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Chromosome instability in yeast and its implications to the study of human cancer

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1. ABSTRACT

One of the most important events during cell division is chromosome segregation, which allows that each daughter cell receives one set of duplicated chromosomes. Any mistakes in chromosome segregation process will result in loss or gain of chromosomes after mitosis, which may lead to either down-regulation of cancer suppressors or up-regulation of oncogenes. Therefore, defects in chromosome segregation may contribute to cancer development. As chromosome segregation is a conserved cellular process, the studies of budding yeast, a genetically tractable model organism, lay the foundation for the understanding of mitosis in human cells. This review summarizes the recent progress in chromosome segregation regulation in budding yeast *S. cerevisiae* and the possible application of the knowledge in cancer treatment.

2. INTRODUCTION

Chromosomes are highly packed DNA that carry genetic information in all eukaryotic cells. Faithful DNA duplication and chromosome segregation are essential for the survival and identity of all living organisms. After DNA duplication, the spindle microtubule emanating from the opposite spindle poles attaches to the kinetochore, a protein complex that assembles on centromeric DNA, resulting in the establishment of chromosome biorientation. The presence of cohesion prevents sister-chromatid separation until biorientation is established on all chromosomes. A separase-dependent cleavage of cohesin triggers anaphase entry. Subsequently, the imposed pulling force by microtubules on chromosomes leads to chromosome segregation.

Any defects in chromosome segregation process will lead to chromosome mis-segregation. As a result, daughter cells receive more or less chromosomes, generating aneuploidy cells. Accumulating evidence indicates that chromosome mis-segregation contributes to birth defects and cancer development (1, 2). The majority of the knowledge of chromosome segregation and its regulation comes from the studies of budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe* because of the convenient genetic and biochemical tools available for these model organisms. This review will focus on the recent progress in chromosome segregation and its regulation in budding yeast and the application of this knowledge in cancer diagnosis and treatment.

3. THE KINETOCHORE-MICROTUBULE INTERACTION IN BUDDING YEAST

3.1. The centromeric dna of budding yeast

The centromere is a uniquely specialized region of the chromosome to which spindle fibers attach during cell division. In budding yeast, centromeric DNA consists of three defined regions named CDE (centromere DNA element) I, II, and III. The 125bp centromere is sufficient to support high-fidelity chromosome segregation (3). The DNA sequence of CDE I, II, and III are conserved among all the 16 chromosomes in budding yeast. Removal of CDEI or CDEI plus parts of CDEII diminishes the quality of the chromosome segregation significantly but does not abolish it (4). Similarly, deletion of CDEI specific binding factor has a minor effect on centromere function. In contrast, deletion of CDEIII results in a complete loss-of-function of centromere. A single-base-pair change in CDEIII has been shown to abolish centromere function (5). Therefore, the 25-bp CDEIII is the most important for faithful chromosome segregation.

3.2. The kinetochore proteins

The kinetochore is a structure that assembles on centromeric DNA, and mediates the attachment of chromosome to the microtubule plus-end of the mitotic spindle. During anaphase, kinetochores maintain plus-end attachment, thereby generating force required for chromosome movement. Budding yeast cells have the simplest kinetochores. However, even these kinetochores consist of more than 60 kinetochore components (6). The assembly of budding yeast kinetochore begins with the binding of the CBF3 (centromere binding factor) complex with CDEIII. The components in CBF3 complex include Ndc10, Ctf13, Skp1, and Cep3, and a mutation in CBF3 proteins results in a complete loss of chromosome-microtubule attachment (7). Although CBF3 binds to centromeric DNA efficiently, CBF3 is not sufficient to mediate kinetochore-microtubule attachment, indicating the presence of other kinetochore proteins essential for the kinetochore-microtubule interaction.

To establish the kinetochore-microtubule connection, some kinetochore proteins have to interact with the microtubule. DASH (Dam1/Duo1, Ask1, Spc34/Spc19, Hsk1) is a microtubule- and kinetochore-associated complex required for proper chromosome segregation and bipolar attachment of sister chromatids to the mitotic spindle. The DASH complex (also called Dam1 or DDD complex) consists of 10 components and forms a ring structure around a single microtubule (8-10). Ndc80 (Ndc80, Nuf2, Spc24, Spc25), COMA (Ctf19p-Okp1p-Mcm21p-Ame1p), and MIND (Mtw1p including Nnf1p-Nsl1p-Dsn1p) complexes are thought to function in the outer kinetochore that bridge the gap between centromere-bound CBF3 and microtubule-associated DASH complex (11, 12). In support this notion, Ndc80, COMA, and MIND complexes are associated with centromeric DNA in a CBF3-dependent manner (12). All kinetochore components, except the DASH complex, associate with centromeric DNA independently of the presence of microtubules, suggesting that the DASH complex could be the last component that associates with kinetochores (8). Therefore, it is speculated that CBF3, Ndc80, MIND, and COMA kinetochore complexes bind to centromeric DNA before kinetochore-microtubule interaction. While the DASH complex associates with centromeric DNA only after the establishment of kinetochore-microtubule interaction. Therefore, the association of DASH with the centromeric DNA is likely to mark the stable kinetochore-microtubule interaction.

3.3. Lateral capture verses end-on binding

Kinetochore-microtubule interactions have been demonstrated to be a multi-step process. In mammalian cells, kinetochores, away from the pole regions, are initially captured by the lateral side, rather than the tip, of a single microtubule extended from the spindle poles. Once captured, kinetochores are transported toward the spindle poles along the surface of a microtubule. During their poleward movement, kinetochores interact with additional microtubules. Eventually each sister kinetochore attaches to the plus end of the microtubule from the opposite poles (13, 14).

Recently, it has been demonstrated that budding yeast shares a similar process. During most of the cell cycle, kinetochores remain in the vicinity of spindle pole bodies (SPBs). When centromeric DNA is being replicated during S-phase, kinetochore proteins are believed to dissociate from the centromere and quickly assemble after the replication of centromeric DNA. Therefore, detached kinetochores are recaptured by microtubules following centromeric DNA duplication. Recent evidence demonstrates that detached kinetochores interact initially with the lateral surfaces of microtubules. The capture requires factors that facilitate nuclear microtubule extension, such as microtubule binding protein Bim1 and Stu2. Also, kinetochore complexes, CBF3, COMA, MIND, and Ndc80, but not DASH, are necessary for the capture (15, 16). Currently, kinetochores are believed to be captured by the lateral side of the microtubule (lateral binding), and subsequently move towards the spindle poles. Finally, the kinetochores establish a firm interaction with the plus-end of microtubules (End-on binding). However, the transition from lateral to end-on binding still remains a poorly understood process. The interaction between DASH and centromere could be the key to this transition.

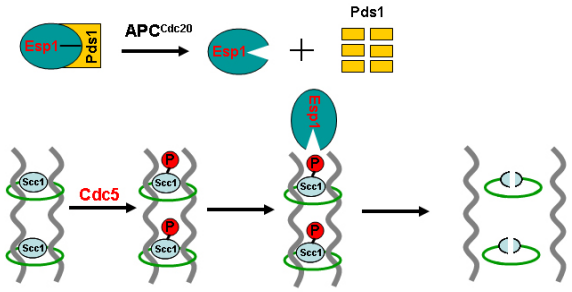

Figure 1. The regulation of anaphase entry in budding yeast.

4. THE INITIATION OF CHROMOSOME SEGREGATION

4.1. Anaphse entry regulation in budding yeast

Chromosome segregation does not happen until all chromosomes are attached to the microtubules emanating from the opposite spindle poles. To ensure that each daughter cell receives exactly one copy of chromosomes, newly synthesized sister chromatids remain linked to each other. A conserved protein complex, cohesin, is required to establish and maintain the link between sisters. The cohesin complex contains two homologous ATP binding proteins, Smc1 and Smc3, in addition to the Scc3 and Scc1/Mcd1 proteins (17, 18). The cohesin associates with chromosomes during DNA replication and remains bound until anaphase entry (18, 19).

Anaphase entry is triggered by the activation of a conserved separase, Esp1, which cleaves cohesin Scc1 and leads to sister chromatid separation (20, 21). Prior to anaphase, Esp1 is kept inactive by the presence of securin protein, Pds1, which binds to Esp1 and inhibits its separase activity (22). Therefore, the key step for anaphase entry is to destroy Pds1 and liberate separase Esp1 for cohesin cleavage. Pds1 protein is subjected to the ubiquitin-mediated proteolysis by anaphase promoting complex (APC), an E3 enzyme (23). Therefore, APC-mediated degradation of Pds1 frees separase Esp1, which triggers anaphase entry by cleaving cohesin Scc1. Another layer of regulation of anaphase entry is achieved through the phosphorylation of cohesin Scc1 by a conserved protein kinase Cdc5. This kinase phosphorylates the serine residues of Scc1 and enhances its cleavage by separase Esp1. Thus cells control anaphase entry through both Pds1 degradation and Scc1 phosphorylation (24) (Figure 1).

4.2. The spindle checkpoint

What will happen to cells if chromosomes fail to be attached by spindle microtubules? Cells have developed a delicate feedback mechanism to prevent cell cycle progression in the presence of unattached chromosomes. In early 90s, two research groups performed genetic screens for mutants that fail to arrest cell cycle progression when the spindle structure is disrupted by benomyl, a commonly used microtubule depolymerizing drug. *MAD1, 2,* and *3* genes were identified from a genetic screen for mutants that fail to form colonies on plates containing 15μg/ml benomyl (25). Another group identified three different genes, *BUB1, 2,* and *3* (Bud Uninhibited in Benomyl), which are required to prevent rebudding in the presence of high concentration of benomyl (26). Both *mad* and *bub* mutants fail to maintain metaphase arrest in response to microtubule perturbation, and these genes are named as spindle checkpoint genes.

Further analysis demonstrates that the spindle checkpoint is required for the cell cycle arrest in response to both spindle disruption and dysfunctional kinetochores. Mutation in Ctf13, one component of CBF3 kinetochore complex, arrests cell cycle at metaphase with short spindle structure (27). Introduction of the spindle checkpoint mutants abolishes the metaphase arrest of *ctf13,* and the double mutant cells exhibit elongated spindle structure when incubated at the non-permissive temperature (28). As both spindle disruption and dysfunctional kinetochore disrupt centromere-microtubule interaction, a more reasonable model is that the defective kinetochore-microtubule interaction activates the spindle checkpoint, which prevents anaphase entry.

4.3. The target of the spindle checkpoint

Anaphase entry is initiated through APC-dependent degradation of anaphase inhibitor Pds1. Cdc20 associates with APC and is essential for Pds1 degradation (29). In a two-hybrid screen for proteins that interact with Cdc20, Mad1, Mad2 and Mad3 were identified (30). Overexpression of Cdc20 allows cells with a depolymerized spindle to leave mitosis. Mutants in Cdc20 that are resistant to the spindle checkpoint no longer bind to Mad proteins, suggesting that Cdc20 is the target of the spindle checkpoint. The current model is that the spindle checkpoint components bind to Cdc20 and block Pds1 degradation in response to the defective kinetochore-microtubule interaction (31).

4.4. Checkpoints that monitor lack of tension

The stable interaction between sister kinetochores and microtubules from the opposite poles generate tension on chromosomes. The spindle checkpoint prevents anaphase entry in response to defective chromosome attachment. However, the primary cell-cycle defect that activates the spindle checkpoint remains controversial. Experiments in several organisms indicate that the spindle checkpoint is activated by either lack of kinetochore-microtubule attachment or defects in the tension exerted by microtubule-generated forces on kinetochores (32).

Two centromere localized proteins, Sgo1 and Ipl1, have been shown to be required to sense the lack of tension and activate the spindle checkpoint (33, 34). Ipl1 is an essential protein kinase, and *ipl1* mutants were isolated as they exhibit increased ploidy (35). Like its counterpart Aurora B in mammalian cells, Ipl1 forms a complex with Bir1 and Sli15. This complex is translocated from centromere to spindles after anaphase onset and it is named chromosome passenger complex (CPC) (36). Further studies indicate that Ipl1 is required to activate the spindle checkpoint in response to conditional inhibition of either replication or sister chromatid cohesion, conditions that

prevent generation of bipolar force and kinetochore tension (33, 37). Spindle checkpoint activation in kinetochore mutants depends on the presence of unattached kinetochores. A recent observation indicates that Ipl1 is required for the generation of unattached chromosomes in kinetochore mutants (38). When Ipl1 function is impaired in these kinetochore mutants, the attachment is restored and the checkpoint was turned off. Therefore, Ipl1 is believed to activate the spindle checkpoint in response to tension defects by creating unattached kinetochores. Although Sgo1 is required to activate the spindle checkpoint in response to the lack of tension, Sgo1 has not been shown to create unattached chromosomes in kinetochore mutants. Therefore, kinetochore mutants depend on Ipl1 to convert the tension defects to unattached chromosomes. More experiments are needed to reveal the molecular function of Sgo1 in tension checkpoint function.

Recent work from Desai lab shed light on the molecular mechanism as how Ipl1 acts as a tension sensor. Using an *in vitro* approach based on the sequence-specific budding yeast centromere, a complex of the chromosome passenger protein Bir1 and Sli15 was identified as they link centromeres to microtubules (39). Both Bir1 and Sli15 are essential for the interaction of CBF3-bound centromere with microtubules. Even though Ipl1 forms a complex with Bir1 and Sli15, Ipl1 does not contribute to the microtubule-centromere linkage, but the targeting and activation of Ipl1 is controlled by Bir1 and Sli15. Elimination of the linkage mediated by Bir1 and Sli15 prevents Ipl1 activation. These observations lead to the hypothesis that Bir1-Sli15-mediated linkage, which bridges centromeres and microtubules, is the tension sensor that relays the mechanical state of centromere-microtubule attachments into local control of Ipl1 kinase activity (39). But the tension checkpoint function of Ipl1 is still elusive at molecular levels.

4.5. Regulation of kinetochore-microtubule interaction by ipl1 kinase

If Ipl1 is required to create unattached chromosomes in response to lack of tension, then Ipl1 should somehow promote the dissociation of kinetochores from microtubules. Given the fact that Ipl1 is a protein kinase, we reason that Ipl1 executes its function by phosphorylating some kinetochore proteins. The identified substrates of Ipl1 include two components of DASH complex, Dam1 and Spc34, as well as a kinetochore protein Ndc80. Mutation of the four Ipl1 phosphorylation sites of Dam1 results in lethality, but mutation in three of the four Ipl1 phosphorylation sites of Dam1 shows synthetic temperature-sensitivity lethality when combined with *spc34(T199A)*. *dam1-3A spc34(T199A)* mutant photocopies the phenotype of *ipl1* mutants and exhibits massive defects in chromosome segregation. In many cases, more than 90% of the DNA was segregated to a single pole after anaphase (40). These observations indicate that Dam1 could be the key substrate of Ipl1 kinase.

Some observations suggest the positive role of Ipl1 kinase in facilitating DASH-centromere interaction, a process crucial for the stable kinetochore-microtubule interaction. First, the association of Dam1-3A mutant protein with centromeric DNA reduces dramatically. Second, the association of Dam1 with centromeres diminishes in *ipl1-2* mutants when incubated at the restrictive temperature. Finally, Dam1 constitutive phosphorylation mutants suppress the centromere association defects of *ipl1-2* mutants (40). Moreover, data from the Biggins lab demonstrates that *dam1-3A spc34(T119A)* mutants activate the spindle checkpoint, indicating kinetochore-microtubule interaction defects (38). Both *ipl1* and DASH mutants exhibit elongated spindles in cells with disrupted DNA synthesis. The similar phenotype of *Ipl1* and DASH mutants indicates the positive role of Ipl1 in DASH-centromere interaction (41). A challenging question is whether Ipl1's role in DASH-centromere interaction correlates with its tension checkpoint function. One possibility is that the phosphorylation of some kinetochore proteins by Ipl1 promotes the initial, but unstable, DASH-kinetochore interaction. The subsequent phosphorylation of other protein(s) by Ipl1 may disrupt the initial interaction, but facilitate a stable DASH-kinetochore interaction. The Ipl1-dependent disruption of the initial DASH-kinetochore interaction may trigger the generation of unattached kinetochores, when some kinetochore protein is not functional. Further studies are required to understand how Ipl1-dependent phosphorylation contributes to the conversion of tension defects to unattached chromosomes at molecular levels.

5. CYCLIN-DEPENDENT KINASE (CDK) AND CHROMOSOME SEGREGATION

5.1. The regulation of cdk activity

Cyclin dependent kinase governs cell cycle progression, and Cdk activity is tightly regulated during cell cycle. In budding yeast, *CDC28* encodes the single cyclin dependent kinase (Cdk1). The association with periodically expressed cyclins activates the Cdk kinase activity and renders the substrate specificity. There are totally 6 B-type cyclins in budding yeast. Among them, *CLB5*, *6* are transcribed in S-phase, and Clb5, 6-associated Cdk1 promotes DNA synthesis by phosphorylating its substrates during S-phase. Whereas, Clb1, 2, 3, 4 are expressed during M-phase and Clb2 plays a key role in chromosome segregation. After mitosis, cells inactivate Cdk in order to initiate the succeeding cell cycle, and this process is called mitotic exit. CDK inactivation promotes the loading of DNA replication complex onto the replication origin, an essential step for the initiation of DNA synthesis during the succeeding cell cycle (42, 43).

5.2. Mitotic cyclin associated cdk1 promotes chromosome segregation

The conclusion that Clb1-4-associated Cdk1 promotes mitosis is based on the phenotype of mutant cells lacking *CLB1-4*. The mutants arrest at G2/M phase with duplicated but unseparated DNA (44). We found that overexpression of the Cdk inhibitory kinase Swe1 arrested cells at G2/M phase with unseparated sister chromatids (Liu and Wang, personal observation). Given the fact that Swe1 inhibits Clb2-Cdk1, but not Clb5-Cdk1 activity (45,

46), mitotic cyclins are believed to be essential for chromosome segregation.

Budding yeast initiates anaphase by activating the APC^{Cdc20} that degrades anaphase inhibitor Pds1. Cdk1 is required to activate APC, and mutants that are impaired in mitotic Cdk1 function have difficulty entering anaphase presumably due to defective phosphorylation of APC components. Mutating the putative Cdk1 phosphorylation sites in three components of APC, Cdc16, Cdc23, and Cdc27, makes the APC resistant to phosphorylation both *in vivo* and *in vitro* (47). The nonphosphorylatable APC has normal activity in G1, but its mitotic activity is compromised. These results indicate that Cdk1 activates the APC in budding yeast to trigger anaphase.

Anaphase inhibitor Pds1 is also a substrate of Cdk1. Sister-chromatid separation at the metaphase-to-anaphase transition is induced by the Separase Esp1-dependent cleavage of Scc1. Separase is associated with Pds1 until the time of anaphase initiation. Pds1 not only inhibits Esp1, but also promotes its nuclear localization (48). Phosphorylation of Pds1 by Cdk1 is important for efficient binding of Pds1 to Esp1, and for promoting the nuclear localization of Esp1. Therefore, Cdk1 also promotes metaphase-to-anaphase transition by facilitating the nuclear localization of separase Esp1 (49). Recently, about 200 Cdk1 substrates have been identified in budding yeast, and some of them are expected to play positive roles in mitosis after phosphorylation by Cdk1 (50).

5.3. Mitotic exit network (men) and poly-ploidy

The components in MEN pathway include protein kinases Cdc5, Cdc15, Dbf2, a GTPase Tem1, a phosphatase Cdc14, and a Dbf2 binding protein Mob1 (51). Among these, phosphatase Cdc14 is the key player, which dephosphorylates CDK1 substrates. As a consequence, Cdc14 induces the degradation of Clb2 and the accumulation of CDK inhibitor, Sic1, that contribute to CDK inactivation (52). During most of the cell cycle stages, Cdc14 is sequestered within the nucleolus. After chromosome segregation, Cdc14 translocates from the nucleolus to the nucleus and dephosphorylates its substrates. All components in the MEN pathway are required for the release of Cdc14 from the nucleolus, suggesting that Cdc14 acts downstream of MEN pathway (53, 54).

The regulation of MEN activity is achieved through the multi-layer control of Tem1, a small GTPase that localizes on the SPB, and acts on the top of the MEN pathway. The entrance of SPB into a daughter cell allows Tem1 to encounter its activator, lte1, and triggers mitotic exit (55). Before that, Tem1 is kept inactive by a two-component GTPase activating factor composed of Bfa1 and Bub2 (56). Activated Cdc5 kinase phosphorylates Bfa1 and results in its dissociation from Bfa1 (57). Therefore, Cdc5 promotes mitotic exit by inactivating Bfa1, the negative regulator of Tem1. We have also identified a new mechanism that inactivates MEN through the induction of Amn1 protein upon MEN activation. Amn1 binds to Tem1 and abolishes its association with the downstream target Cdc15 (58). In *bfa1Δ* or *bub2Δ* mutants, Tem1 is constitutively active, and the mutant cells exit mitosis in the presence of spindle disruption or mis-orientation, generating poly-ploidy cells (55, 56, 59-61). Taken together, the cooperation of Tem1's cellular localization, Bfa1/Bub2 GAP activity, and cell cycle expressed Amn1 limits the functional window of MEN at late M and early G1 phase. Premature activation of MEN will allow cells exit mitosis without finishing chromosome segregation, resulting in poly-ploidy cells. A long standing question is whether mammalian cells have the conserved mitotic exit network to negatively regulate Cdk activity. Although mammalian cells have been shown to have the well conserved Cdc14 phosphatase, the key component of MEN, other MEN components remain to be identified.

5.4. Fear pathway and chromosome segregation

The FEAR pathway contributes to the initial release of Cdc14 from the nucleolus during early anaphase (62). This network is comprised of Cdc5 kinase, the separase Esp1, the kinetochore-associated protein Slk19, and Spo12. Anaphase inhibitor Pds1 may negatively regulate mitotic exit by inhibiting FEAR pathway through Esp1. Cdc14 release has been shown to be blocked in cells that overproduce non-degradable Pds1 (63, 64). Cdc14 associates with a nucleolar localized protein Net1/Cfi1 and this association is essential for the nucleolar localization of Cdc14 (54, 65). Recent evidence indicates that the FEAR network promotes Net1 phosphorylation by Cdk1. These phosphorylations appear to be required for Cdc14 release at early anaphase, raising the possibility that the FEAR controls Cdc14 release by up-regulating Net1 phosphorylation (see PP2A section) (66). Hence, cells control mitotic exit in two steps. At the onset of anaphase, activated FEAR network induces the partial release of Cdc14 from the nucleolus. Activated MEN then maintains Cdc14 in its released state during late anaphase and telophase.

Recent evidence indicates that the function of FEAR is not limited to mitotic exit regulation. FEAR-dependent Cdc14 release also regulates the localization of chromosome passenger complex (CPC) (67). Before anaphase entry, the chromosome passenger complex (Sli15, Bir1, and Ipl1) localizes at kinetochores presumably because of the function of CPC in the establishment of kinetochore-microtubule interaction. During anaphase, this complex is translocated to the spindle microtubule, resulting in the stabilization of spindle structure. Interestingly, phosphatase Cdc14 regulates the localization of the CPC complex. Cdc14 dephosphorylates Sli15, and thereby directs the complex to the spindle. Activation of Cdc14 by FEAR pathway is sufficient for Sli15 dephosphorylation and relocalization. The significance of FEAR-induced CPC translocation remains unclear. On possibility is that Cdc14 modulates spindle midzone structure through CPC.

Before metaphase, microtubules show high dynamic instability, which is thought to aid the 'search and capture' of chromosomes for bipolar alignment on the spindle. Microtubules become more stable at the onset of

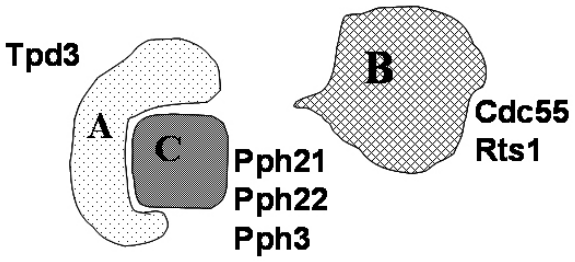

Figure 2. The components of PP2A enzyme.

anaphase, and this change in microtubule behavior is also triggered by FEAR dependent Cdc14 release (68). Released Cdc14 dephosphorylates Ask1, a component of DASH complex, and promotes microtubule stability. Recent evidence suggests the role of Cdc14 in dephosphorylating Fin1 and Ase1, two microtubule-binding proteins that facilitate spindle stabilization and elongation (69, 70). It will be interesting to clarify whether the dephosphorylation of Ase1 and Fin1 depends on FEAR-induced Cdc14 release. Together, Cdc14 mediated dephosphorylation of Ask1 and Sli15 (or other proteins) favors chromosome segregation during anaphase. Although FEAR is not essential for the viability, previous data indicates that *slk19Δ* and *spo12Δ* mutants exhibit noticeable mitotic defects (71, 72). These observations support the conclusion that FEAR-dependent Cdc14 release facilitates chromosome segregation.

6. PROTEIN PHOSPHATASE 2A (PP2A) AND CELL CYCLE REGULATION

6.1. PP2A and sister chromatid separation

PP2A is an abundant cellular phosphatase that regulates a significant array of cellular events. This holoenzyme consists of a catalytic subunit, C, and two regulatory subunits, A and B (Figure 2). In budding yeast, *PPH21*, *PPH22*, and *PPH3* genes encode the catalytic subunits. *TPD3* encodes the A regulatory subunit, while *CDC55* and *RTS1* encode the B and B'-regulatory subunits, respectively (73). *cdc55* mutant was originally identified as a cold sensitive mutant that exhibited hyphal growth when incubated at 12°C (74). Further evidence indicates that the abnormal bud morphology is attributable to the elevated protein levels of Swe1, a protein kinase that phosphorylates and down-regulates Cdk1 kinase activity (75).

cdc55 mutant was also isolated from a genetic screen for mutants that exhibited synthetic lethal phenotype when combined with *ctf13-30,* a kinetochore mutant, indicating the role of Cdc55 in chromosome segregation regulation (76). *cdc55Δ* mutants are also sensitive to nocodazole, a microtubule depolymerizing drug. In the presence of nocodazole, *cdc55Δ* mutants exhibit separate sister chromatids, suggesting the negative role of Cdc55 in anaphase entry (77). Loss of function of Tpd3, the A-regulatory subunit of PP2A, also exhibit separated sister chromatids in the presence of nocodazole (78). Moreover, deletion of the two catalytic subunits, *PPH21* and *PPH22* results in sensitivity to nocodazole (79). Therefore, the loss of function of PP2A results in prematurely separated sister chromatids. Although *swe1Δ* deletion suppresses the cold sensitivity and abnormal bud morphology of *cdc55Δ* mutants, *swe1Δ* is unable to suppress the premature sister-chromatid separation (47, 80), suggesting that PP2A regulates sister chromatid separation independently of Swe1.

One interesting observation is that *cdc55Δ* suppresses the temperature sensitivity of *cdc20-1* (76). As APC^{Cdc20} is essential for Pds1 degradation, APC is likely to be up-regulated in *cdc55Δ* mutants. If that is the case, Pds1 protein levels should be down-regulated in *cdc55Δ* mutants. We examined Pds1 protein levels in *cdc55Δ* mutants in the presence of nocodazole and found that the mutant cells failed to keep high levels of Pds1. Consistently, cohesin Scc1 is cleaved in nocodazole treated *cdc55Δ* mutant cells due to the activation of separase Esp1 (78). Therefore, the hyper-active APC in PP2A mutants is responsible for the premature sister-chromatid separation in the presence of spindle damage.

In the presence of DNA damage, *cdc55Δ* mutant cells also separate sister chromatids, but without noticeable decrease of Pds1 or cohesin Scc1 levels. Further analysis demonstrates that *cdc55Δ* mutants lose cohesion along the entire chromosomes when the spindle is damaged. In contrast, separation of sister chromatids is limited to the centromeric regions in *cdc55Δ* mutant cells after DNA damage. Whether the separated sisters in *cdc55* mutant cells is a result of cohesion-establishment failure or premature sister separation remains unclear. These data suggest that PP2A regulates sister chromatid cohesion in Pds1-dependent and independent manners (78). Further study is required to identify the PP2A substrates involved in sister-chromatid separation.

6.2. PP2A and mitotic exit

PP2A also plays a negative role in mitotic exit. We carried out a genetic screen for genes that are toxic to *cdc5-1* mutants when overexpressed (79). Genes that encode the B-regulatory subunit (Cdc55) and the three catalytic subunits (Pph21, Pph22 and Pph3) of PP2A were isolated from this screen. In addition to *cdc5-1*, overexpression of *CDC55*, *PPH21* or *PPH22* is also toxic to other temperature sensitive mutants that display defects in mitotic exit. Consistent with this observation, deletion of *CDC55* partially suppresses the temperature sensitivity of these mutants. Strikingly, in the presence of spindle poison nocodazole, PP2A mutant cells display released Cdc14 and some *cdc55Δ* cells exit mitosis and reduplicate DNA, resulting in poly-ploidy cells (79, 81). Moreover, PP2A is also required to prevent Cdc14 release in DNA-damage-arrested cells (82). Further studies indicate that $PP2A^{Cdc55}$ dephosphorylates Net1 and prevents the dissociation of Cdc14 from Net1. Interestingly, Separase Esp1 is required for the inactivation of $PP2A^{Cdc55}$, raising the possibility that $PP2A^{Cdc55}$ serves a target of FEAR pathway (83).

7. CELL CYCLE DEFECTS, ANEUPLOIDY, AND CANCER

7.1. Chromosome mis-segregation and cancer

The molecular mechanisms ensuring accurate chromosome segregation during mitosis are critical to maintain chromosome stability. Mis-regulation in this process results in aneuploidy, a condition in which the number of chromosomes is abnormal due to extra or missing chromosomes. Aneuploidy may contribute to cancer development by reducing the expression of tumor suppressors or by amplifying oncogenes. Nearly all solid tumors exhibit chromosome instability at the chromosomal level (84). Recent works demonstrate that chromosome instability (CIN) and aneuploidy, long considered late progression events of established tumor, are in fact early molecular changes in premalignant stages of human breast, bladder, and most of the aggressive prostate cancers (85, 86). Therefore, mis-regulation of chromosome segregation will generate aneuploidy cells that are predisposed to cancer.

7.2. Aurora kinase and cancer

The counterparts of Ipl1 kinase in human cells include Aurora kinase A, B and C. Aurora-B and ICENP (the budding yeast Sli15 homologue) colocalize on human chromosomes and regulate chromosome segregation (87). Exogenous overexpression of wild-type Aurora-B produces multinuclearity in human cells. In long-term culture of Aurora-B-overexpressing cells, multiple nuclei are occasionally fused, and then an increased ploidy and aneuploidy are induced (88). Therefore, the overexpression of Aurora-B contributes to multinuclearity and increased ploidy. Moreover, Aurora-B overexpression is also demonstrated in oral, non-small cell lung carcinoma, prostate cancer, thyroid carcinoma, colon carcinoma, ovarian, and breast cancers (89).

In budding yeast, Ipl1 kinase regulates chromosome segregation by phosphorylating kinetochore protein Dam1, Spc34 and Ndc80. Recent data indicate that Aurora B kinase phosphorylates Ndc80 (also called Hec1), and reduces the affinity of Ndc80 complex with the microtubule in mammalian cells (90, 91). Also, mitotic-centromere-associated kinesin (MCAK) has been shown to be a substrate of Aurora B, and the phosphorylation of MCAK regulates its microtubule depolymerase activity (92, 93). All the Aurora-B-dependent phosphorylation facilitates the release of improper microtubule attachments. Therefore, high levels of Aurora B might lead to aneuploidy cells by promoting the detachment of chromosomes from microtubules. However, detail studies are required to pinpoint the substrate(s) of Aurora B kinase that are directly involved in aneuploidy. As overexpression of Aurora kinases have been demonstrated in the majority of human cancers, Aurora kinases are ideal targets of new anti-cancer drugs.

7.3. Polo-like kinaseS (PLK) and cancer

In budding yeast, Cdc5 kinase is required for both anaphase entry and mitotic exit. Plk1, 2, and 3 are the human homologues of budding yeast Cdc5, and these kinases play pivotal roles in the regulation of cell cycle progression. In mitotic cells, Plks associate with spindle poles and kinetochores, suggesting these kinases may play a role in kinetochore assembly and kinetochore-microtubule interaction. Plk1, the best characterized family member among mammalian Plks, strongly promotes the progression of cells through mitosis. Plk1 is found to be overexpressed in a variety of human tumors and its expression correlates with cellular proliferation and prognosis of tumor patients. When constitutively expressed in NIH3T3 cells, Plk1 causes the formation of an oncogenic focus. These transformed cells form tumors in nude mice (94). Plk1 overexpression is tightly correlated with the development of CIN and aneuploidy observed in neoplasias (95).

Extensive studies have shown that Plk1 expression is elevated in non-small-cell lung cancer, head and neck cancer, esophageal cancer, gastric cancer, melanomas, breast cancer, ovarian cancer, endometrial cancer, colorectal cancer, gliomas, and thyroid cancer. Plk1 gene and protein expression has been proposed as a new prognostic marker for many types of malignancies, and Plk1 is also a potential target for cancer therapy (89).

7.4. The spindle checkpoint and cancer

The mitotic checkpoint guards against chromosome mis-segregation by delaying cell-cycle progression through mitosis until all chromosomes have successfully made spindle-microtubule attachments. Defects in the mitotic checkpoint generate aneuploidy and might facilitate tumorigenesis, but more severe disabling of checkpoint signaling is a possible anticancer strategy. Mutations in spindle checkpoint components that monitor kinetochore-microtubule interaction have been identified in cancer cells.

Chief components of the human spindle checkpoint include *MAD1, MAD2, BUB1, BUB3, BUBR1* and *MPS1*. These genes are crucial for the maintenance of correct chromosome number during cell division, and defects in the spindle checkpoint are implicated in the generation of aneuploidy, which occurs frequently in human cancers. The Volgelstein laboratory initially shows that the Bub1 is mutated in ~ 5% colorectal tumors (96). Reduced expression of Mad2 was reported in ovarian and gastric cancer tissues (97, 98). The recent report that germline mutation in another spindle checkpoint gene BUBR1 is associated with inherited predispositions to cancer strongly supports a causal link between CIN and cancer development (99).

7.5. PP2A and cancer

Like budding yeast, human PP2A holoenzyme exists in several trimeric forms consisting A, B, and C subunits. Different observations contribute to the fundamental hypothesis that PP2A is a tumor suppressor (100). The first piece of evidence comes from the observation that PP2A is the cellular target of okadaic acid. Okadaic acid is the main marine toxin implicated in the diarrhetic poisoning in human after consumption of contaminated bivalve mollusks. Since okadaic acid is a

tumor promoter and a specific inhibitor of PP2A, PP2A is proposed as a tumor suppressor (101). Okadaic acid is able to induce abnormal mitosis *in vitro* in human Hela cells (102). However, it remains untested whether premature chromosome segregation and mitotic exit contribute to the abnormal mitosis in the presence of okadaic acid.

The direct evidence supporting the tumor suppressor function of PP2A comes from the observation that the PP2A-Aβ gene, which encodes the beta isoform of the A subunit of PP2A, is mutated in 15% of primary lung tumors (103). Moreover, at least four PP2A Aα isoform somatic mutants have been identified in human tumors, including a glutamic acid-to-aspartic acid (E64D) substitution in a lung carcinoma, a glutamic acid-to-glycine substitution (E64G) in a breast carcinoma, an arginine-to-tryptophan substitution (R418W) in a malignant melanoma, and a frame-shift mutation at nucleotide position 652 in a breast carcinoma (104). Each of these PP2A Aα mutants are defective in their binding to specific B and/or C subunits (105). In addition to these Aα point mutations, decreased expression of Aα has been reported in 43% of human brain tumors (106), and in the human breast cancer cell line MCF-7 (107). These PP2A Aα mutants exhibited defects in binding to other PP2A subunits and impaired phosphatase activity (108). Recent work indicates that perturbation of the B-regulatory subunit of PP2A also contribute to cancer development (109, 110). Suppressing a specific PP2A subunit, B56γ, converts immortal HEK cells into tumorigenic cells under some specific conditions (111).

The introduction of small tumor (small-t) antigen of simian virus 40 (SV40) leads to the experimental transformation of growth arrested human cells (112). SV40 small t forms complexes with and inhibits PP2A, and this interaction also plays a critical role in human cell transformation (113). All these observations support the tumor suppressor function of PP2A. Accumulating evidence indicates the negative role of PP2A in anaphase entry and mitotic exit in budding yeast, therefore, further analysis is required to clarify whether the negative roles of PP2A in cell cycle control are conserved in mammalian cells. Moreover, more experiments are necessary to determine whether the cancer suppressor function of PP2A is attributable to its negative role in cell cycle control.

8. CONCLUSIONS

Aneuploidy, or abnormal chromosome content, is the most common characteristic of human solid tumors. Tumor cells become aneuploidy as a result of aberrant mitotic divisions. Up or down-regulation of any proteins that are involved in mitosis may result in chromosome segregation defects. Aneuploidy might contribute to tumor formation by altering transcription of tumor suppressors or oncogene products. Understanding of chromosome segregation regulation provides new targets for cancer therapy. For example, inhibitors of Aurora kinase and Plk1 could be use to prevent the growth of cancer cells that overproduce these kinases (89).

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10. REFERENCES

1. Hassold, T. & P. Hunt: To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet*, 2, 280-91 (2001)
2. Rajagopalan, H. & C. Lengauer: Aneuploidy and cancer. *Nature*, 432, 338-41 (2004)
3. Cottarel, G., J. H. Shero, P. Hieter & J. H. Hegemann: A 125-base-pair CEN6 DNA fragment is sufficient for complete meiotic and mitotic centromere functions in Saccharomyces cerevisiae. *Mol Cell Biol*, 9, 3342-9 (1989)
4. Cumberledge, S. & J. Carbon: Mutational analysis of meiotic and mitotic centromere function in Saccharomyces cerevisiae. *Genetics*, 117, 203-12 (1987)
5. Hegemann, J. H., J. H. Shero, G. Cottarel, P. Philippsen & P. Hieter: Mutational analysis of centromere DNA from chromosome VI of Saccharomyces cerevisiae. *Mol Cell Biol*, 8, 2523-35 (1988)
6. McAinsh, A. D., J. D. Tytell & P. K. Sorger: Structure, function, and regulation of budding yeast kinetochores. *Annu Rev Cell Dev Biol*, 19, 519-39 (2003)
7. He, X., D. R. Rines, C. W. Espelin & P. K. Sorger: Molecular analysis of kinetochore-microtubule attachment in budding yeast. *Cell*, 106, 195-206 (2001)
8. Li, Y., J. Bachant, A. A. Alcasabas, Y. Wang, J. Qin & S. J. Elledge: The mitotic spindle is required for loading of the DASH complex onto the kinetochore. *Genes Dev*, 16, 183-97 (2002)
9. Westermann, S., A. Avila-Sakar, H. W. Wang, H. Niederstrasser, J. Wong, D. G. Drubin, E. Nogales & G. Barnes: Formation of a dynamic kinetochore- microtubule interface through assembly of the Dam1 ring complex. *Mol Cell*, 17, 277-90 (2005)
10. Westermann, S., H. W. Wang, A. Avila-Sakar, D. G. Drubin, E. Nogales & G. Barnes: The Dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends. *Nature*, 440, 565-9 (2006)
11. Westermann, S., I. M. Cheeseman, S. Anderson, J. R. Yates, 3rd, D. G. Drubin & G. Barnes: Architecture of the budding yeast kinetochore reveals a conserved molecular core. *J Cell Biol*, 163, 215-22 (2003)
12. De Wulf, P., A. D. McAinsh & P. K. Sorger: Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. *Genes Dev*, 17, 2902-21 (2003)
13. Hayden, J. H., S. S. Bowser & C. L. Rieder: Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. *J Cell Biol*, 111, 1039-45 (1990)
14. Rieder, C. L. & S. P. Alexander: Kinetochores are transported poleward along a single astral microtubule

during chromosome attachment to the spindle in newt lung cells. *J Cell Biol*, 110, 81-95 (1990)
15. Tanaka, K., N. Mukae, H. Dewar, M. van Breugel, E. K. James, A. R. Prescott, C. Antony & T. U. Tanaka: Molecular mechanisms of kinetochore capture by spindle microtubules. *Nature*, 434, 987-94 (2005)
16. Tanaka, T. U., M. J. Stark & K. Tanaka: Kinetochore capture and bi-orientation on the mitotic spindle. *Nat Rev Mol Cell Biol*, 6, 929-42 (2005)
17. Guacci, V., D. Koshland & A. Strunnikov: A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. *Cell*, 91, 47-57 (1997)
18. Michaelis, C., R. Ciosk & K. Nasmyth: Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*, 91, 35-45 (1997)
19. Uhlmann, F. & K. Nasmyth: Cohesion between sister chromatids must be established during DNA replication. *Curr Biol*, 8, 1095-101 (1998)
20. Uhlmann, F., F. Lottspeich & K. Nasmyth: Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature*, 400, 37-42 (1999)
21. Uhlmann, F., D. Wernic, M. A. Poupart, E. V. Koonin & K. Nasmyth: Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell*, 103, 375-86 (2000)
22. Ciosk, R., W. Zachariae, C. Michaelis, A. Shevchenko, M. Mann & K. Nasmyth: An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell*, 93, 1067-76 (1998)
23. Cohen-Fix, O., J. M. Peters, M. W. Kirschner & D. Koshland: Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev*, 10, 3081-93 (1996)
24. Alexandru, G., F. Uhlmann, K. Mechtler, M. A. Poupart & K. Nasmyth: Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. *Cell*, 105, 459-72 (2001)
25. Li, R. & A. W. Murray: Feedback control of mitosis in budding yeast. *Cell*, 66, 519-31 (1991)
26. Hoyt, M. A., L. Totis & B. T. Roberts: S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, 66, 507-17 (1991)
27. Doheny, K. F., P. K. Sorger, A. A. Hyman, S. Tugendreich, F. Spencer & P. Hieter: Identification of essential components of the S. cerevisiae kinetochore. *Cell*, 73, 761-74 (1993)
28. Wang, Y. & D. J. Burke: Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast Saccharomyces cerevisiae. *Mol Cell Biol*, 15, 6838-44 (1995)
29. Visintin, R., S. Prinz & A. Amon: CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science*, 278, 460-3 (1997)
30. Hwang, L. H., L. F. Lau, D. L. Smith, C. A. Mistrot, K. G. Hardwick, E. S. Hwang, A. Amon & A. W. Murray: Budding yeast Cdc20: a target of the spindle checkpoint. *Science*, 279, 1041-4 (1998)
31. Lew, D. J. & D. J. Burke: The spindle assembly and spindle position checkpoints. *Annu Rev Genet*, 37, 251-82 (2003)
32. Pinsky, B. A. & S. Biggins: The spindle checkpoint: tension versus attachment. *Trends Cell Biol*, 15, 486-93 (2005)
33. Biggins, S. & A. W. Murray: The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Genes Dev*, 15, 3118-29 (2001)
34. Indjeian, V. B., B. M. Stern & A. W. Murray: The centromeric protein Sgo1 is required to sense lack of tension on mitotic chromosomes. *Science*, 307, 130-3 (2005)
35. Chan, C. S. & D. Botstein: Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. *Genetics*, 135, 677-91 (1993)
36. Wheatley, S. P., A. Carvalho, P. Vagnarelli & W. C. Earnshaw: INCENP is required for proper targeting of Survivin to the centromeres and the anaphase spindle during mitosis. *Curr Biol*, 11, 886-90 (2001)
37. Tanaka, T. U., N. Rachidi, C. Janke, G. Pereira, M. Galova, E. Schiebel, M. J. Stark & K. Nasmyth: Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell*, 108, 317-29 (2002)
38. Pinsky, B. A., C. Kung, K. M. Shokat & S. Biggins: The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. *Nat Cell Biol*, 8, 78-83 (2006)
39. Sandall, S., F. Severin, I. X. McLeod, J. R. Yates, 3rd, K. Oegema, A. Hyman & A. Desai: A Bir1-Sli15 complex connects centromeres to microtubules and is required to sense kinetochore tension. *Cell*, 127, 1179-91 (2006)
40. Cheeseman, I. M., S. Anderson, M. Jwa, E. M. Green, J. Kang, J. R. Yates, 3rd, C. S. Chan, D. G. Drubin & G. Barnes: Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell*, 111, 163-72 (2002)
41. Bachant, J., S. R. Jessen, S. E. Kavanaugh & C. S. Fielding: The yeast S phase checkpoint enables replicating chromosomes to bi-orient and restrain spindle extension during S phase distress. *J Cell Biol*, 168, 999-1012 (2005)
42. Lengronne, A. & E. Schwob: The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G (1). *Mol Cell*, 9, 1067-78 (2002)
43. Noton, E. & J. F. Diffley: CDK inactivation is the only essential function of the APC/C and the mitotic exit network proteins for origin resetting during mitosis. *Mol Cell*, 5, 85-95 (2000)
44. Fitch, I., C. Dahmann, U. Surana, A. Amon, K. Nasmyth, L. Goetsch, B. Byers & B. Futcher: Characterization of four B-type cyclin genes of the budding yeast Saccharomyces cerevisiae. *Mol Biol Cell*, 3, 805-18 (1992)
45. Liu, H. & Y. Wang: The function and regulation of budding yeast Swe1 in response to interrupted DNA synthesis. *Mol Biol Cell*, 17, 2746-56 (2006)
46. Hu, F. & O. M. Aparicio: Swe1 regulation and transcriptional control restrict the activity of mitotic cyclins toward replication proteins in Saccharomyces

cerevisiae. *Proc Natl Acad Sci U S A*, 102, 8910-5 (2005)
47. Rudner, A. D. & A. W. Murray: Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex. *J Cell Biol*, 149, 1377-90 (2000)
48. Jensen, S., M. Segal, D. J. Clarke & S. I. Reed: A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. *J Cell Biol*, 152, 27-40 (2001)
49. Agarwal, R. & O. Cohen-Fix: Phosphorylation of the mitotic regulator Pds1/securin by Cdc28 is required for efficient nuclear localization of Esp1/separase. *Genes Dev*, 16, 1371-82 (2002)
50. Ubersax, J. A., E. L. Woodbury, P. N. Quang, M. Paraz, J. D. Blethrow, K. Shah, K. M. Shokat & D. O. Morgan: Targets of the cyclin-dependent kinase Cdk1. *Nature*, 425, 859-64 (2003)
51. D'Amours, D. & A. Amon: At the interface between signaling and executing anaphase--Cdc14 and the FEAR network. *Genes Dev*, 18, 2581-95 (2004)
52. Visintin, R., K. Craig, E. S. Hwang, S. Prinz, M. Tyers & A. Amon: The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Molecular Cell*, 2, 709-18 (1998)
53. Shou, W., J. H. Seol, A. Shevchenko, C. Baskerville, D. Moazed, Z. W. Chen, J. Jang, H. Charbonneau & R. J. Deshaies: Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell*, 97, 233-44 (1999)
54. Visintin, R., E. S. Hwang & A. Amon: Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature*, 398, 818-23 (1999)
55. Bardin, A. J., R. Visintin & A. Amon: A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell*, 102, 21-31 (2000)
56. Pereira, G., T. Hofken, J. Grindlay, C. Manson & E. Schiebel: The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol Cell*, 6, 1-10 (2000)
57. Hu, F., Y. Wang, D. Liu, Y. Li, J. Qin & S. J. Elledge: Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell*, 107, 655-65 (2001)
58. Wang, Y., T. Shirogane, D. Liu, J. W. Harper & S. J. Elledge: Exit from exit: resetting the cell cycle through Amn1 inhibition of G protein signaling. *Cell*, 112, 697-709 (2003)
59. Bloecher, A., G. M. Venturi & K. Tatchell: Anaphase spindle position is monitored by the BUB2 checkpoint. *Nat Cell Biol*, 2, 556-8 (2000)
60. Daum, J. R., N. Gomez-Ospina, M. Winey & D. J. Burke: The spindle checkpoint of Saccharomyces cerevisiae responds to separable microtubule-dependent events. *Curr Biol*, 10, 1375-8 (2000)
61. Wang, Y., F. Hu & S. J. Elledge: The Bfa1/Bub2 GAP complex comprises a universal checkpoint required to prevent mitotic exit. *Curr Biol*, 10, 1379-82 (2000)
62. Stegmeier, F., R. Visintin & A. Amon: Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. *Cell*, 108, 207-20 (2002)
63. Cohen-Fix, O. & D. Koshland: Pds1p of budding yeast has dual roles: inhibition of anaphase initiation and regulation of mitotic exit. *Genes Dev*, 13, 1950-9 (1999)
64. Shirayama, M., A. Toth, M. Galova & K. Nasmyth: APC (Cdc20) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. *Nature*, 402, 203-7 (1999)
65. Shou, W., J. H. Seol, A. Shevchenko, C. Baskerville, D. Moazed, Z. W. Chen, J. Jang, A. Shevchenko, H. Charbonneau & R. J. Deshaies: Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell*, 97, 233-44 (1999)
66. Azzam, R., S. L. Chen, W. Shou, A. S. Mah, G. Alexandru, K. Nasmyth, R. S. Annan, S. A. Carr & R. J. Deshaies: Phosphorylation by cyclin B-Cdk underlies release of mitotic exit activator Cdc14 from the nucleolus. *Science*, 305, 516-9 (2004)
67. Pereira, G. & E. Schiebel: Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. *Science*, 302, 2120-4 (2003)
68. Higuchi, T. & F. Uhlmann: Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. *Nature*, 433, 171-6 (2005)
69. Khmelinskii, A., C. Lawrence, J. Roostalu & E. Schiebel: Cdc14-regulated midzone assembly controls anaphase B. *J Cell Biol* (2007)
70. Woodbury, E. L. & D. O. Morgan: Cdk and APC activities limit the spindle-stabilizing function of Fin1 to anaphase. *Nat Cell Biol*, 9, 106-12 (2007)
71. Parkes, V. & L. H. Johnston: SPO12 and SIT4 suppress mutations in DBF2, which encodes a cell cycle protein kinase that is periodically expressed. *Nucleic Acids Res*, 20, 5617-23 (1992)
72. Zeng, X., J. A. Kahana, P. A. Silver, M. K. Morphew, J. R. McIntosh, I. T. Fitch, J. Carbon & W. S. Saunders: Slk19p is a centromere protein that functions to stabilize mitotic spindles. *J Cell Biol*, 146, 415-25 (1999)
73. Evans, D. R. & M. J. Stark: Mutations in the Saccharomyces cerevisiae type 2A protein phosphatase catalytic subunit reveal roles in cell wall integrity, actin cytoskeleton organization and mitosis. *Genetics*, 145, 227-41 (1997)
74. Healy, A. M., S. Zolnierowicz, A. E. Stapleton, M. Goebl, A. A. DePaoli-Roach & J. R. Pringle: CDC55, a Saccharomyces cerevisiae gene involved in cellular morphogenesis: identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. *Mol Cell Biol*, 11, 5767-80 (1991)
75. Yang, H., W. Jiang, M. Gentry & R. L. Hallberg: Loss of a protein phosphatase 2A regulatory subunit (Cdc55p) elicits improper regulation of Swe1p degradation. *Mol Cell Biol*, 20, 8143-56 (2000)
76. Wang, Y. & D. J. Burke: Cdc55p, the B-type regulatory subunit of protein phosphatase 2A, has multiple functions in mitosis and is required for the kinetochore/spindle checkpoint in Saccharomyces cerevisiae. *Mol Cell Biol*, 17, 620-6 (1997)
77. Minshull, J., A. Straight, A. D. Rudner, A. F. Dernburg, A. Belmont & A. W. Murray: Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. *Curr Biol*, 6, 1609-20 (1996)
78. Tang, X. & Y. Wang: Pds1/Esp1-dependent and -independent sister chromatid separation in mutants defective for protein phosphatase 2A. *Proc Natl Acad Sci U S A*, 103, 16290-5 (2006)

79. Wang, Y. & T. Y. Ng: Phosphatase 2A Negatively Regulates Mitotic Exit in Saccharomyces cerevisiae. *Mol Biol Cell*, 17, 80-9 (2006)
80. Rudner, A. D., K. G. Hardwick & A. W. Murray: Cdc28 activates exit from mitosis in budding yeast. *J Cell Biol*, 149, 1361-76 (2000)
81. Yellman, C. M. & D. J. Burke: The Role of Cdc55 in the Spindle Checkpoint Is through Regulation of Mitotic Exit in Saccharomyces cerevisiae. *Mol Biol Cell*, 17, 658-66 (2006)
82. Liang, F. & Y. Wang: DNA damage checkpoints inhibit mitotic exit by two different mechanisms. *Mol Cell Biol*, 27, 5067-78 (2007)
83. Queralt, E., C. Lehane, B. Novak & F. Uhlmann: Downregulation of PP2A (Cdc55) Phosphatase by Separase Initiates Mitotic Exit in Budding Yeast. *Cell*, 125, 719-32 (2006)
84. Rajagopalan, H., M. A. Nowak, B. Vogelstein & C. Lengauer: The significance of unstable chromosomes in colorectal cancer. *Nat Rev Cancer*, 3, 695-701 (2003)
85. Visscher, D. W., T. L. Wallis & J. D. Crissman: Evaluation of chromosome aneuploidy in tissue sections of preinvasive breast carcinomas using interphase cytogenetics. *Cancer*, 77, 315-20 (1996)
86. Krause, S., G. Feil, T. Beiter, H. Pressler, K. M. Schrott & K. H. Bichler: Examination of tumorigenesis of precursor lesions in bladder cancer by *in situ* hybridization. *Urol Int*, 72, 118-22 (2004)
87. Adams, R. R., D. M. Eckley, P. Vagnarelli, S. P. Wheatley, D. L. Gerloff, A. M. Mackay, P. A. Svingen, S. H. Kaufmann & W. C. Earnshaw: Human INCENP colocalizes with the Aurora-B/AIRK2 kinase on chromosomes and is overexpressed in tumour cells. *Chromosoma*, 110, 65-74 (2001)
88. Tatsuka, M., H. Katayama, T. Ota, T. Tanaka, S. Odashima, F. Suzuki & Y. Terada: Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. *Cancer Res*, 58, 4811-6 (1998)
89. Li, J. J. & S. A. Li: Mitotic kinases: the key to duplication, segregation, and cytokinesis errors, chromosomal instability, and oncogenesis. *Pharmacol Ther*, 111, 974-84 (2006)
90. Deluca, J. G., W. E. Gall, C. Ciferri, D. Cimini, A. Musacchio & E. D. Salmon: Kinetochore microtubule dynamics and attachment stability are regulated by hec1. *Cell*, 127, 969-82 (2006)
91. Cheeseman, I. M., J. S. Chappie, E. M. Wilson-Kubalek & A. Desai: The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell*, 127, 983-97 (2006)
92. Ohi, R., M. L. Coughlin, W. S. Lane & T. J. Mitchison: An inner centromere protein that stimulates the microtubule depolymerizing activity of a KinI kinesin. *Dev Cell*, 5, 309-21 (2003)
93. Andrews, P. D., Y. Ovechkina, N. Morrice, M. Wagenbach, K. Duncan, L. Wordeman & J. R. Swedlow: Aurora B regulates MCAK at the mitotic centromere. *Dev Cell*, 6, 253-68 (2004)
94. Smith, M. R., M. L. Wilson, R. Hamanaka, D. Chase, H. Kung, D. L. Longo & D. K. Ferris: Malignant transformation of mammalian cells initiated by constitutive expression of the polo-like kinase. *Biochem Biophys Res Commun*, 234, 397-405 (1997)
95. Eckerdt, F., J. Yuan & K. Strebhardt: Polo-like kinases and oncogenesis. *Oncogene*, 24, 267-76 (2005)
96. Cahill, D. P., C. Lengauer, J. Yu, G. J. Riggins, J. K. Willson, S. D. Markowitz, K. W. Kinzler & B. Vogelstein: Mutations of mitotic checkpoint genes in human cancers. *Nature*, 392, 300-3 (1998)
97. Kim, H. S., K. H. Park, S. A. Kim, J. Wen, S. W. Park, B. Park, C. W. Gham, W. J. Hyung, S. H. Noh, H. K. Kim & S. Y. Song: Frequent mutations of human Mad2, but not Bub1, in gastric cancers cause defective mitotic spindle checkpoint. *Mutat Res*, 578, 187-201 (2005)
98. Wang, X., D. Y. Jin, R. W. Ng, H. Feng, Y. C. Wong, A. L. Cheung & S. W. Tsao: Significance of MAD2 expression to mitotic checkpoint control in ovarian cancer cells. *Cancer Res*, 62, 1662-8 (2002)
99. Hanks, S., K. Coleman, S. Reid, A. Plaja, H. Firth, D. Fitzpatrick, A. Kidd, K. Mehes, R. Nash, N. Robin, N. Shannon, J. Tolmie, J. Swansbury, A. Irrthum, J. Douglas & N. Rahman: Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. *Nat Genet*, 36, 1159-61 (2004)
100. Janssens, V., J. Goris & C. Van Hoof: PP2A: the expected tumor suppressor. *Curr Opin Genet Dev*, 15, 34-41 (2005)
101. Bialojan, C. & A. Takai: Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. *Biochem J*, 256, 283-90 (1988)
102. Ghosh, S., N. Paweletz & D. Schroeter: Effects of okadaic acid on mitotic HeLa cells. *J Cell Sci*, 103 (Pt 1), 117-24 (1992)
103. Wang, S. S., E. D. Esplin, J. L. Li, L. Huang, A. Gazdar, J. Minna & G. A. Evans: Alterations of the PPP2R1B gene in human lung and colon cancer. *Science*, 282, 284-7 (1998)
104. Calin, G. A., M. G. di Iasio, E. Caprini, I. Vorechovsky, P. G. Natali, G. Sozzi, C. M. Croce, G. Barbanti-Brodano, G. Russo & M. Negrini: Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene*, 19, 1191-5 (2000)
105. Ruediger, R., H. T. Pham & G. Walter: Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. *Oncogene*, 20, 10-5 (2001)
106. Colella, S., H. Ohgaki, R. Ruediger, F. Yang, M. Nakamura, H. Fujisawa, P. Kleihues & G. Walter: Reduced expression of the Aalpha subunit of protein phosphatase 2A in human gliomas in the absence of mutations in the Aalpha and Abeta subunit genes. *Int J Cancer*, 93, 798-804 (2001)
107. Suzuki, K. & K. Takahashi: Reduced expression of the regulatory A subunit of serine/threonine protein phosphatase 2A in human breast cancer MCF-7 cells. *Int J Oncol*, 23, 1263-8 (2003)
108. Chen, W., J. D. Arroyo, J. C. Timmons, R. Possemato & W. C. Hahn: Cancer-associated PP2A Aalpha subunits induce functional haploinsufficiency and tumorigenicity. *Cancer Res*, 65, 8183-92 (2005)
109. Rangarajan, A. & R. A. Weinberg: Opinion: Comparative biology of mouse versus human cells:

modelling human cancer in mice. *Nat Rev Cancer*, 3, 952-9 (2003)
110. Rangarajan, A., S. J. Hong, A. Gifford & R. A. Weinberg: Species- and cell type-specific requirements for cellular transformation. *Cancer Cell*, 6, 171-83 (2004)
111. Chen, W., R. Possemato, K. T. Campbell, C. A. Plattner, D. C. Pallas & W. C. Hahn: Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell*, 5, 127-36 (2004)
112. Bikel, I., X. Montano, M. E. Agha, M. Brown, M. McCormack, J. Boltax & D. M. Livingston: SV40 small t antigen enhances the transformation activity of limiting concentrations of SV40 large T antigen. *Cell*, 48, 321-30 (1987)
113. Pallas, D. C., L. K. Shahrik, B. L. Martin, S. Jaspers, T. B. Miller, D. L. Brautigan & T. M. Roberts: Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. *Cell*, 60, 167-76 (1990)

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