Tousled-mediated Activation of Aurora B Kinase Does Not Require Tousled Kinase Activity in Vivo*

Gary M. Riefler‡, Sharon Y. R. Dent§, and Jill M. Schumacher§

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From the ‡Departments of Molecular Genetics and §Biochemistry and Molecular Biology, the Program in Genes & Development, and the §Center of Excellence for Epigenetic Research, M. D. Anderson Cancer Center, University of Texas, Houston, Texas 77030

The Aurora kinases comprise an evolutionarily conserved protein family that is required for a variety of cell division events, including spindle assembly, chromosome segregation, and cytokinesis. Emerging evidence suggests that once phosphorylated, a subset of Aurora substrates can enhance Aurora kinase activity. Our previous work revealed that the Caenorhabditis elegans Tousled-like kinase TLK-1 is a substrate and activator of the AIR-2 Aurora B kinase in vitro and that partial loss of TLK-1 enhances the mitotic defects of an air-2 mutant. However, given that these experiments were performed in vitro and with partial loss of function alleles in vivo, a necessary step forward in our understanding of the relationship between the Aurora B and Tousled kinases is to prove that TLK-1 expression is sufficient for Aurora B activation in vivo. Here, we report that heterologous expression of wild-type and kinase-inactive forms of TLK-1 suppresses the lethality of temperature-sensitive mutants of the yeast Aurora B kinase Ipl1. Moreover, kinase-dead TLK-1 associates with and augments the activity of Ipl1 in vivo. Together, these results provide critical and compelling evidence that Tousled has a bona fide kinase-independent role in the activation of Aurora B kinases in vivo.

Mitotic chromosome segregation must occur with high fidelity so that appropriate chromosome number is maintained. Indeed, a hallmark of cancer cells is aneuploidy, the inheritance of an unequal number of chromosomes (1, 2). Thus, understanding the function of proteins that contribute to mitosis, as well as the regulatory cross-talk between mitotic players, is of considerable importance.

Although many proteins work together to orchestrate mitosis, the Aurora family kinases are critical regulators of multiple aspects of cell division (3–5). Whereas yeast cells harbor one Aurora-like kinase, Ipl1 in budding yeast and Ark1 in fission yeast (6, 7), metazoans have at least two highly conserved family members, Auroras A and B. Aurora A is associated with mitotic centrosomes and is required for mitotic entry, centrosome maturation, spindle assembly, and chromosome segregation (5, 8–11). Aurora B displays a dynamic localization during mitosis, associating with chromosomes in prophase, concentrating at the inner centromere at metaphase, and abruptly relocating to the central spindle and cleavage furrow at anaphase (3, 12, 13). This dynamic behavior is shared with other "chromosomal passengers," including INCENP (3), survivin, and borealin. Together, these proteins form the highly conserved chromosomal passenger complex that regulates kinetochore/microtubule attachment and cytokinesis (13).

Interestingly, the phosphorylation of a subset of Aurora A and B substrates results in a concomitant increase in Aurora kinase activity (14–21). Some of these substrate activators appear to impart controlled spatial and temporal Aurora activation. For instance, TPX2, the first identified activator of Aurora A, is required to localize Aurora A to spindle microtubules and, upon phosphorylation, dictates a conformational change in the kinase that increases activity (14, 15). INCENP, a component of the chromosomal passenger complex, is phosphorylated by Aurora B and is required to both correctly target and activate Aurora B (16, 17, 19). Recently, our laboratory reported that the Caenorhabditis elegans Tousled-like kinase is likely to be a second substrate activator of Aurora B (20).

The founding member of the Tousled kinase family was identified in Arabidopsis as a gene product required for normal flower and organ development (22). Subsequent studies revealed that Tousled-like proteins are serine/threonine kinases that are most highly expressed and active during S-phase and phosphorylate the chromatin assembly factor Asf1 in vitro (23–28). The human Tousled-like kinase Tlk1 has been linked recently to DNA repair pathways because Tlk1 activity is decreased upon DNA damage or replication fork stalling in an ATM- and Chk1-dependent manner (29, 30).

Interestingly, histone H3 has been reported to be a second substrate of the Tousled-like kinases (25, 28, 31). Experimental evidence includes histone H3 kinase activity associated with recombinant Ttk1 or Ttk1 immunoprecipitated from human tissue culture cells and that heterologous expression of human Tlk1 in budding yeast could rescue the lethality of a temperature-sensitive (ts) allele of the Aurora-like kinase ipl1 (31). The

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2 To whom correspondence should be addressed: Dept. of Molecular Genetics, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 1006, Houston, TX 77030. Fax: 713-834-6339; E-mail: jschumac@mdanderson.org.

3 The abbreviations used are: INCENP, inner centromere protein; WT, wild-type; ts, temperature-sensitive; IP, immunoprecipitate; MYBP, myelin basic protein; KD, kinase-dead; GST, glutathione S-transferase.
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TLK-1-dependent increase in ipl1 ts mutant viability was interpreted to be due to the substitution of TLK1-dependent mitotic H3S10 (histone H3 serine 10) phosphorylation by TLK1-dependent H3S10 phosphorylation (31).

In our studies of C. elegans TLK-1, we found no evidence that TLK-1 could directly phosphorylate H3S10 in vitro or in vivo (27). Instead, we found that TLK-1 is a substrate and in vitro activator of Aurora B, a highly conserved mitotic H3S10 kinase (20, 32). Importantly, the activation of C. elegans Aurora B in vitro is not dependent on TLK-1 kinase activity (20). To test whether kinase-dead TLK-1 is sufficient to activate Aurora B in vivo, we assayed whether expression of a kinase-dead TLK-1 mutant could suppress the lethality of two ipl1 ts alleles. Our results reveal that TLK-1 directly associates with and activates the Ipl1 kinase independently of TLK-1 kinase activity. Hence, we conclude that TLK-1 is sufficient for Aurora B activation in vivo and that TLK-1 has separable kinase-dependent and kinase-independent functions in the eukaryotic cell cycle.

EXPERIMENTAL PROCEDURES

Yeast Strains, Plasmids, and Growth Conditions—The yeast strains used in this study were SBY1730 (ura3-1, leu2-3,112, his3-11, bar1, can1-100, ade2-1, IPL1-myc13:KAN) (from S. Biggins, Fred Hutchinson Cancer Center, Seattle, WA), DBY5301 (a ade2, his3-A 200, ura3-52, leu2Δ101::URA3::leu2Δ102, lys2Δ101::HIS3::lys2-Δ102ipl1-1D2), and DBY9462 (a corresponding isogenic wild-type strain to DBY5301), CAY1410D (a ura3-52, lys2-801, his3-A 200, leu2-3,112, ipl1-1), and CAY9146B (a corresponding isogenic wild-type strain to CAY914-10D but α mating type). The DBY and CAY1410D strains were provided by C. Chan (University of Texas at Austin). Yeast cells were propagated according to standard procedures in either rich medium (yeast peptone dextrose) or appropriate selective medium (synthetic complete).

The entire coding sequences of C. elegans TLK-1 (C07A9.3) and ICP-1 (Y39G10AR.13) were cloned into XbaI and NotI sites of the yeast expression vector pYC2NTB or pYESNTB (Invitrogen) to create translational fusions with a V5 epitope tag. TLK-1 mutants (S634A, S634E, and kinase-dead (KD) D802A) were PCR-amplified from previously described constructs (20) and subcloned into the XbaI and NotI sites of pYC2NTB or pYESNTB. The construction of GST-ASF-1 was described previously (20). All constructs were verified by automated DNA sequencing (M. D. Anderson Cancer Center DNA Analysis Core Facility).

Immunoprecipitation and Western Blotting—Cell extracts were prepared from 500 ml of cells grown in Ura− medium + 2% galactose for 6 h to induce V5-TLK-1 expression (from a starting A600 of 0.4). Cells were collected by centrifugation, washed once with water, and resuspended in 5 ml of lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride). Cell suspensions were flash-frozen in liquid nitrogen and ground into powder in a coffee mill with dry ice. After thawing on ice, cellular debris was pelleted by centrifugation at 5000 × g for 10 min. Supernatants were then clarified by incubation with 100 μl of protein G-Sepharose (GE Healthcare) at 4 °C for 1 h. V5-TLK-1 was immunoprecipitated with 2 μg of anti-V5 monoclonal antibody (Invitrogen) overnight at 4 °C. V5-TLK-1 immunoprecipitates (IPs) were isolated by adding 25 μl of protein G-Sepharose at 4 °C for 2 h. Bound material was washed five times with lysis buffer, resuspended, and boiled in 25 μl of loading buffer. Proteins were resolved by 10% SDS-PAGE and transferred to nitrocellulose. The membranes were blocked for 30 min in Tris-buffered saline supplemented with 0.1% Tween 20 and 2% bovine serum albumin followed by overnight incubation with mouse anti-Myc monoclonal antibody 9E10 (Santa Cruz Biotechnology) at a final dilution of 1:1000. After incubation with horseradish peroxidase-conjugated anti-mouse secondary antibodies (Bio-Rad), proteins were detected by chemiluminescence (GE Healthcare). Following detection of Myc-Ipl1 with anti-Myc monoclonal antibody 9E10, membranes were stripped (2.2 mM glycine, pH 4.0, and 0.5 mM NaCl) and reprobed with anti-V5 antibody at a final dilution of 1:5000.

Kinase Assays—Immunoprecipitation of V5-TLK-1 was performed as described above. Concurrently, Myc-Ipl1 was immunoprecipitated from parallel cultures with 1 μg of anti-Myc antibody overnight at 4 °C. Myc-Ipl1 IPs were isolated and washed as described above for V5-TLK-1 IPs. After the final wash, a 27.5-gauge needle was used to remove all traces of wash buffer from the protein G-Sepharose pellets. The V5-TLK-1 IPs were then resuspended in 20.5 μl of kinase reaction buffer (20 mM HEPES, pH 7.6, 5 mM EGTA, 1 mM dithiothreitol, and 25 mM β-glycerophosphate), whereas the Myc-Ipl1 IPs were resuspended in 60 μl of the same buffer. Increasing amounts of the Myc-Ipl1 IPs were transferred to individual tubes, and the final volumes were adjusted to 20.5 μl by addition of kinase buffer. 500 ng of myelin basic protein (MYBP; Sigma) and a mixture of 30 μCi (3 Ci/μmol) of [γ-32P]ATP, 10 nM unlabeled ATP, and 7.5 mM magnesium chloride were added, and each reaction was incubated at room temperature for 15 min. The samples were boiled in loading buffer, separated by SDS-PAGE, and transferred to nitrocellulose, and 32P incorporation into MYBP was assessed by phosphorimaging. MYBP protein loading was determined by Ponceau S staining (Sigma). Western blotting with anti-V5 and anti-Myc antibodies was performed as described above.

Phosphorylation of GST-ASF-1 was assayed by immunoprecipitation of V5-TLK-1 and V5-TLK-1 KD as described above followed by kinase assays with GST-ASF-1 substituted for MYBP. 32P incorporation into GST-ASF-1 was assessed by phosphorimaging, and GST-ASF-1 protein loading was determined by Ponceau S staining (Sigma). Western blotting with anti-V5 and anti-Myc antibodies was performed as described above.

Phosphorylation of MYBP by Ipl1 in the presence of V5-TLK-1 was calculated as [(32P-MYBP/MYBP load) − (32P-MYBP for vector alone/MYBP load)](MYc-Ipl1 load) × (average V5 load/V5 load per lane). Phosphorylation of MYBP by Ipl1 in the absence of V5-TLK-1 was calculated as (32P-MYBP/MYBP load)/(MYc-Ipl1 load).
RESULTS

*C. elegans* TLK-1 Rescues the Lethality of *ipl1* ts Yeast Mutants—Our previous results revealed that *C. elegans* TLK-1 is phosphorylated by the Aurora B kinase AIR-2 at serine 634 in vitro and in vivo (20). AIR-2 phosphorylation of TLK-1 induces a positive feedback loop, resulting in increased AIR-2 kinase activity in vitro (20). Importantly, the TLK-1-mediated increase in AIR-2 kinase activity is independent of TLK-1 kinase activity (20). Given that Aurora B is highly conserved in structure (supplemental Fig. 1) and function (33, 34), we tested whether *C. elegans* TLK-1 is sufficient to rescue the activity of a ts allele of the sole *Saccharomyces cerevisiae* Aurora kinase, *Ipl1*. To this end, an *ipl1* ts mutant strain, *ipl1-1* (6), and an isogenic wild-type (WT) strain were transformed with a low-copy plasmid expressing a galactose-inducible V5-TLK-1 fusion protein. *ipl1-1* cells grown in glucose and harboring an empty expression vector were completely viable at 30 °C but exhibited defective growth at 35 °C compared with a vector-transformed isogenic WT strain (Fig. 1, A and B). Growth in galactose resulted in slightly slower growth of both strains at 30 °C but severely exacerbated *ipl1-1* lethality at 35 °C (Fig. 1, A and B).

The slight growth defects of the WT and *ipl1-1* strains grown in galactose at 30 °C were rescued by the presence of WT TLK-1 (Fig. 1A), as was the growth defect of *ipl1-1* cells cultured in glucose- or galactose-containing medium at 35 °C (Fig. 1B). The rescue in glucose medium at 35 °C suggests that expression of TLK-1 is somewhat leaky in the absence of galactose. Altogether, these results are consistent with previous studies showing that human Tlk1 can suppress the growth defects of *ipl1* ts yeast mutants (31). However, because TLK-1-dependent enhancement of AIR-2 kinase activity in vitro did not require TLK-1 kinase activity (20), we hypothesized that kinase-dead TLK-1 would also be able to rescue *ipl1* ts growth. Expression of a kinase-dead version of TLK-1 (TLK-1KD) from the same low-copy galactose-inducible yeast expression vector used in the experiments above suppressed *ipl1-1* growth defects at 30 and 35 °C (Fig. 1B). Interestingly, TLK-1KD could also suppress the growth defects of a stronger *ipl1* ts allele, *ipl1-2* (Fig. 2) (6). These results argue against the model that human Tlk1 is suppressing *ipl1* ts mutant growth defects via direct phosphorylation of H3S10 (31). Instead, these data are consistent with TLK-1 having a kinase-independent role in the activation of Ipl1/Aurora B kinases (20).

Another feature of our model is that AIR-2 phosphorylation of TLK-1 serine 634 further activates AIR-2 (20). To test the contribution of TLK-1 serine 634 to Ipl1 kinase activity in vivo, the growth of wild-type and *ipl1-1* yeast cells transformed with full-length TLK-1 harboring site-directed mutations of serine 634 to alanine (S634A) or glutamic acid (S634E) were compared (Fig. 1, A and B). The growth of WT and *ipl1-1* cells transformed with TLK-1(S634E) in all conditions tested (30 or 35 °C, in glucose or galactose) was comparable with that of cells transformed with WT TLK-1 (Fig. 1, A and B). In contrast, growth defects of *ipl1-1* cells in the same growth conditions were not rescued by the presence of the TLK-1(S634A) expression plasmid (Fig. 1, A and B). Together, these data suggest that phosphorylation of TLK-1...
Serine 634 has a critical role in TLK-1-dependent activation of Ipl1/Aurora B kinases in vivo (20).

C. elegans ICP-1/INCENP also suppresses ipl1 ts mutant lethality—Because heterologous expression of the AIR-2 activator TLK-1 suppresses ipl1 ts lethality, we tested whether expression of a second AIR-2 activator, ICP-1 (17), would similarly suppress ipl1 mutant growth defects. Indeed, expression of C. elegans ICP-1 greatly suppressed the growth defect of ipl1-2 cells (Fig. 2). These results suggest that the mechanisms underlying Aurora B activation are highly conserved and can be substituted across species, analogous to the activation of the yeast Cdc28 kinase by mammalian cyclins (35, 36).

**TLK-1 Associates with Ipl1 in Yeast**—Our model predicts that TLK-1-dependent suppression of ipl1 ts lethality is due to a direct association between Ipl1 and TLK-1, which results in an increase in Ipl1 kinase activity. To determine whether TLK-1 and Ipl1 physically interact, we expressed V5-tagged WT TLK-1, TLK-1KD, TLK-1(S634A), and TLK-1(S634E) in a yeast strain in which the endogenous *IPL1* gene was replaced by Myc-tagged Ipl1 (under the control of the endogenous *IPL1* promoter) (37). Protein extracts were made from these Myc-Ipl1/V5-TLK-1 strains after growth in galactose at 30 °C. V5-TLK-1 protein complexes were immunoprecipitated with a V5-specific antibody and assayed for the presence of Myc-Ipl1 and V5-TLK-1 (Fig. 3A). Myc-Ipl1 was present in each V5-TLK-1 complex (WT, KD, S634A, and S634E) but was not immunoprecipitated from Myc-Ipl1 cells transformed with the V5 tag alone (Fig. 3A, **VECTOR**). Hence, Ipl1 binding is not dependent on the presence of serine 634 or the kinase activity of TLK-1, suggesting that the TLK-1 serine 634 and kinase-dead mutations are not grossly defective in protein folding or stability.

To determine whether the TLK-1 and Ipl1 interaction is cell cycle-dependent, the Myc-Ipl1 strain transformed with the WT V5-TLK-1 construct was synchronized by arrest in G1. Aliquots taken at 0, 1, 2, and 3 h after release into fresh medium were visually assayed for the presence of large buds (indicative of mitosis) and subjected to lysis followed by either Western analysis with V5- and Myc-specific antibodies as in (8) or immunoprecipitation with a V5-specific antibody followed by Western analysis with V5- and Myc-specific antibodies (C). Extracts from each time point had equivalent amounts of protein as visualized by Ponceau S staining (**Load**). There was also a corresponding increase in the amount of TLK-1-associated Ipl1 (Fig. 3C). These results suggest that TLK-1 is stabilized during mitosis, perhaps because of its association with or phosphorylation by Ipl1.

**TLK-1 Augments the Kinase Activity of Ipl1-containing Complexes in Vivo**—To determine whether the suppression of ipl1 ts lethality by TLK-1 is due to increased Ipl1 kinase activity, we compared Myc-Ipl1 kinase activity in the presence and absence of kinase-dead TLK-1. TLK-1KD harbors a missense mutation in a conserved amino acid within the kinase domain of TLK-1. We have shown previously that this protein lacks kinase activity
when purified from bacteria (20), and kinase assays of V5-TLK-1 and V5-TLK-1KD yeast IPs with recombinant ASF-1 revealed that TLK-1KD kinase activity was greatly compromised (supplemental Fig. 2). To determine whether Myc-Ipl1 kinase activity is increased in the presence of TLK-1KD, V5-TLK-1KD complexes were immunoprecipitated from Myc-Ipl1 cells and assayed for associated kinase activity using MYBP as a substrate. Levels of V5-TLK-1 and Myc-Ipl1 were assayed by Western blotting with V5- and Myc-specific antibodies, respectively (Fig. 4A). The degree of MYBP phosphorylation was compared with Myc-Ipl1 complexes immunoprecipitated with anti-Myc antibody from cells transformed with the V5 tag alone (Fig. 4, A and B). TLK-1KD IPs showed a significant increase (~6–8-fold) in MYBP phosphorylation compared with Myc-Ipl1 IPs (Fig. 4, A and B). However, in this experiment, the phosphorylation status of TLK-1 serine 634 did not appear to influence Ipl1 kinase activity because TLK-1KD(S634A) and TLK-1KD(S634E) IPs displayed phospho-MYBP levels that were not statistically different from TLK-1KD IPs (Fig. 4, A and B). From these experiments, we conclude that TLK-1 physically interacts with and enhances the kinase activity of Ipl1.

**DISCUSSION**

Here, we report that expression of *C. elegans* TLK-1, a substrate and *in vitro* activator of the *C. elegans* Aurora B homolog AIR-2, enhances the kinase activity and function of budding yeast Ipl1 *in vivo*. As corroborating *in vivo* evidence for our previous finding that TLK-1 is an activator of the Aurora B kinase *in vitro* (20), we found that TLK-1 can suppress the growth defect of ipl1 ts yeast mutants. Furthermore, TLK-1 and Ipl1 associate with one another, and Ipl1 immunoprecipitated with TLK-1 has a 6–8-fold increase in kinase activity compared with Ipl1 in the absence of TLK-1. Importantly, TLK-1 kinase activity is not required for suppression of ipl1 ts lethality or augmentation of Ipl1 kinase activity *in vivo*.

Tousled kinases are highly expressed in interphase cells (23, 27), and their levels drop as cells enter mitotic prophase. However, staining of *C. elegans* embryos with a phospho-TLK-1 Ser634-specific antibody revealed that phosphorylated TLK-1 perdures through metaphase and then drops to undetectable levels at anaphase (20). These results suggest that Ser634 phosphorylation may stabilize TLK-1, a conclusion that is supported by the high level of TLK-1 in mitotic yeast extracts.

We showed previously that phosphorylation of TLK-1 Ser634 is necessary for AIR-2/Aurora B kinase activation *in vitro* (20). Consistent with these observations, the growth defect of ipl1-1 cells was not rescued by phosphorylated mutant TLK-1 (TLK-1(S634A)), but was rescued by WT and phosphomimetic (TLK-1(S634E)) TLK-1. However, Ipl1 kinase activity associated with phosphorylated mutant TLK-1KD complexes was similar to that associated with TLK-1KD with an intact Ser634. Possible reasons for this discrepancy are as follows. 1) The Ipl1 kinase assays are less sensitive than the ipl1 ts suppression assays. 2) In the suppression assays, TLK-1(S634A) may inhibit the association of Ipl1 with its substrates, and this is not recapitulated in the kinase assays. 3) TLK-1 may augment Ipl1 kinase activity with respect to a set of critical substrates, which is reflected in the *ipl1*-1 growth assay, but not by MYBP phosphorylation with purified complexes. Regardless of the exact molecular mechanism, the data presented here support the role of TLK-1 as an activator of Aurora B. Recently, a Tousled-like kinase was found to genetically interact with, and to be an *in vitro* substrate of, the sole Aurora kinase in trypanosomes (28). Further studies will determine whether this interaction augments Aurora kinase activity in this and other organisms.

Very little is known about downstream functions of the Tousled kinase, except that Tousled might influence chromatin assembly and structure (24, 26). For instance, the chromatin assembly factor Asf1 is an *in vitro* substrate of the Tousled kinase in many different organisms (24–26, 28), yet the *in vivo*
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The mechanism of Aurora kinase activation by substrate activators is beginning to be unveiled. Aurora A is activated by a growing list of substrates, including Ajuba, TPX2, Maskin, and Bora (15, 18, 21, 43). Activation of Aurora A by TPX2 involves a modest alteration of the conformation of the activation loop of Aurora A such that a fully extended conformation is generated (14, 44). Subsequent phosphorylation of Aurora A at Thr288 further activates the kinase (45). TPX2 also inhibits binding of protein phosphatase 1 to Aurora A (15, 46). To date, only two Aurora B substrate activators have been reported. Activation of Aurora B by INCENP is a two-step process in which the full kinase activity of Aurora B is achieved by binding INCENP and then by phosphorylation of conserved residues within the carboxyl-terminal IN-box domain (16, 17, 19). The mechanism by which TLK-1 enhances Aurora B activity remains to be determined. However, it is clear from our studies that this functional interaction does not require TLK-1 kinase activity. Hence, we conclude that Tousled kinase has separable kinase-dependent and kinase-independent roles in the eukaryotic cell cycle. Importantly, Aurora substrate activators do not appear to share motifs that are useful for the identification of additional activators. Because C. elegans TLK-1 and ICP-1 both suppress the growth defect of ipl1 yeast mutants, this assay will provide a robust screen for additional metazoan activators of the Aurora B kinase.

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REFERENCES

1. Nguyen, H. G., and Ravid, K. (2006) J. Cell. Physiol. 208, 12–22
2. Li, J. I., and Li, S. A. (2006) Pharmacol. Ther. 111, 974–984
3. Carmena, M., and Earnshaw, W. C. (2003) Nat. Rev. Mol. Cell Biol. 4, 842–854
4. Adams, R. R., Carmena, M., and Earnshaw, W. C. (2001) Trends Cell Biol. 11, 49–54
5. Ducat, D., and Zheng, Y. (2004) Exp. Cell Res. 301, 60–67
6. Chan, C. S., and Botstein, D. (1993) Genetics 135, 677–691
7. Petersen, J., Paris, J., Willer, M., Philippe, M., and Hagan, I. M. (2001) J. Cell Sci. 114, 4371–4384
8. Schumacher, J. M., Ashcroft, N., Donovan, P. J., and Golden, A. (1998) Development (Camb.) 125, 4391–4402
9. Hannak, E., Kirkham, M., Hyman, A. A., and Oegema, K. (2001) J. Cell Biol. 155, 1109–1116
10. Hachet, V., Canard, C., and Gonczy, P. (2007) Dev. Cell 12, 531–541
11. Porter, N., Audhya, A., Maddox, P. S., Green, R. A., Dammermann, A., Desai, A., and Oegema, K. (2007) Dev. Cell 12, 515–529
12. Schumacher, J. M., Golden, A., and Donovan, P. J. (1998) J. Cell Biol. 143, 1635–1646
13. Vader, G., Medema, R. H., and Lens, S. M. (2006) J. Cell Biol. 173, 833–837
14. Bayliss, R., Sardon, T., Vernos, I., and Conti, E. (2003) Mol. Cell 12, 851–862
15. Eyers, P. A., Erikson, E., Chen, L. G., and Maller, J. L. (2003) Curr. Biol. 13, 691–697
16. Sessa, F., Mapelli, M., Ciferri, C., Tarricone, C., Areces, L. B., Schneider, T. R., Stukenberg, P. T., and Musacchio, A. (2005) Mol. Cell 18, 379–391
17. Bishop, J. D., and Schumacher, J. M. (2002) J. Biol. Chem. 277, 27577–27580
18. Hirota, T., Kunitoku, N., Sasayama, T., Marumoto, T., Zhang, D., Nitta, M., Hatakeyama, K., and Saya, H. (2003) Cell 114, 585–598
19. Honda, R., Korner, R., and Nigg, E. A. (2003) Mol. Biol. Cell 14, 3325–3341
20. Han, Z., Riefler, G. M., Saam, J. R., Mango, S. E., and Schumacher, J. M. (2005) Curr. Biol. 15, 894–904
21. Hutterer, A., Berndik, D., Wirtz-Peitz, F., Zigmam, M., Schleiffer, A., and Knoblach, J. A. (2006) Dev. Cell 11, 147–157
22. Roe, J. L., Nembhauser, J. L., and Zambryski, P. C. (1997) Plant Cell 9, 335–353
23. Sillje, H. H., Takahashi, K., Tanaka, K., Van Houwe, G., and Nigg, E. A. (1999) EMBO J. 18, 5691–5702
24. Sillje, H. H., and Nigg, E. A. (2001) Curr. Biol. 11, 1068–1073
25. Ehsan, H., Reichheld, J. P., Durfee, T., and Roe, J. L. (2004) Plant Physiol. 134, 1488–1499
26. Carrera, P., Moshkin, Y. M., Gronke, S., Sillje, H. H., Nigg, E. A., Jackle, H., and Karch, F. (2003) Genes Dev. 17, 2578–2590
27. Han, Z., Saam, J. R., Adams, H. P., Mango, S. E., and Schumacher, J. M. (2003) Curr. Biol. 13, 1921–1929
28. Li, Z., Gourguechon, S., and Wang, C. C. (2007) J. Cell Sci. 120, 3883–3894
29. Groth, A., Lukas, J., Nigg, E. A., Sillje, H. H., Wernstedt, C., Bartek, J., and Hansen, K. (2003) EMBO J. 22, 1676–1687
30. Krause, D. R., Jonnalagadda, I. C., Gatei, M. H., Sillje, H. H., Zhou, B. B., Nigg, E. A., and Khanna, K. (2003) Oncogene 22, 5927–5937
31. Li, Y., DeFatta, R., Anthony, C., Sunavala, G., and De Benedetti, A. (2001) Oncogene 20, 726–738
32. Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., Lin, R., Smith, M. M., and Allis, C. D. (2000) Cell 102, 279–291
33. Ruchaud, S., Carmena, M., and Earnshaw, W. C. (2007) Nat. Rev. Mol. Cell Biol. 8, 798–812
34. Ke, Y. W., Dou, Z., Zhang, J., and Yao, X. B. (2003) Cell Res. 13, 69–81
35. Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M., and Roberts, J. M. (1991) Cell 66, 1217–1228
36. Lew, D. J., Dulic, V., and Reed, S. I. (1991) Cell 66, 1197–1206
37. Pinsky, B. A., Kung, C., Shokat, K. M., and Biggins, S. (2006) Nat. Cell Biol. 8, 78–83
38. Eisenberg, J. C., and Shilatifard, A. (2006) Curr. Opin. Genet. Dev. 16, 184–190
39. Workman, J. L. (2006) Genes Dev. 20, 2009–2017
40. Korber, P., Barbaric, S., Luckenbach, T., Schmid, A., Schermer, U. J., Blaschke, D., and Horz, W. (2006) J. Biol. Chem. 281, 5539–5545
41. Adkins, M. W., Howar, S. R., and Tyler, J. K. (2004) Mol. Cell 14, 657–666
42. Schwabish, M. A., and Struhl, K. (2006) Mol. Cell 22, 415–422
43. Pascault, G., Delcros, J. G., Cremet, J. Y., Prigent, C., and Arlot-Bonne mains, Y. (2005) J. Biol. Chem. 280, 13415–13423
44. Cheetham, G. M., Knettel, R. M., Coll, J. T., Renwick, S. B., Swenson, L., Weber, P., Lippke, J. A., and Austen, D. A. (2002) J. Biol. Chem. 277, 42419–42422
45. Walker, A. O., Seghezzi, W., Korver, W., Sheung, J., and Lees, E. (2000) Oncogene 19, 4906–4916
46. Katayama, H., Zhou, H. L., Tatsuka, M., and Sen, S. (2001) J. Biol. Chem. 276, 46219–46224