent on cell cycle progression of HeLa cells synchronized at S phase with thymidine or at M phase with nocodazole (Fig. 5). Aik protein level peaked by double thymidine block (Fig. 5A). 32P-Labeled human aik mRNA was hybridized with a band of 2.4-kb transcript using RNAs extracted from HeLa cells synchronized at and released from S phase. The aik mRNA increased after the release, peaked at S–12 h, and then decreased. Since mitotic index of HeLa cells peaks at 12–13 h after the release from S phase block (34), high expression of aik mRNA was observed at G2/M phase, but expression was low in G1 and S phase (Fig. 5A).

Aik protein level was examined by Western blotting to see whether it is also dependent on cell cycle progression of HeLa cells synchronized at S phase with thymidine or at M phase with nocodazole (Fig. 5). Aik protein level peaked by...
The results of protein expression shown in Fig. 5, Aik detected in the precipitates with preimmune antisera. Considering the results of protein expression shown in Fig. 5, Aik kinase activity showed a good correlation with the protein contents.

Subcellular Localization of Aik at Spindle Pole Region during M Phase—Clues to the function of Aik kinase were sought by examining subcellular localization in HeLa cells using indirect immunofluorescent microscopy (Fig. 7). Anti-α-tubulin antibody and DAPI staining revealed limited localization of Aik in centrosomes of mitotic cells. Further careful analysis of these cells from prophase to anaphase showed Aik localized not only in centrosome but also spreading to the spindle pole region. However, it was not detected in this region in telophase and interphase HeLa cells at all. In COS7 cells and melanoma cell line MMG1, similar centrosomal localization of Aik was also observed (data not shown).

**DISCUSSION**

We have identified a novel human gene encoding a serine/threonine kinase designated as aik. The deduced Aik amino acid sequence contains all of the amino acids conserved in serine/threonine kinases and is most closely related to members of the Aurora/Ipl1 family protein kinases, which are known to be required for normal chromosome segregation in Drosophila (11) and yeast (29). Temperature-sensitive mutants of Ipl1 cause chromosome number alteration (10). Temperature-sensitive mutants of aurora form monopolar spindle and cannot segregate the chromosomes (11). The conservation of amino acid sequences through entire sequences between Aik and two kinases in Xenopus suggests that closely related kinases to Aik must exist in vertebrates. The sequence homology strongly suggests that Aik is a member of the Aurora/Ipl1 family protein kinases conserved from yeast to human and that it might play a role in chromosome segregation as do Aurora and Ipl1.

The tissue distribution of aik examined by Northern blotting showed that expression was very high in testis (Fig. 3) and weak bands were detected in skeletal muscle, thymus, and spleen. Expression of Aik at protein levels was examined in some cell lines by Western blotting analysis. All of the proliferating cells examined expressed Aik protein with HeLa cells showing the highest level (Fig. 4). A computer search of protein sequence data base (GenBank™) revealed the existence of Aik transcripts in several infant tissues such as brain (accession no. Z43879) or liver spleen (accession no. N64029). These results indicate the expression of Aik in some proliferating cells, suggesting its possible involvement in cell proliferation. The expression of mouse STK-1 is high in the proliferating tissues of testis, spleen, small intestine, and colon (30). The difference of the expression pattern between Aik and STK-1 may suggest some functional difference between them.

Since the phenotypes of IPL1 and aurora mutants are abnormalities in chromosome segregation, it is conceivable that the homologous kinase Aik may also function at M phase. The present investigation provided evidence supporting this notion.

![Aik protein level in various human cell lines.](image1)

![Cell cycle-dependent expression of Aik.](image2)

![Cell cycle-dependent activation of Aik kinase.](image3)
Aik is predominantly expressed in a subset of proliferating tissues or cells. Cell cycle-dependent expression of Aik was observed in HeLa cells by Northern and Western blotting. The levels of both are low in G1/S but high in G2/M (Fig. 5). Since this activity appeared to be correlated with the protein levels, it is conceivable that it is at least partly regulated by the changes in protein content. These results suggest that Aik plays a role in cell cycle regulation at M phase in relation to the function of the centrosome/spindle pole region. As the N-terminal portion of Aik has little homology with Aurora, Ipl1, or STK-1, it might also be possible that the N-terminal region acts to regulate the kinase activity.

Aik is localized to centrosome and spindle pole from prophase to anaphase (Fig. 7). This subcellular localization as well as the expression profile during cell cycle suggests that Aik has a role(s) in centrosome or spindle organization at M phase. Although the subcellular localization of Aurora and Ipl1 has not been well studied, the phenotypes of these mutants suggest that the localization of these proteins may also be at centrosome or spindle pole body and may be involved in spindle organization. Thus, the localization of Aik suggests that it may function in spindle formation like Aurora.

Various centrosomally localized protein kinases have been identified in vertebrate cells, including Cdc2 (12), Plk1 (13), Fyn (14), Src (15), and A kinase (16). Some of the kinases were suggested to function as regulators of microtubule organization and spindle formation. Ectopic expression of mutant cyclin B2 in HeLa cells causes cell division arrest with multiple mitotic spindle poles (17). In a recent study, one of the substrates of Cdc2 kinase was identified to be a kinesin-like motor protein and to regulate the centrosomal localization (8). Plk1 is phosphorylated and activated by Cdc2 kinase (28). Aik was phosphorylated in M phase (data not shown), suggesting that the protein kinase cascade containing Aik may play a part in centrosomal function.

Complementation experiments were performed to examine whether Aik is a functional homolog of Ipl1. Low copy number plasmid harboring Aik coding region was transformed to Saccharomyces cerevisiae ipl1 mutant, but Aik failed to complement the mutant phenotype (data not shown). Although the sequence homology and the subcellular localization of Aik suggested that these two kinases might have similar functions, this was not the case. It is conceivable that because of the difference in amino acid sequence between Aik and Ipl1, a substrate or a regulatory protein cannot interact with Aik in yeast cells. Some human homologs of yeast proteins are known to lack the possibility to complement the yeast mutants, so that we cannot exclude the possibility that Aik is not a functional human homolog of Ipl1. It remains to be elucidated whether Aik plays the same role as Ipl1 in human.

Since Aik has protein kinase activity, the question arises what the physiological substrate(s) is for it. Several centrosomal components have been shown to be phosphorylated and to control the rate of microtubule dynamic behavior at the centrosome. Furthermore, phosphorylation regulates the function of a microtubule-based motor protein such as kinesin-like protein (8). The substrate of Aik has not been identified, nor has that for Aurora or Ipl1. Our experiments showed a high molecular weight phosphorylated band in the in vitro kinase assay, suggesting that a putative Aik substrate was co-immunoprecipitated and phosphorylated with the enzyme. The candidate substrate protein was co-immunoprecipitated only when it was extracted from M phase-blocked cells (data not shown). The substrate of Aik kinase may be a protein that participates in centrosome function.

We are now investigating the kinetics of Aik kinase and identifying substrates to elucidate the function of the protein.

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REFERENCES

1. Kellogg, D. R., Oegema, K., Raff, J., Schneider, K. & Alberts, B. M. (1995). Mol. Biol. Cell 6, 1675-1684
2. Vandre, D. D., Davis, F. M., Rao, P. N. & Borisy, G. G. (1984). Proc. Natl. Acad. Sci. U. S. A. 81, 4439-4443
3. Centonze, V. E. & Borisy, G. G. (1990). J. Cell Sci. 95, 405-411
4. Verde, F., Berrez, J. M., Anthony, C. & Karsenti, E. (1991). J. Cell Biol. 112, 1177-1187
5. Kuriyama, R. & Maekawa, T. (1992). Exp. Cell Res. 202, 345-354
6. Buendia, B., Draetta, G. & Karsenti, E. (1992). J. Cell Biol. 116, 1431-1442
7. Blangy, A., Lane, H. A., d’Herin, P. M., Kress, M. & Nigg, E. A. (1995). Cell 83, 1159-1169
8. Axton, J. M., Dombradi, V., Cohen, P. T. & Glover, D. M. (1990). Cell 63, 33-46
9. Chan, C. S. & Botstein, D. (1993). Genetics 135, 677-691
10. Glover, D. M., Leibowitz, M. H., McLean, D. A. & Parry, H. (1995). Cell 81, 95-105
11. Bailly, E., Doree, M., Nurse, P. & Bornens, M. (1989). EMBO J. 8, 3985-3995
12. Golsteyn, R. M., Mundt, K. K., Fry, A. M. & Nigg, E. A. (1995). J. Cell Biol. 129, 1617-1628
13. Ley, S. C., Marsh, M., Bebbington, C. R., Proudfoot, K. & Jordan, P. (1994). J. Cell Biol. 125, 639-649
14. David-Pfeuty, T., Bagrodia, S. & Shalloway, D. (1993). J. Cell Sci. 105, 613-628
15. Nigg, E. A., Schafer, G., Hilt, H. & Epenberger, H. M. (1985). Cell 41, 1039-1051
16. Gallant, P. & Nigg, E. A. (1992). J. Cell Biol. 117, 213-224
17. Kitada, K., Johnson, A. L., Johnston, L. H. & Sugino, A. (1993). Mol. Cell. Biol. 13, 4445-4457
18. Okhara, H., Hagan, I. M. & Glover, D. M. (1995). Genes Dev. 9, 1059-1073
19. Gallant, P. & Nigg, E. A. (1992). J. Cell Sci. 95, 405-411
20. Verde, F., Berrez, J. M., Anthony, C. & Karsenti, E. (1991). J. Cell Biol. 112, 1177-1187
21. Vandre, D. D., Davis, F. M., Rao, P. N. & Borisy, G. G. (1984). Proc. Natl. Acad. Sci. U. S. A. 81, 4439-4443
22. Centonze, V. E. & Borisy, G. G. (1990). J. Cell Sci. 95, 405-411
23. Verde, F., Berrez, J. M., Anthony, C. & Karsenti, E. (1991). J. Cell Biol. 112, 1177-1187
24. Kuriyama, R. & Maekawa, T. (1992). Exp. Cell Res. 202, 345-354
25. Buendia, B., Draetta, G. & Karsenti, E. (1992). J. Cell Biol. 116, 1431-1442
26. Blangy, A., Lane, H. A., d’Herin, P. M., Kress, M. & Nigg, E. A. (1995). Cell 83, 1159-1169
27. Axton, J. M., Dombradi, V., Cohen, P. T. & Glover, D. M. (1990). Cell 63, 33-46
28. Chan, C. S. & Botstein, D. (1993). Genetics 135, 677-691
29. Glover, D. M., Leibowitz, M. H., McLean, D. A. & Parry, H. (1995). Cell 81, 95-105
30. Bailly, E., Doree, M., Nurse, P. & Bornens, M. (1989). EMBO J. 8, 3985-3995
31. Golsteyn, R. M., Mundt, K. K., Fry, A. M. & Nigg, E. A. (1995). J. Cell Biol. 129, 1617-1628
32. Ley, S. C., Marsh, M., Bebbington, C. R., Proudfoot, K. & Jordan, P. (1994). J. Cell Biol. 125, 639-649
33. David-Pfeuty, T., Bagrodia, S. & Shalloway, D. (1993). J. Cell Sci. 105, 613-628
34. Nigg, E. A., Schafer, G., Hilt, H. & Epenberger, H. M. (1985). Cell 41, 1039-1051
35. Gallant, P. & Nigg, E. A. (1992). J. Cell Biol. 117, 213-224
36. Kitada, K., Johnson, A. L., Johnston, L. H. & Sugino, A. (1993). Mol. Cell. Biol. 13, 4445-4457
37. Okhara, H., Hagan, I. M. & Glover, D. M. (1995). Genes Dev. 9, 1059-1073
38. Gallant, P. & Nigg, E. A. (1992). J. Cell Sci. 95, 405-411
39. Verde, F., Berrez, J. M., Anthony, C. & Karsenti, E. (1991). J. Cell Biol. 112, 1177-1187
40. Vandre, D. D., Davis, F. M., Rao, P. N. & Borisy, G. G. (1984). Proc. Natl. Acad. Sci. U. S. A. 81, 4439-4443
41. Centonze, V. E. & Borisy, G. G. (1990). J. Cell Sci. 95, 405-411
42. Verde, F., Berrez, J. M., Anthony, C. & Karsenti, E. (1991). J. Cell Biol. 112, 1177-1187
43. Kuriyama, R. & Maekawa, T. (1992). Exp. Cell Res. 202, 345-354
44. Buendia, B., Draetta, G. & Karsenti, E. (1992). J. Cell Biol. 116, 1431-1442
45. Blangy, A., Lane, H. A., d’Herin, P. M., Kress, M. & Nigg, E. A. (1995). Cell 83, 1159-1169
46. Axton, J. M., Dombradi, V., Cohen, P. T. & Glover, D. M. (1990). Cell 63, 33-46
47. Chan, C. S. & Botstein, D. (1993). Genetics 135, 677-691
48. Glover, D. M., Leibowitz, M. H., McLean, D. A. & Parry, H. (1995). Cell 81, 95-105
49. Bailly, E., Doree, M., Nurse, P. & Bornens, M. (1989). EMBO J. 8, 3985-3995
50. Golsteyn, R. M., Mundt, K. K., Fry, A. M. & Nigg, E. A. (1995). J. Cell Biol. 129, 1617-1628
51. Ley, S. C., Marsh, M., Bebbington, C. R., Proudfoot, K. & Jordan, P. (1994). J. Cell Biol. 125, 639-649
52. David-Pfeuty, T., Bagrodia, S. & Shalloway, D. (1993). J. Cell Sci. 105, 613-628
53. Nigg, E. A., Schafer, G., Hilt, H. & Epenberger, H. M. (1985). Cell 41, 1039-1051
54. Gallant, P. & Nigg, E. A. (1992). J. Cell Biol. 117, 213-224
