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MINIREVIEW

Spindle Checkpoint Control in Budding Yeast

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Eukaryotic cell cycle progresses via four phases, G1, S, G2, and M in that order. Each phase can start only after the previous phase has been completed. The molecular mechanism on how this is accomplished was first genetically studied by Weinert and Hartwell (77) using *S. cerevisiae* and they designated this phenomenon checkpoint control. The first example of the checkpoint was the DNA damage checkpoint; the cell cycle arrests at G2 until the damage in DNA is repaired. Since then checkpoint controls have been found in other than DNA damage such as those checking replication errors, spindle integrity, nuclear position, etc. Each checkpoint has been found to be a key point of cell cycle regulation, therefore, checkpoint study became a central subject in cell cycle investigation. Here we concentrate our attention on the spindle checkpoint in *S. cerevisiae*.

Progression of mitosis

Mitosis is a mechanism to correctly divide the replicated genetic material into two daughter cells. Mitosis is started by activation of MPF, a complex consisting of Cdk1 and mitotic cyclin, and ends by abolishing the MPF activity by destruction of the mitotic cyclin or by accumulating the Cdk inhibitor, Sic1. Another key step of mitosis is the metaphase-anaphase transition which is stimulated by destruction of an anaphase inhibitor, Pds1 (80, 81), by ubiquitin-dependent proteolysis. The spindle checkpoint is a monitoring system of these processes to ensure the correct partitioning of the replicated chromosomes. The sister chromatids remain unseparated until the onset of anaphase by a protein complex called cohesin consisting of Scc1 and Smc (24, 49, 51). The stage in which every chromatid is caught by a spindle is metaphase, and then Pds1 is ubiquitinylated by anaphase promoting complex (APC^{Cdc20}) followed by degradation by the 26S proteasome (14). Pds1 forms a complex with Esp1 which is kept inactive until Pds1 is degraded. Freed Esp1 cuts Scc1, a component of cohesin and then sister chromatids begin to separate (70). This is the key step called the metaphase-anaphase transition. In anaphase, the spindle elongates along the mother-daughter axis to deliver the replicated chromosomes to two daughter cells. When spindles are fully elongated and chromosomes are partitioned, Clb2 is ubiquitinylated by APC^{Cdh1} and degraded by the 26S proteasome and Cdk inhibitor Sic1 is accumulated, thereby collapsing Cdk activity. This is the end of mitosis. Cytokinesis occurs soon after.

Microtubules and SPB

Budding yeast has two types of microtubules, cytoplasmic microtubules and spindles. The main components of microtubules are α tubulin and β tubulin and tubulin monomers and microtubules are in a dynamic equilibrium. At the nucleation center of microtubules, γ tubulin functions as a primer of polymerization. A spindle pole body (SPB) functions as a microtubule organizing center. SPB is a protein complex embedded in the nuclear membrane and consists of three lamellae, outer plaque, central plaque, and inner plaque (1, 6) (Fig. 1). Nucleation of cytoplasmic microtubules occurs at the cytoplasmic face of the SPB and spindle assembly starts at the inner plaque of the SPB. One end of the cytoplasmic microtubules is obviously at the SPB and the other end is on the cell cortex. The cytoplasmic microtubules play a role in determining the nuclear position in the cell, which in turn plays an impotant role in correct partitioning of genetic materials. How do cytoplasmic microtubules find a correct attachment site on the cell cortex? The factors functioning in this process have been elucidated (40, 42). Kar9 and Bim1 play pivotal roles here. Kar9 is localized to a bud tip and Bim1 is localized on microtubules. Kar9 does not have affinity to microtubules but to Bim1. Microtubules accomodating Bim1 explore the cortex and are fixed when they are captured by Kar9 on the cortex via Bim1 on microtubules. Two types of spindles are known; one is pole to pole spindles that traverse the nucleus and the

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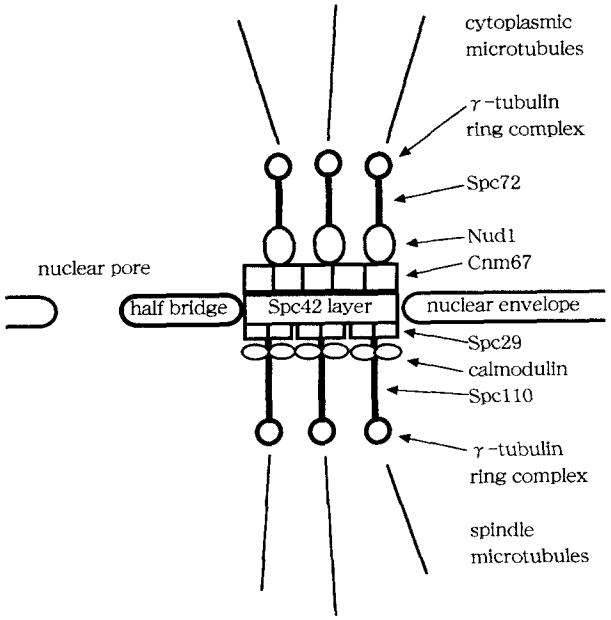

Fig. 1. A model of a spindle pole body of budding yeast.

other connects SPB and kinetochores of chromosomes. Spindles are thought to find kinetochores by search and capture mechanism like cytoplasmic microtubules that find the attachment site on the cortex (28, 50).

The assembly of cytoplasmic microtubules is started at either the outer plaque or the half bridge (Fig. 1), whereas spindles emanate from the inner plaque. The SPB is duplicated at the beginning of S phase and one of them moves to the opposite site during S phase. The SPB not only acts as the microtubule assembly center but also affords a site of assembly, or a site of action, of many proteins that function at mitotic exit and cytokinesis (See later).

Mutants defective in the spindle checkpoint

Chromosomal separation must start only after the spindle apparatus has been completed, suggesting the presence of the spindle checkpoint mechanisms. This concept was developed from characterization of yeast mutants showing sensitivity to spindle poisons. Two sets of mutants, *mad* (mitosis arrest deficient) mutants and *bub* (budding uninhibited by benzimidazoles) mutants were isolated (29, 44). All of these mutants quickly lost their viability upon exposure to a spindle poison, such as benomyl or nocodazole. In wild-type cells when exposed to such a drug, the cell cycle stops at and stays in the M phase, thereby remaining viable. In contrast, *mad* or *bub* mutants continue their cell cycle and pass through M phase even in the presence of the drug and consequently die. They can not hold their cell cycle at M phase when their spindles have been damaged. Three *mad* mutants (*mad1*, *mad2*, and *mad3*) and three *bub* mutants (*bub1*, *bub2*, and *bub3*) have been reported. *MPS1* is another spindle checkpoint gene which has been identified as a gene functioning in SPB duplication (78). A spindle checkpoint is activated by a spindle poison at a concentration causing apparently no damage to the spindle structure. Furthermore, overexpression of the components of the checkpoint can arrest cells with normal spindles in mitosis (27). Thus a spindle checkpoint is unlikely to be activated by an abnormal spindle structure. For the spindle checkpoint, kinetochore or cell cortex, the another end point of spindles, also plays an important role (See later).

The Mad2 pathway

Hardwick *et al.* (27) demonstrated that Mps1 is likely located at the top of the spindle checkpoint pathway; overexpression of *MPS1* in the absence of spindle damage caused cell cycle arrest, and this was dependent on the *MAD* and *BUB* genes. How do the *MAD* and *BUB* genes work in the spindle checkpoint pathway? The connection between kinetochore and the spindle checkpoint is predicted from the facts that anaphase is inhibited until bipolar attachments between all kinetochores and spindles are achieved (52, 56). Tension between kinetochores and centrosomes is thought to be required for progression to mitosis since arrest induced by a single unattached chromosome in insect cells can be overcome by applying tension on the kinetochore of the unattached chromosome (45).

In budding yeast, involvement of kinetochores in the spindle checkpoint pathway was examined genetically (76). A temperature-sensitive mutant of the *CTF13* gene encoding a component of the centromere binding protein complex CBF3 was delayed at G2/M at a restrictive temperature. The delay was dependent on *BUB1*, *BUB3*, *MAD1*, and *MAD2* but not on *BUB2*. Furthermore, the low concentration of nocodazole induced a cell cycle delay in the *ctf13* mutant cells in a spindle checkpoint-dependent manner. Altogether, the antimitotic effect of nocodazole mediated by *MAD1*, *MAD2*, BUB1, and *BUB3* is due to inhibition of spindle-kinetochore interactions.

Sequence analysis and characterization of *MAD1* (26), *MAD2* (11), *BUB1* (57), and *BUB3* (29) were conducted. The checkpoint functions of frog and human homologues of *MAD1* and *MAD2* were shown to be conserved (10, 11, 35, 46) and Mad1 and Mad2 were shown to be localized to unattached kinetochores in tissue culture cells. *BUB1* encodes a protein kinase that binds to and phosphorylates Bub3 (57) and mouse (67) and fission yeast (4) Bub1 homologues. Bub1 has been localized to unattached kinetochores in a Bub3-dependent manner (66). The localization of Mad and Bub proteins at the kinetochores is consistent with the idea that these checkpoint genes monitor kinetochore-spindle interactions (76). Then what are the molecular mechanisms by which spindle/kinetochore defects are monitored and what does send a signal to induce a cell cycle delay? These questions are still open

but one could believe that the signal may be sent to Mps1 and Bub1 (20) when kinetochore/spindle interactions are defective, then Mps1 and Bub1 may phosphorylate Mad and/or Bub proteins to execute spindle chekpoint functions.

Understanding of how the spindle checkpoint arrests the cell cycle at metaphase has progressed in recent years due to a development of the understanding of ubiquitin-dependent proteolysis and the molecular mechanisms of sister chromatid separation. The spindle checkpoint blocks sister chromatid separation by inhibiting the APC. Mad2 and Mad3 bind to Cdc20, an essential activator of the APC, thereby inhibiting APC activity (30). The interaction between Mad2 and Cdc20 is conserved in vertebrates (17, 37). Cdc20 appears to target proteins, such as Pds1 and Clb5, for ubiquitinylation by the APC (59, 72). That Cdc20 is a target of the spindle checkpoint was further substantiated by the fact that *cdc20* mutant defective in Mad2 association allows cells to escape from the mitotic arrest (30, 38). Thus this spindle checkpoint pathway is designated the Mad2 pathway. Destruction of Pds1 is necessary to trigger sister chromatid separation (12, 14, 81) and mitotic exit (13, 68). A *cdc20* mutant arrests in metaphase with high Pds1 and Cdk levels, showing that inhibition of Cdc20 by the spindle checkpoint would be sufficient to prevent both sister chromatid separation and the destruction of the mitotic cyclins that are required for exit from mitosis.

What are the roles of other Mad and Bub proteins in regulation of Cdc20 activity? To address this question, interactions between proteins functioning in the spindle checkpoint were examined. Mad1 and Mad2 form a tight complex (9). Bub1 and Bub3 also form a tight complex (57). Mad3 is a homologue of Bub1 but it does not have a kinase domain (25). Mad1, Mad2, and Mad3 were shown to interact with Cdc20 by the two-hybrid system (30). Mad2 and Mad3 coprecipitated with Cdc20 at all stages of the cell cycle. Mad2 binds to Cdc20 in a Mad1-dependent manner and binding of Mad3 to Cdc20 is absolutely dependent on Mad2 (30). Molecular dissection of Mad3 reveals that it has two sites for binding to both Cdc20 and to Bub3. Binding motifs for these two proteins are conserved in Bub1 and, as expected, Bub1 bound Cdc20 and Bub3 (25). Mad3-Bub3, Bub1-Bub3, and Mad1-Mad2 complexes are constitutively present in cells. It is possible that these are intermediates to the formation of the checkpoint proteins at kinetochores. Interestingly, some of these gene products are not seen in the Cdc20 immunoprecipitates; Bub1 and Mad1 are not present in the complex. As shown in Xenopus (10), Mad1 recruits Mad2 to kinetochores and Bub3 may recruit both Bub1 and Mad3 to kinetochores (66). It should be noted that Mps1/Mad1 and Bub1/Bub3 are kinase/substrate complexes. A plausible interpretation for the Mad2 spindle checkpoint pathway is as follows; microtubule-free kinetochores attract

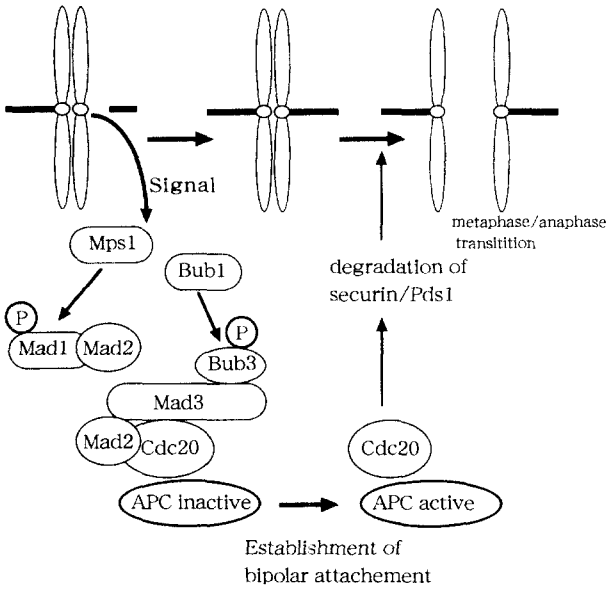

Fig. 2. The Mad2 pathway.

Mad1 and Bub3, which in turn, load Mad2 and Mad3 on Cdc20 to inhibit its activity (Fig. 2).

As for the activation mechanisms of the Mad2 spindle checkpoint, many questions are left to be solved. How do checkpoint proteins receive signals from unattached chromosomes? Mps1 is thought to be an upstream component of the spindle checkpoint, however, little information is available about its intracellular localization and function. Mob1, a Mps1 binding protein, is localized to SPB in M phase and to the bud neck at late anaphase (Yoshida and Toh-e, unpublished). Given that Mps1 localizes to the SPB, then a question arises how signals at the SPB and kinetochores are connected.

Mitotic exit and the Bub2 spindle checkpoint pathway

There are genes, a mutation in any of which results in mitotic arrest at late anaphase or telophase at its restrictive temperature. A typical phenotype of the arrest is shown in Fig. 3; nuclei has been separated but spindles remain intact and Cdk activity is kept high. These mutants can not exit from mitosis; therefore, the responsible genes showing such a phenotype are collectively called the MEN (Mitotic Exit Network) genes (Table 1). The MEN proteins contain a small GTPase, its regulators, a protein phosphatase, and protein kinases. All of these proteins are eligible to function in a signal transduction pathway by a phosphorylation-dephosphorylation cascade.

Unveiling of the function of these genes and of the interactions between them has been a demand in cell cycle research. A key regulator is the small GTPase Tem1, which has been isolated as a multicopy suppressor of the cold sensitivity of the *lte1* deletion (60), the wild-type of which contains a motif of a guanine nucleotide exchange factor of Ras-type GTPase (61). A possible

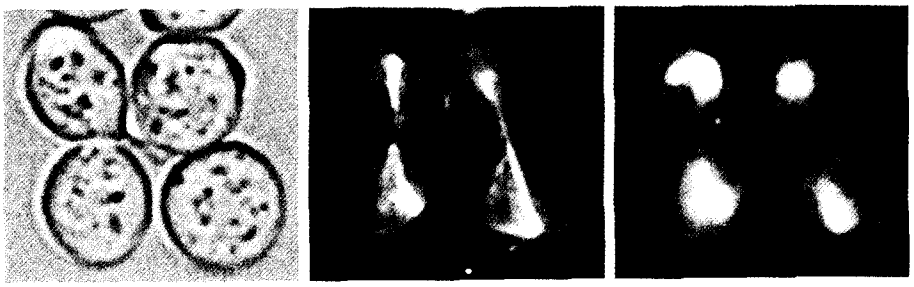

Fig. 3. Phenotype of a mutant arrested at late anaphase/telophase. A *nud1-44 GFP-TUB1* temperature-sensitive mutant cells arrested at 37°C for 3 hours were fixed with 5% formaldehyde for 10 minutes at the room temperature. DNA was stained by DAPI and microtubulues were visualized by GFP fluorescence.

downstream effector of Tem1 is Cdc15 kinase (2, 62). *CDC15* encodes a serine/threonine protein kinase and its temperature-sensitive mutant is a classical *CDC* mutant showing mitotic arrest. The most downstream in this cascade is *CDC14*, encoding a dual protein phosphatase (75), since an extra copy of *CDC14* bypasses a defect in many of the other members of the MEN (32, 62, 72). Cdc14 phosphatase can dephosphorylate proteins phosphorylated by Cdk (72), therefore Cdc14 should be sequestrated somewhere in the cell as far as phospho-proteins produced by Cdk must be present. Actually Cdc14 is localized to the nucleolus during G1, S, and early anaphase whereas it is released and dispersed all over the nucleus (and partly in the cytoplasm) at late anaphase (73). The regulatory mechanism for the change of localization of Cdc14 was elucidated. A revertant from a *cdc15-2* temperature-sensitive mutant was used for cloning the responsible gene and a new gene designated *NET1* was identified. Visintin *et al.* (73) isolated *CFI1/NET1* by two-hybrid screening using *CDC14* as bait. Net1 is a nucleolar protein and forms a complex with Sir2 and Cdc14, called a RENT (Regulator of Nucleolar Silencing and Telophase) complex. In the absence of Net1 activity, Cdc14 is constitutively released from the nucleolus, thereby dephosphorylating Cdh1, Swi5, and Sic1 (31, 72,). Dephosphorylation of Cdh1 activates APC to degrade Clb2, a mitotic cyclin, thereby inactivating Cdk. Another way for cells to exit mitosis is inhibition of Cdk activity by an Cdkl inhibitor, Sic1 (69). Swi5 is a transcription factor which stimulates the expression of *SIC1*. Dephosphorylated Swi5 translocates into the nucleus and activates *SIC1* expression. Phosphorylated Sic1 is a target of ubiquitinylation by SCF ubiquitin ligase and is quickly degraded by the 26S proteasome (53, 71). In contrast, the dephosphorylated form of Sic1 is stable so that it accumulates and inhibits Cdk. The consequence of the release of Cdc14 from the nucleolus is a collapse of Cdk activity, thereby allowing the cells to exit mitosis (Fig. 4).

Tem1 and Cdc15 are upstream members of the MEN. Genetic interaction between these two genes was known already (62) and recently these gene products were found to interact physically (2, 3). The yeast two hybrid screening using Tem1 as bait picked up a DNA fragment encoding a part of Cdc15 (2). The *in vivo* interaction between Tem1 and Cdc15 was comfirmed by the experiment showing that Tem1 immunoprecipitates contained Cdc15 (2, 3). Connection between the MEN and the spindle checkpoint was hinted at by the work on septum formation in fission yeast.

The *cdc7*$^{+}$ gene of fission yeast is a *CDC15* kinase gene homologue and essential for septation (19). Another gene *spg1*$^{+}$ is a *TEM1* homologue and also essential for septation (58). A mutation in the *S. pombe cdc16*$^{+}$ gene leads to the formation of multiple septa without cytokinesis

Table 1. Regulators of the MEN

gene	product	function
CDC14	protein phosphatase	component of the nucleolar RENT complex; dephosphorylates Cdh1, Sic1 and Swi5
CDC5	protein kinase	*S. cerevisiae* homologue of polo kinase family; activator of APC^{Cdh1}; localizes to the SPB
DBF2	protein kinase	component of the CCR4 transciptional complex; binds to Mob1; homologous to Dbf20 of *S. cerevisiae;* localizes to the SPB
MOB1	novel protein	component of the CCR4 transcriptional complex; binds to Dbf2 and Mps1 kinase; localizes to the SPB
TEM1	Ras-type GTPase	localizes to the SPB; binds to Cdc15
CDC15	protein kinase	localizes to the SPB; binds to Tem1
LTE1	GEF	probable GTP/GDP exchange factor for Tem1
NUD1	SPB component	component of the outer plaque of SPB; binds to Bub2/Bfa1 complex and Cdc15

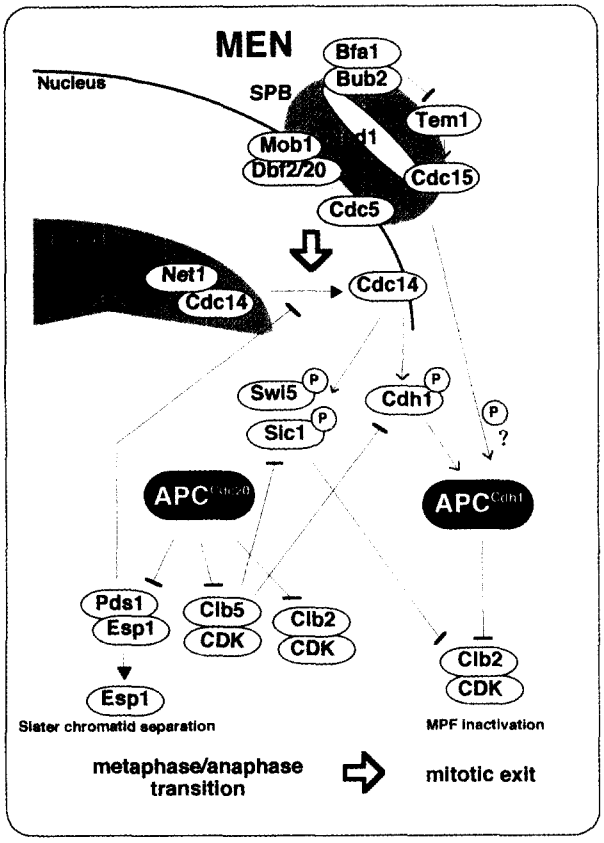

Fig. 4. A model of mitotic exit network.

(18). The nucleotide sequence of the *cdc16*$^+$ gene revealed that it is a homologue of *BUB2* of *S. cerevisiae* and acts as a Spg1GTPase activating protein with Byr4 (23, 65). Spg1, Cdc16, and Byr4 form a complex (23). These three proteins localize to SPBs. Spg1 stays at the SPBs throughout the cell cycle but Cdc16 and Byr4, which localize to the SPB in G2 phase and early mitosis, come off from one of the SPBs in late anaphase, resulting in the activation of Spg1 on the SPB which had lost the GAP protein. The activated Spg1 attracts Cdc7 to the SPB with Spg1-GTP which signals for septation. A homologue of *byr4*$^+$, *BFA1*, was found in the *Saccharomyces cerevisiae* genome by homology search and was shown to be involved in the Bub2 spindle checkpoint pathway (41, 43). Now, four gene products functioning at the septation of fission yeast have homologues in budding yeast although the time of their functions apparently different; the genes in fission yeast act at cytokinesis and those of budding yeast at exit from mitosis.

That Tem1 functions downstream of the Bub2 was demonstrated by the following observations. (i) Overproduction of Tem1 in the wild-type cells rendered them sensitive to nocodazole and this sensitivity was found to be due to a defect in the spindle chekpoint; cells rebudded and died, (ii) nocodazole sensitivity displayed by the *bub2* mutant was dependent on functional Tem1, (iii) overexpression of Bfa1 caused mitotic arrest at late anaphase like MEN mutants, and (iv) this arrest was canceled by introducing the activated form of Tem1, Tem1-1. It was shown that Bub2, Bfa1, and Tem1 form a complex *in vivo* (54). These proteins were found to be localized to the SPB. Bfa1 and Tem1 are localized to the SPB in a Bub2-dependent manner, however, a small amount of Tem1 stays at the SPB in the absence of Bub2 and localization of Bub2 to the SPB is dependent on Bfa1 (54). Interestingly, Bfa1 and Tem1 distribute unevenly between two SPBs at late anaphase when each of them entered into a separate compartment (3, 54). Tem1 and Bfa1 are abundantly present at the SPB in the daughter cell. In fission yeast, SPBs are asymmetric and that containing Spg1-GTP recruits Cdc7. It is quite possible that the same things happen to the SPBs of budding yeast. Then the question is which SPB contains activated Tem1. Lte1 which has been believed to be an activator of Tem1 localizes to the surface of a daughter cell until nuclear division and it exits both mother and daughter cells after spindles are dissoluted (3, 54). This observation is favorable to the idea that the SPB in the daughter cell contains Tem1-GTP. On the other hand, GAP, a negative regulator of Tem1, localizes to the SPB in the daughter cell, suggesting this SPB could contain Tem1-GDP. Therefore, localization of Cdc15 in budding yeast, given that Tem1-GTP recruits Cdc15, will afford us an important clue about which SPB contains Tem1-GTP and the mechanism underlying the exit from mitosis. Cenamor *et al.* (8) reported that Cdc15 localized to the SPB in a daughter cell at late anaphase and then disappeared from the SPB in a Cdc14-dependent manner. It is possible that cycling of Tem1 between the GTP form and the GDP form is necessary for its biological function. Asakawa *et al.* (2) demonstrated that inactive Tem1 did not interact with Cdc15, implying that the activated form of Tem1 can bind to Cdc15. Since Tem1-Cdc15 interaction does not seem to affect kinase activity, the regulation of Cdc15 by Tem1 may be conducted at the level of determination of intracellular localization.

It is quite clear that SPB plays an important role in cell cycle regulation especially at the spindle checkpoint control. This notion was further substantiated by examining the phenotypes displayed by the mutant of the SPB component. Nud1 is a component of the cytoplasmic face of the SPB and a *nud1* temperature-sensitive mutant is defective in the outer plaque (1). At a restrictive temperature, *nud1*-ts mutant cells were arrested at late anaphase/telophase like the MEN mutants (1). Since the *nud1-44* mutant was suppressed by overproduction of Sic1 or Cdc15 (Yoshida and Toh-e, unpublished), it is likely arrested at a state in which Cdk activity remains high. Therefore, *NUD1* belongs to the MEN genes.

Gruneberg *et al.* demonstrated that Nud1 interacted

with and accomodated Spc72, a core component of SPB, at the outer plaque of the SPB. Spc72 acts at the start of γ tubuline polymerizing either at the outer plaque or at the half bridge. Nud1 is necessary not only for establishment of cytoplasmic microtubules but also for accomodation of non-SPB proteins at the SPB. Bub2, Bfa1, and Tem1 localize to the SPB in a Nud1-dependent manner, indicating that those proteins localize to the cytoplasmic face of the SPB (15). Furthermore, Nud1 was found to physically interact with Bub2 and Bfa1, suggesting that Nud1 acts as a landmark of loading Bub2 and other proteins to the SPB.

The localization of Bub2 suggests that the Bub2 spindle checkpoint monitors a state of cytoplasmic microtubules. In the case of the Mad2 checkpoint, the system monitors spindle-kinetochore interaction. Cytoplasmic microtubules connect the SPB and the cell cortex to keep the proper position and orientation of the nucleus. Thus the Bub2 checkpoint likely monitors the cytoplasmic microtubule-cell cortex interaction. This is probably the case; using *kar9Δ bub2Δ* (15), *dyn1Δ bub2Δ* (5), *spc72*-ts *bub2Δ*, and *bim1Δ bub2Δ* (54), the Bub2 pathway is necessary to arrest the cell cycle in responding to a defect in cytoplasmic microtubule-cell cortex interaction. In *kar9Δ* or *dyn1Δ* cells, the nucleus is completely sequestered in mother cells and mitosis is halted at late anaphase. Since this mitotic arrest was not seen in the *kar9Δ bub2Δ* (15) cells and *dyn1Δ bub2Δ* (5) cells, it is possible that Bub2 monitors the spindle position (Fig. 5).

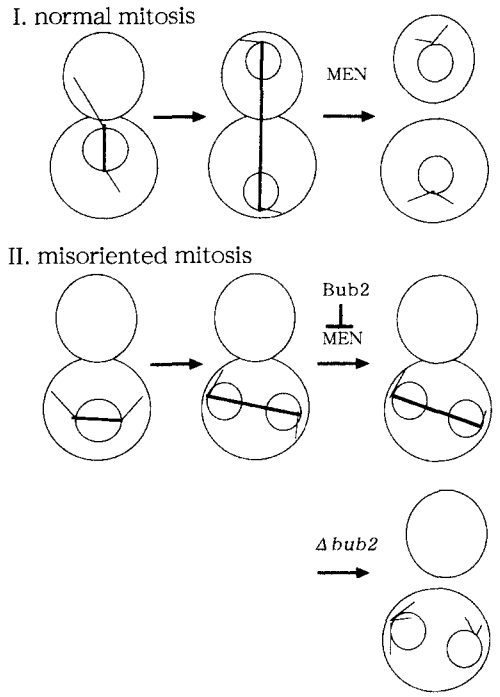

Fig. 5. The Bub2 pathway.

To keep the order of two APC functions.

There are two spindle checkpoint mechanisms working to regulate M phase progression, one is the Mad2 pathway regulating the metaphase-anaphase transition and the other the Bub2 pathway controlling the exit from mitosis. Ubiquitin dependent proteolysis is the target of the spindle checkpoint; the Mad2 pathway targets the regulator of APC, Cdc20, and the Bub2 pathway the release of Cdc14 from the nucleolus which dephosphorylates Cdh1, Swi5, and Sic1, resulting in upregulation of ubiquitin-dependent proteolysis. How is this order, APC^{Cdc20} first and then APC^{Cdh1}, guaranteed? One of the candidates for this role was shown to be Pds1. Cohen-Fix and Koshland (13) demonstrated that indestructible Pds1 inhibited dephosphorylation of Cdh1 thereby inhibiting the activation of APC^{Cdh1} in late mitosis, which resulted in inhibition of Clb2 destruction. This phenomenon was depen-dent on the activity of APC but was not seen in G1 where APC^{Cdh1} had already been activated. Pds1 is thonght to act on the pathway, probably MEN, which activates APC in late mitosis. Tinker-Kulberg and Morgan (68) showed that Pds1 blocks cyclin destruction in late anaphase by a mechanism that is independent of sister chromatid separation. These features of Pds1 may provide it with a sophisticated way to keep the order of the two APC functions.

It is believed that Cdc20 is an activator of APC acting at early mitosis, however, Cdc20 seems to have multiple roles in mitosis; in additon to the role in metaphase-anaphase transition, Cdc20 can degrade Clb2 cyclin, when abundantly present, in an APC-and MEN-dependent manner (47, 82). Shirayama *et al.* (63) demonstrated that Clb5 persisted in late anaphase in *pds1Δ cdc20Δ* cells and inhibited exit from mitosis. According to their interpretation, the balance of a phosphorylation level by Cdk kinase and Cdc14 phosphatase would determine exit from mitosis. In the wild-type cells, Pds1 and Clb5 are degraded roughly at the onset of metaphase-anaphase transition (14). Cdc20 and Cdc5, regulators of APC, are also unstable proteins and are degraded by APC-dependent proteolysis (64). Pds1 and Clb5 are ubiquitinylated by APC^{Cdc20}, whereas Cdc5 and Cdc20 are ubiquitinylated by APC^{Cdh1}. In contrast to Pds1 and Clb5, Cdc20 and Cdc5 persist until late anaphase like Clb2. The alternating sequence of accumulation and destruction of these cell cycle regulators seems to ensure the order of M phase progression.

Mitotic exit and Cytokinesis

One of the obvious features displayed by a MEN mutant is a defect in cytokinesis. Jimenez *et al.* (34) found and analyzed a mutant of *cdc15*, *cdc15-lyt1*, to show that the mutant kept at a restrictive temperature for a longer period, 6 hours, escaped from the arrest and re-replicated without completion of cytokinesis and died. Contribution of Cdc15 to cytokinesis is further supported by the obser-

vation that recruitment of IQGAP-like protein and actin, both required for cytokinesis, to the bud neck was dependent on Cdc15 (48). Cdc15 localizes not only to the SPB but also to the bud neck at late anaphase (79), implying a role of Cdc15 in cytokinesis. Besides Cdc15, Dbf2 and Mob1 were shown to be localized to the SPB and changed their location to the bud neck at late anaphase. Interestingly, the neck localization of Dbf2 and Mob1 is dependent on Cdc14 (22). In this context, it should be noted that release of Cdc15 from the SPB depended on Cdc14 (8). Since the bud neck localization of MEN proteins is restricted to a short period in late anaphase, these proteins must have some special tasks in cytokinesis. Uncovering substrate(s) of these protein kinases will give us a clue for their roles in cytokinesis. The end of mitosis and cytokinesis are temporary close events. Fission yeast has a set of homologous genes of MEN of budding yeast. In the former yeast, these genes function exclusively at cytokinesis but in the latter yeast they play essential role in mitosis and at cytokinesis. Comparative studies of the function of these genes in two organisms will be a promising approach to understanding the mechanism connecting mitosis and cytokinesis.

Concluding remarks

Mitosis is a mechanism to partition the replicated genetic material to the daughter cells in eukaryotes. In mitosis, a series of events must proceed in order. To regulate this process, eukaryotic cells developed the surveillance system called spindle checkpoint. Budding yeast has two spindle checkpoint pathways; the Mad2 pathway which monitors spindle-kinetochore interaction and the Bub2 pathway which monitors cytoplasmic microtubules. The target of the Mad2 pathway is well defined, whereas it is still elusive how the Bub2 pathway passes a signal to the downstream effector although the output is known to be the release of Cdc14 from the nucleolus. Tem1 and Cdc15, key players of the Bub2 pathway, localize to the SPB throughout the cell cycle and in late anaphase, respectively, and may generate a signal to be transduced to downstream effector(s). Release of Cdc14 from the nucleolus is dependent on Tem1 and on other members of MEN (73). Interestingly, Cdc15 is dephosphorylated by Cdc14 and becomes a more efficient mitotic activator (33). A dephosphorylated form of Cdc15 showed no change in enzyme activity but bypassed the temperature-sensitivity of *tem1-3*, *dbf2-2*, and *cdc5-1*, but not that of *cdc14-1*. It is of interest to know how the MEN proteins which localize to the SPB transmit the checkpoint signal to the nucleolus.

Mps1 is believed to be the most upstream component of the spindle chekpoint systems (21, 27). Given that activation of Mps1 triggers spindle checkpoint mechanisms, how do cells discriminate signals of the Mad2 pathway from that of the Bub2 pathway? Mps1 binds and phosphorylates Mad1 which activates the Mad2 pathway, whereas no Mps1 substrate executing the Bub2 checkpoint is known. Mob1 binds to Mps1 and is phosphorylated by Mps1 kinase, however, Mob1 does not seem to transmit the signal of the Bub2 checkpoint. Rather, Mob1 plays a role in a late stage of mitosis with Dbf2. Nonetheless, the presence of two pathways of spindle checkpoint doubly ensures the correct partitioning of replicated chromosomes.

Many genes belonging to the spindle checkpoint pathways are conserved in the eukaryotes (11, 16, 35, 36, 39, 46, 55, 67). Interestingly, checkpoint genes of animals, at least some, are essential (16, 36). In contrast, none of the yeast counterparts are essential. A defect in the spindle checkpoint causes a chromosomal instability, thereby inducing cancer. Cahill *et al.* (7) showed that some cancers displaying chromosomal instability were associated with mutations in the *BUB1* gene. The molecular mechanism underlying the spindle checkpoint dissected using yeast will contribute to understanding the development of cancers.

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