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The Spindle Assembly Checkpoint and Its Defects in Human Cancer

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ABSTRACT Chromosome segregation is one of the most complex, conserved and astonishingly accurate processes whose inner molecular details have just beginning to be understood. These events are controlled by the spindle assembly checkpoint, which is mediated by the Bub-Mad pathway and finally Ub-mediated degradation of the regulators of sister chromatid cohesion by APC/C. This pathway also bifurcates into two – one is the Mad1, Mad2, Mad3, Bub1, Bub3 and the other is Bub2, Bfa1 pathway. Recent studies have implicated hMad2, as one of the key regulator of the pathway. Any defect in the pathway is supposed to lead to aneuploidy in tumor cell.

INTRODUCTION

Cell cycle checkpoints play a crucial role in the fidelity of flow of genetic information. They scrutinize and maintain the genomic integrity and monitor the other aspects of cell division. Checkpoints first attempt to correct the deleterious events of cell cycle by making some temporary delay. If successful, it allows the cell to divide normally. Failing to do so, however, it sends the cell to apoptotic pathway for elimination. Malfunction of checkpoints is catastrophic and allows cells to grow without any control. Checkpoints operate mainly at three distinct points in the cell cycle. Before initiating the DNA synthesis proper integrity of the template DNA and the availability of the metabolic pools are checked during the G1/S checkpoints. The G2/M (DNA damage checkpoint) ensures the correctness of copy writing of chromosomes and the spindle assembly checkpoint ensures the proper and equal distribution of the condensed genetic information into two daughter cells (Novak et al.1998). Any defect in these checkpoints will cause genomic instability or loss of genomic information in the daughter cells and thus aberrant cells will be generated. The spindle assembly checkpoint is controlled by the Bub-Mad pathway and finally ubiquitin (Ub) - mediated degradation of regulators of sister chromatid cohesion by APC/C (Amon 1999). This checkpoint also bifurcates into two distinct pathways – one involves the *Mad1*, *Mad2*, *Mad3*, *Bub1*, *BubR*1and *Bub3* gene products and the other uses *Bub2* and *Bfa1* gene products, of which the first one bears the main responsibility to halt the cell cycle upon spindle damage. A number of recent studies reveal that *Mad2* is the key regulator of this checkpoint and plays the central role. This review examines how the protein components in these pathways interact to each other and finally pause, but not stop the cell cycle to allow time to re-attach the spindle to kinetochore or repair the damaged spindle.

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THE SPINDLE ASSEMBLY CHECKPOINT

On completion of the repair processes by the repair machinery of the cell, G2/M checkpoint is automatically inactivated due to lack of DNA damage signal. Already duplicated centrosomes separate and give rise two mitotic spindle poles that organize the mitotic apparatus and cells enter into mitosis. At prophase, the whole genome condenses into numbers of chromosomes (specific for each organism) and they start gathering on the metaphase plate .Two spindle pole bodies emanates mitotic microtubule called spindle and the chromosomes start attaching with them through highly specialized region known as kinetochore. This correct attachment of all kinetochores to microtubule is crucial before sister chromatid-separation. Otherwise, unattached kinetochore will give rise to aneuploidy or chromosome loss in daughter cells.

The spindle assembly checkpoint monitors the proper attachment of all chromosomes and halts the onset of anaphase sensing any unattached kinetochore. Various components of the spindle assembly checkpoint and their probable functions are given in Table 1. The mechanism, by which the checkpoint senses and captures the unattached kinetochore, repairs them by halting the cell cycle progression and then triggers the mitotic exit is beginning to be understood.

THE SENSORY MECHANISM

Two models have been proposed for sensing the unattached kinetochore, (a) absence of tension at kinetochore and (b) the existence of unattached kinetochore (Somasundaram 2000). Model (a) is supported by the work of Li and Nicllas in mantis spermatocytes (Li and Nicllas 1995). In these cells a monoattached bivalent prevents progression into anaphase. When tension is applied from the unattached side of chromosomes with a micro-needle, cells proceed into anaphase. The lack of kinetochore microtubule attachment is characterized by reactivity to a monoclonal antibody, 3F3/2 (a phosphoantigen). When tension is applied with a micro-needle it eliminates the binding of the antibody; which reveals that the lack of physical tension is translated into a chemical signal that

Table 1: Spindle Checkpoint components and their complexes

Components	*Structural Features*	*Expression level*	*Comments*
Mad1	Coiled-coil	Not cell cycle regulated	Recruits Mad2 at kinetochores, bringing from cytoplasm to nucleus.
Mad2	Horma domain	Not cell cycle regulated	Binds Mad1 and Cdc20
BubR1/Mad3	Serine-Threonine kinase	Not cell cycle regulated	Binds Bub3, capable of autophosphorilation. Yeast Mad3 lacks the kinase domain
Bub1	Serine-Threonine kinase	Not cell cycle regulated	Reported substrates include BUb3, Mad1 and adenomatous poliposis coli.
Bub3	Seven WD40 repeats	Not cell cycle regulated	Interacts with Bub3-binding domain in Bub1 and BubR1
Mps1	Dual-specificity kinase	Cell cycle regulated	Recruits checkpoint proteins at kinetochores; Reported substrates include Mad1, Spc110; Also required for spindle pole-body-duplication in budding yeast.
CENP-E	Kinesin-like plus-end-microtubule directed motor	Cell cycle regulated	Substrate of MAP kinase. Interact with and BubR1
Ipl1	Aurora kinase	—	Sense Tension at kinetochores and ensure bipolar orientation.
Zw10	—	—	Complexes to Rod and localize dynein at kinetochore
Rod	—	—	Complexes to Zw10 and localize dynein at kinetochore
Mitogen Activated protein kinase	Kinase	Cell cycle regulated	Many types of signal-transduction, activates p90RSK, which in tern activatesBub1 during Xenopus oocyte maturation.

Complexes	*Comments*
Mad1-Mad2	Locate at nuclear pore complex in interphase. Very stable except at kinetochores, where it might be turned into a high-turnover complex
Mad2-CDC20	Forms invitro using purified components, but invivo complex formation need Mad1. Inhibits ability of Cdc20 to activate APC.
Bub3-Bub1, Bub3-	Bub3 might form constitutive interactions with Bub1 and BubR1/Mad3. Bub3 purifies with BubR1, BubR1/Mad3 suggesting that the complex is stable.
Mad2-Cdc20-BubR1/Mad3-Bub3	In budding yeast and HeLa cells it may be crucial for checkpoint inhibition by APC.
BubR1-CENP-E	Stoichiometric complex and possible link between attachment machinery to the spindle checkpoint.
BubR1-CDC20	Forms in vitro with purified components, inhibits the ability of Cdc20 to activate APC.
Zw10-Rod	Direct interaction is not yet characterized, but these two purify in a complex.

leads to phosphorylation of kinetochore-associated proteins, and the related signal transduction. However, the loss of tension is not sufficient to localize the spindle checkpoint components Mad2 and Bub3 (Somasundaram 2000). On the other hand support for the second model came from the fact that upon treatment of cells with a microtubule-depolymerizing drug, taxol, loss of tension at kinetochore occurs but it does not lead to any apparent problem to microtubule-kinetochore attachment (Amon 1999). The phosphoantigen 3F3/2 localizes to all kinetochores in taxol-treated cells, but the signaling elements Mad2 and Bub3 only localize to kinetochores that have not attached to microtubules (Gorbsky and Ricketts1993.). This explanation is also supported by the work of Martinez-Exposito (Martinez-Exposito1999). They showed that Bub3 is present at low levels on the kinetochores of normal metaphase chromosomes but it accumulates at high level on the kinetochores of lagging chromosomes that are not under tension. Actually it appears that both the pathways are necessary and no one is sufficient for the complete signaling. This signal transudation pathway is coupled to motor machinery that is involved in the movement of the daughter chromatids. CENP-E, a kinesin like plus end directed motor has been implicated in chromosome congression and metaphase alignment, and more recently in checkpoint signaling. (Chan et al. 1998; Chan et al. 1999; Yao et al. 2000; Abrieu et al. 2000). It forms a stoichiometric complex with BubR1 in HeLa cells, which provided the first link between microtubule attachment and the spindle checkpoint machinery (Yao et al. 2000).

THE KEY STEPS

The sensory mechanism starts the 'wait anaphase' signal from an unattached kinetochore and it triggers the accumulation of the checkpoint components that comprises the Bub-Mad families of proteins. Mps1 phosphorylates Mad1 and the corresponding structural changes enable it to bind Mad2 in cytoplasm (Sironi et al. 2002). Afterwards, this complex is transported to nucleus and is recruited to kinetochore by the assistance of other checkpoint components (Iwanaga et al. 2002). This starts the temporal delay in the cell cycle progression and allows more time to reattach the spindle to unattached kinetochore. Subsequently spindle attachment replaces the checkpoint complexes and Mad1-Mad2 complex is broken down to liberate free Mad2 to activate Cdc20 (Chung et al. 2002). The Mad2-Cdc20 activates the APC/C, an ubiquitin ligase protein complex and Cdc20 remained in complex with activated APC/C (Musacchio et al. 2002). This Cdc20 bound activated APC/C complex ubiquitinates securin of the securin-separase complex liberating free separase. Free separase is next activated upon phosphorylation by polo kinase and degrades cohesin, the glue like proteins that kept the sister chromatids together. Finally, the pull towards the pole starts the sister separation and subsequent anaphase transition (Fig.1).

Bub1 and Mps1 function upstream of all other signaling components and their function are interdependent. It is thought that Mad2 is signalling either of the two phenomena. It is tempting to speculate that CENP-E could be a tension-sensing molecule because kinesin-like proteins undergo microtubule-dependent conformational changes that lead to the production of force. Conversely, it is plausible that the application of tensile forces on a motor protein could cause it to undergo conformational changes that allosterically affect its ability to interact with other proteins that are involved in checkpoint signaling. (McEwen et al. 2001).

ROLE OF KINASES IN CHECKPOINT SIGNALING

Thus, two types of signals exist in higher eukaryotes that activate the spindle checkpoint. Similarly, at least two complementary pathways of spindle checkpoint exist by which it pauses the cell cycle temporarily. (Fig. 2). Protein phosphorylation plays an important role in transmitting these signals. Genetic studies in budding yeast identified two protein kinases MPS1 and Bub1. MPS1 specifically phosphorylates Mad1 in response to activation of spindle checkpoint in a Bub1, Bub3 and Mad2 dependent but Mad3 and Bub2 independent manner (Somasundaram 2000). This suggests that Bub1, Bub3 and Mad2 function upstream and Bub2, Mad3 function downstream or parallel to phospho-Mad1 pathway in the signaling cascade (Somasundaram 2000.). But how Bub1, BubR1, Bub3, and other proteins work in this

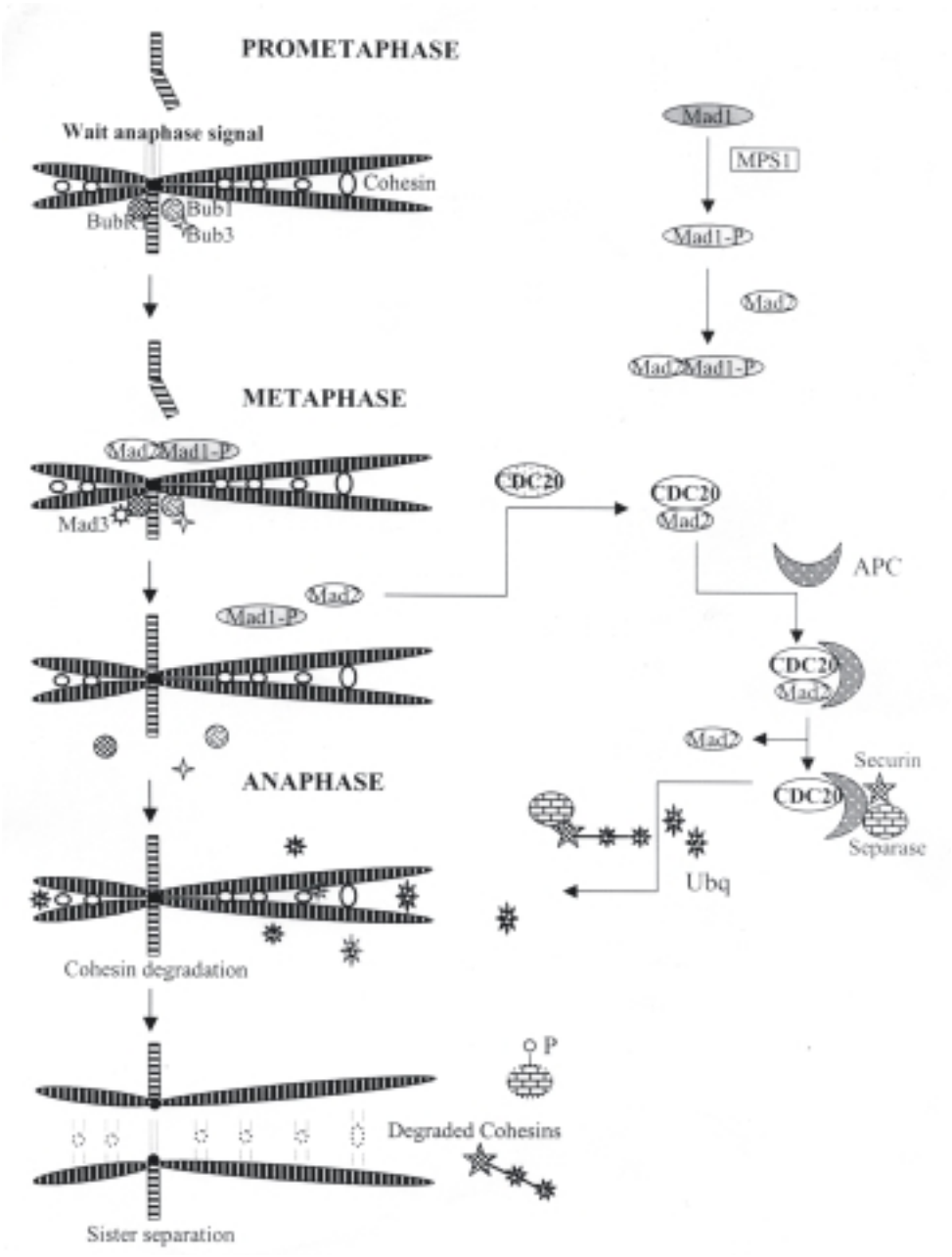

Fig.1. Key steps in the regulation of mitotic progression
At prometaphase two copies of the homologous chromosomes are hold together by complexes of proteins known as cohesins. At the onset of anaphase this protein complexes are disrupted and the components are degraded by ubiqutin mediated proteolysis. Phosphorylated Mad1 binds Mad2 and carry it from cytoplasm to kinetochore in the nucleus in response to unattached spindle. Upon reattachment of spindle Mad2 and other components are set free. Mad2-CDC20 complex then activates the APC/C complex. It triggers securing ubiquitination, separase activation, and finally degradation of cohesins, thus leads to metaphase anaphase transition.

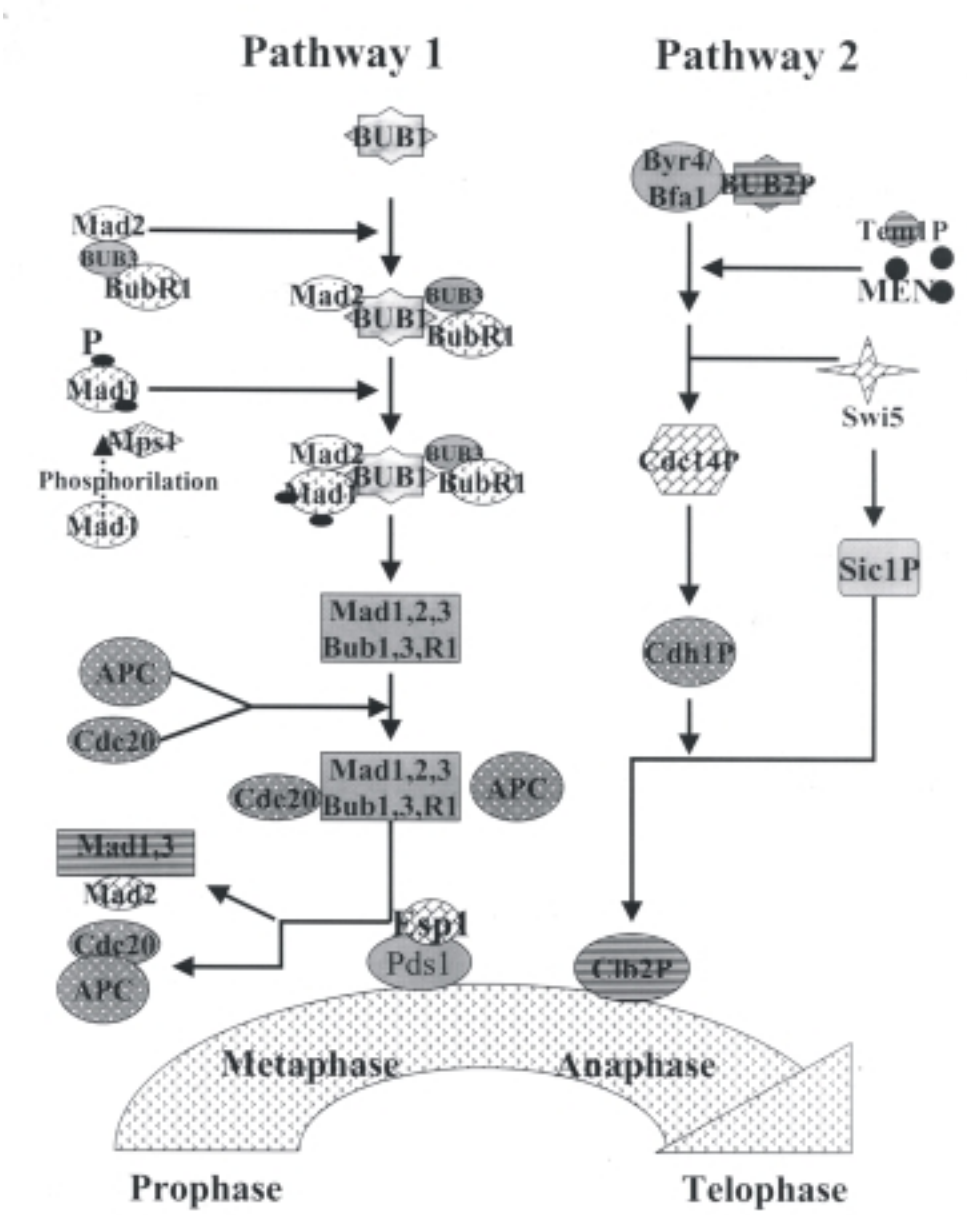

Fig.2. Bifurcation of the Spindle Assembly Checkpoint and the Genetic Interactions
Two parallel pathways control the spindle checkpoint. There are two distinct sets of gene products (Bub, Mad and other) that interact in these two pathways. The Bub1, Bub3, BubR1, Mad1, Mad2, Mad3, Mps1 pathway finally control the checkpoint by Pds1/Sp1 complex. On the other hand Bub2, Bfa4, Byr1control by Clb2. These two pathways are complementary, but they are activated and operated by different set of gene products.

process to activate Cdc20? It is known that Mad1-Mad2 forms a complex with other checkpoint proteins such as Bub1, BubR1, Bub3 etc but exact nature of these interactions are poorly understood. But it is suspected that they are operating through some phosphorylation-dephosphorylation switch. Though Bub1 and BubR1 are a part of a common complex, BubR1 is phosphorylated in absence of spindle damage where as Bub1 is phosphorylated in response to spindle damage. In fact Bub1 shows phosphorylation to some extent in every cell, and bind kinetochore when it enters mitosis. Subsequently it is dephosphorylated automatically on spindle attachment during cell cycle progression and detaches from kinetochore. However, BubR1 remains bound as it is activated in response to change in tension (Taylor et al. 2002; Chen 2002).

MAD2, THE KEY ELEMENT

Mad2 have extremely dynamic role in the spindle assembly checkpoint, because it is the component of both the 'wait anaphase' signal and the catalytic machinery that is required to generate this signal. Two related interactions of Mad2 are with Cdc20 and Mad1. Mad1 and Mad2 form a tight complex that is essential for spindle checkpoint. This complex plays a crucial role in inhibition of APC activity. Localization of Mad2 to kinetochore depends on microtubule attachment, not on the tension signal (Waters et al. 1998) .It is reported that Mad2 associates transiently with APC/Cdc20 complex during mitosis and prevents the degradation of inhibitors of mitotic exit machinery (Li and Benezera 1996; Wassmann and Benezra 1998). Chen et al showed that both free Mad2 and Mad1 bound Mad2 are necessary for proper spindle checkpoint to operate (Chung and Chen 2002). In his model he showed that N-terminal of Mad1 binds to kinetochore and C-terminal binds to Mad2 to give 1:1 complex. This data was further supported by an independent work of Jeang et al. who showed that 2 Leucine zipper domain situated at the C-terminal of Mad1 are crucial for Mad2 binding and an internal NLS sequence in Mad1 direct the Mad1-Mad2 complex to the nucleus, where the complex binds with unattached kinetochore and checkpoint is activated (Iwanaga et al. 2002). These findings establish that Mad1 and CDC20 are competitive Mad2 ligands and identify Mad1 as both a positive regulator and a competitive inhibitor of the Mad2-Cdc20 complex. This contradictory role might be explained by the hypothesis that Mad1 bound Mad2 is the only source of Mad2, available for Cdc20 binding, despite the free Mad2 pool of cytoplasm (Chung and Chen 2002). The remarkable stability of Mad1-Mad2 might prevent Mad2 from reaching Cdc20 when checkpoint is inactivated (Chen et al. 1998; Jin et al.1998; Chen et al.1999; Sironi et al. 2001). Kinetochore localized assembly factors could recognize the Mad1-Mad2 complex and favor subsequent Mad2 exchange onto Cdc20. The Bub1-Bub3 complex for instance, forms a complex with Mad1, disruption of which abrogates the checkpoint (Chung and Chen 2002). Thus a Mad1-Mad2 complex has different fates depending on the state of the checkpoint. A low turnover of cytosolic Mad1-Mad2 complex could be recruited to kinetochores and turned into a high turnover complex that releases Mad2 to Cdc20, while replenishing itself with Mad2 from a cytosolic pool. A conformational change in the tetrameric assembly might be required for Mad2 release (Sironi et al. 2002), and it is possible that a phosphorylation-dephosphorylation switch regulates this transition. Human Mad2 is phosphorylated during mitosis (Pines 2002). Mps1 phosphorylates Mad1 in budding yeast (Hardwick 1996), whereas hMad1 is a substrate of hBub1 in vitro. Mitotic phosphorylation of hMad1 and XMad1 has not been observed but this might be due to the transient nature of the modification. As an alternative mechanism, it has been suggested that oligomerization of Mad2 is important for the checkpoint function (Fang et al. 1998), but recent evidences has questioned this. (Sironi et al. 2002).

DESTRUCTION OF THE WAIT ANAPHASE SIGNAL

Overall the pathway discussed above responds when chromosome detachment occurs. But how is the inhibition of the APC relieved? In a dynein-mediated pathway (not discussed here) checkpoint proteins are moved from kinetochore to spindle poles, which is one possible mechanism of preventing the production of APC inhibitory complexes. In addition several Mad2 complexes have been reported to be disassembled before mitotic exit (Ikui et al. 2002; Wassmann and Benezra 1998) and irreversible

APC activation might ensue after the release of BubR1-Bub3 and Mad2. Cdc20/APC then ubiquitinates Pds1/Cut2 leading to its destruction of cohesions and cells are allowed to pass through anaphase. (Somasundaram 2000). Increased affinity of mitotic APC for CDC20, possibly as a consequence of APC or Cdc20 phosphorylation is also important for the recruitment step. After the recovery of ideal conditions, Bub2/Byr1/Bfa1 pathway is also inactivated and cytokinesis starts after completion of chromosome segregation (Rong 1999).

CHECKPOINT DEFECTS AND TUMOR PROGRESSION

Most human cancers are characterized by genomic instability and many of them develope defects in cell cycle control as part of the process of multistep carcinogenesis. One of the hallmarks of cancer cells is aneuploidy. Any subtle defect in spindle checkpoint, i.e mutation in any of its key regulators may lead to such aneuploidy in developing tumour cells. The types of chromosome instability and genome rearrangements found in yeast are also found in cancer cells leading to the speculation that they arise through similar mechanisms. Determination of mitotic index in breast and colorectal cancer cell lines to assay the efficiency of mitotic arrest in response to spindle damage reveals inefficient spindle checkpoint function (Iwanaga et al. 2002). In budding yeast, the Mad and Bub proteins do not contribute equally to chromosome segregation fidelity. Deletion of either *Bub1* or *Bub3* has the greatest effect and these two proteins might have roles in chromosome segregation in addition to checkpoint function. Although the spindle checkpoint is not essential for growth in yeast, two reported knockout mouse (Mad2 and Bub3) show less embryonic viability and frequent tumorigenesis (Dobles 2000; Kalitsis 2000). After 5-6 days these embryos accumulate mitotic errors and undergo apoptosis. Additionally, as already mentioned, there is a cytosolic pool of free Mad2, which might be important to maintain a high turnover rate of Mad2 at the spindle, because its decrease has been shown to impair the checkpoint (Chung and Chen 2002). Importance of the maintenance of critical level of Mad2 in the cell was also revealed from the fact that $Mad2^{+/-}$ mice develop lung cancer at high rates after long latencies, connecting defects in spindle checkpoint and tumorigenesis (Michel and Banezra 2001.). Finally mutations in *Bub1, BubR1, Bub3, Mad1, Mad2,* genes have been reported in various types of cancers albeit in lower frequency (Nomoto and Takahasi 1999; Takahasi et al. 1999; Cahill et al. 1998; Reis 2001).

IMPORTANCE AND FUTURE DIRECTIONS

Starting from the last decade a large body of information on key molecules of spindle assembly checkpoint has been accumulated. However, a great deal remains to be understood on the molecular basis of the signaling mechanisms operating at spindle assembly checkpoint. The spindle assembly checkpoint ensures the proper and equal distribution of the condensed genetic information into two daughters. Any defect in this checkpoint will allow genomic instability or loss of genomic information leading to generation of transformed cells. Knowledge on the defects of spindle assembly checkpoint genes in various cancers is just beginning to be unfolded. Thus, future research will be directed towards understanding the signaling mechanisms in more detail using powerful techniques of florescence microscopy and biochemical reconstitution experiments and various genomic techniques will be used to decipher the molecular defects in the spindle check point genes in various types of tumours.

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