Mitotic Checkpoints, Genetic Instability, and Cancer

M. DOBLES AND P.K. SORGER

Massachusetts Institute of Technology, Department of Biology, Cambridge, Massachusetts 02139

Accurate propagation of the genome is a key event in cell proliferation. Errors in DNA metabolism such as incomplete DNA repair and inappropriate recombination are known to contribute to human cancer (de Klein et al. 1982; Leach et al. 1993; Parsons et al. 1993). We are interested in determining the tumorogenic effects of errors in the microtubule-based processes that physically separate chromosomes during mitosis, a process we refer to as chromosome segregation. The idea that inaccurate segregation promotes cancer is not new. The vast majority of tumor cells are aneuploid (Hartwell and Kastan 1994) and many colorectal cancer cells, when grown in vitro, are characterized by unstable chromosome number (Lengauer et al. 1997). One way that chromosome instability may contribute to cancer is by increasing the frequency at which recessive mutations in tumor suppressor genes are uncovered. Despite the appeal of this idea, it has as yet little experimental support, in part because the molecular mechanisms of chromosome segregation are much less well understood than those of DNA metabolism.

Mechanistically, we can distinguish several different ways in which chromosome number can be altered, including re-replication without mitosis, failure to assemble a bipolar spindle, and incomplete chromosome-microtubule attachment. We are particularly interested in the latter, because it is monitored by a spindle checkpoint that appears to have a critical role in linking chromosome movement to the Cdc2-driven cell cycle (Hoyt et al. 1991; Li and Murray 1991; Rieder et al. 1994). This mitotic surveillance system is sensitive to the state of chromosome-microtubule linkage and functions to prevent the initiation of anaphase if even a single chromosome has failed to achieve correct bivalent linkage to the spindle (Li and Nicklas 1995; Rieder et al. 1995). Mutations in spindle checkpoint components are known to cause chromosome instability in a variety of organisms (Bernard et al. 1998; Basu et al. 1999; Kitagawa and Rose 1999; Dobles et al. 2000), and they have been found in at least some human tumors (Cahill et al. 1998). Because spindle checkpoint proteins mediate the response of cells to antimicrotubule drugs, their deletion also makes cells hypersensitive to drugs that interfere with microtubule function, drugs that include the important human chemotherapeutics paclitaxel and the vinca alkaloids. Our current research is focused on trying to understand the connections between spindle checkpoint proteins, chromosome instability, and oncogenic transformation in mouse and human cells.

Despite the obvious molecular differences between the processes that sense and respond to errors in DNA metabolism and errors in chromosome segregation, several lines of evidence suggest that DNA damage and mis-segregation are linked in cells. First, Drosophila carrying a deletion of the spindle checkpoint gene bub1 accumulate DNA damage and chromosome breaks (Basu et al. 1999). Second, antimicrotubule drugs that interfere with chromosome segregation in mammalian cells induce p53-dependent cell cycle arrest that appears to be linked to the accumulation of DNA damage (Lanni and Jacks 1998). Third, mutations in the DNA repair gene Brca2 synergize with mutations in bub1 to transform mouse cells (Lee et al. 1999). These results suggest that lesions in the microtubule-based processes that function during mitosis to move chromosomes cause not only loss or gain of whole chromosomes, but also DNA damage that probably consists of double-strand breaks generated by the physical shearing of DNA.

In this paper, we discuss the checkpoint mechanisms in mammalian cells that ensure the accuracy of chromosome segregation by linking chromosome-microtubule attachment to division, and we inquire whether checkpoint mutations have a role in tumor progression, with emphasis on some of our own experiments. We are particularly interested in answers to the following: What are the mechanisms by which cells ensure accurate segregation of chromosomes at mitosis? What mutations in cancer cells give rise to chromosome instability, and is instability tumor-promoting? Are some of the consequences of chromosome missegregation caused by DNA damage?

THE SPINDLE ASSEMBLY CHECKPOINT

Accurate chromosome segregation can occur only if all chromosomes achieve a state of bivalent attachment prior to the onset of anaphase. One of the two sisters in each pair of chromatids must attach to microtubules emanating from one spindle pole and the other sister must attach to microtubules from the other pole. Attachment is mediated by kinetochores, DNA-protein complexes that assemble at centromeres. Bivalent attachment ensures that the two sets of sisters move to opposite poles at anaphase and thus that each daughter cell inherits a complete copy of the genome (Nicklas 1997). The presence of even a single kinetochore unattached to microtubules is sufficient to activate the checkpoint and arrest a cell in mitosis (Rieder et al. 1995).

A pathway linking anaphase initiation to bivalent chromosome attachment must have three components: a sensor for chromosome-microtubule linkage, a signal transmission system, and an output onto the cell cycle...
machinery that induces cell cycle arrest. The genes involved in the spindle checkpoint were first identified in the budding yeast *Saccharomyces cerevisiae* and include *MAD1-3* (mitotic-arrest-deficient), *BUB1-3* (budding uninhibited by benzimidazole), *MPS1*, and several other genes (Hoyt et al. 1991; Li and Murray 1991; Weiss and Winey 1996). All of these genes, with the exception of *BUB2*, appear to function in a common pathway acting at the metaphase to anaphase transition (Hardwick et al. 1996; Alexandru et al. 1999). *BUB2* functions in a second pathway that controls mitotic exit (Alexandru et al. 1999; Fraschini et al. 1999; Li 1999). Regulation of the cell cycle by the mitotic checkpoint (the *MAD-BUB* pathway) appears to involve the binding of Mad2 (and possibly other checkpoint proteins) to Cdc20 (Li et al. 1997; Fang et al. 1998; Hwang et al. 1998; Kim et al. 1998; Wassmann and Benezra 1998; Chan et al. 1999; Hardwick et al. 2000). Cdc20 is an essential activator of the anaphase promoting complex (APC), a multiprotein ubiquitin conjugating complex that targets key cell cycle proteins for proteolytic destruction (Visintin et al. 1997; Shirayama et al. 1998). The binding of Mad2 to Cdc20-APC inhibits the degradation of proteins such as securins whose destruction is necessary for the initiation of anaphase and mitotic exit (Cohen-Fix et al. 1996; Yamamoto et al. 1996; Alexandru et al. 1999).

The mitotic checkpoint appears to sense incorrect spindle formation by monitoring kinetochore-microtubule binding. In a cell arrested in mitosis by the presence of a single unattached chromosome, laser ablation of the unattached kinetochore releases the cell from arrest (Rieder et al. 1995). Many checkpoint proteins, including Mad1, Mad2, Bub1, Bub1R, and Bub3, localize specifically to unattached kinetochores (Li and Benezra 1996; Taylor and McKeon 1997; Chan et al. 1998; Jin et al. 1998; Martinez-Exposito et al. 1999). Our lab has studied Bub3p, a WD-domain protein that binds to the Bub1/R1 protein kinases. We find that Bub3 is present on all kinetochores in HeLa and 3T3 cells during prophase before microtubule-chromosome attachment occurs. About 60–70% of Bub3 is released once microtubules have attached, but a significant amount remains bound to the kinetochore until the end of anaphase. When cells in metaphase are treated with nocodazole to depolymerize microtubules, Bub3 accumulates at very high levels on all kinetochores (Martinez-Exposito et al. 1999). Thus, the association and dissociation of checkpoint proteins on kinetochores is reversible and is sensitive to the state of microtubule attachment.

It is not clear what molecular event is being monitored by the checkpoint. One possibility is that the checkpoint simply senses whether or not microtubule-binding sites on a kinetochore are fully occupied. A second possibility is that the checkpoint monitors the tension imposed on bi-valently attached sister kinetochores by opposing microtubule-dependent pulling forces. Support for the idea that tension plays a part in checkpoint signaling derives from classic experiments in which cells arrested in metaphase with an unattached kinetochore can be induced to reenter the cell cycle by imposing tension on the maloriented chromosome with a microneedle (this experiment has been possible only in meiotic praying mantid cells) (Li and Nicklas 1995). In addition, we and other investigators have found that bivalently attached yeast and mammalian chromatids become significantly stretched by microtubule-dependent forces and that these forces are strong enough to substantially deform chromatin (Fig. 1). However, whereas Bub3 accumulates at high levels on kinetochores when spindle microtubules are depolymerized, the elimination of spindle tension by acute taxol treatment (which stabilizes microtubules and eliminates tension on kinetochores) does not cause a similar rise in kinetochore-associated Bub3 (Martinez-Exposito et al. 1999). This shows that a major determinant of the amount of Bub3 at kinetochores is the extent of microtubule attachment, but it does not imply that Bub3 is not also involved in a tension-sensitive checkpoint signal.

The relevance for checkpoint biology of treating cells with high levels of nocodazole is unclear. Mammalian cells do not normally experience conditions that would lead to complete microtubule depolymerization, and the mitotic checkpoint is unlikely to have evolved to deal with this artificially extreme state. We have therefore examined Bub3 localization in cells with one or two mal-oriented chromosomes but relatively normal microtubule organization. This can be accomplished by treating cells with very low doses of taxol (or nocodazole) for approximately 18 hours to subtly impair microtubule dynamics. In these cells, we found that the great majority of chromosomes were bivalently attached, but some were unable to congress to the metaphase plate and thus remained misaligned. High Bub3 levels were present on the kinetochores of these lagging chromosomes and, in cases in which a chromosome was mono-oriented, high levels of Bub3 were found selectively on the unattached kinetochore. Quantitative analysis of a series of lagging chromosomes suggested that, as the number of kinetochore-bound microtubules increases, the levels of kinetochore-bound Bub3 fall (Martinez-Exposito et al. 1999). These findings strongly support the idea that Bub3 is involved in generating or transducing a signal that is sensitive to the state of kinetochore-microtubule binding. How the signal is transmitted to Mad2 to inhibit APC is not yet known, but it almost certainly involves a cascade of the Bub1/R1 and Mps1 kinases (Hardwick et al. 1996).

**ESSENTIAL NATURE OF THE SPINDLE CHECKPOINT**

The classical view of checkpoints is that they are nonessential and are activated only in response to the occasional problem (Hartwell and Weinert 1989). This view is consistent with the finding that mitotic checkpoint genes are dispensable for normal growth in *S. cerevisiae* and become essential only when microtubule function is impaired by mutation or drug treatment (Hoyt et al. 1991; Li and Murray 1991). However, the localization of checkpoint proteins to kinetochores during prophase in normally growing animal cells suggests that the checkpoint may function during all mitoses and not only when errors have occurred in spindle assembly. Indeed, recent exper-
iments in *Schizosaccharomyces pombe* and several animal species have demonstrated a very high rate of chromosome missegregation in cells lacking checkpoint function (Fig. 2) (Bernard et al. 1998; Basu et al. 1999; Kitagawa and Rose 1999; Dobles et al. 2000). We have shown recently that knocking out the *mad2* gene in mice causes

Figure 1. Chromatin stretching at centromeres of bivalently oriented chromosomes in HeLa and *S. cerevisiae* cells. (a) Plot of Bub3 signal intensity relative to kinetochore-to-kinetochore distance for an untreated prophase HeLa cell (green points), a metaphase cell (blue points), and a cell with metaphase morphology treated for 30 min with 10 µm of taxol (red points). Peak signal intensities in the Bub3p channel are reported without background correction. (b) HeLa cell treated with 20 nM taxol for 16 hr showing lagging chromosomes in which only one kinetochore has attached to microtubules. A lagging chromosome is circled, and the kinetochore-to-kinetochore distance is indicated by the arrow. Chromosomes stain blue, microtubules red, and Bub3 yellow (a pseudo-color for FITC). (c) Metaphase *S. cerevisiae* cells carrying an Spc42-GFP tag marking the spindle poles (P) and a chromatin-targeted GFP tag 2 kb (~2kbCHIV Tag) or 38 kb (~38ChV Tag) from the centromere. Distances between sister chromatids are indicated. The separation of the chromosome tags reflects a transient sister separation in metaphase and is a consequence of microtubule-imposed tension. (d) Plot of distance between each sister chromatid and the reference spindle pole body for a cell with a ~2ChIV Tag. A single green line is shown when the sisters are together and two lines when they are separated. Spindle length is shown above in the darker line. This movie begins just after the spindle poles have separated and ends approximately 10 min prior to anaphase. (a,b, Adapted, with permission, from Martinez-Exposito et. al. 1999 [copyright National Academy of Sciences]; c,d, adapted, with permission, from He et. al. 2000 [copyright Cell Press].)
The disruption of mitotic checkpoints causes early mitotic exit because the disruptions are lethal. However, the timing of mitosis has been examined in tissue culture cells injected with anti-Mad2 antibodies and in cells expressing dominant-negative fragments of Bub1. In both cases, the duration of mitosis is shorter and chromosome missegregation is increased (Taylor and McKeon 1997; Gorbsky et al. 1998). Careful analysis of the time between the final chromosome-microtubule attachment and the onset of anaphase in PtK1 cells suggests the existence of two controls over the duration of mitosis: an intrinsic timing control (presumably dependent on the Cdc2-driven cell cycle engine) and an additional control imposed by the mitotic checkpoint. The first control appears to provide sufficient time for most chromosomes to attach to microtubules, but without the second control, there exists a high probability that some chromosomes will remain unattached when anaphase starts and will not be properly segregated (Rieder et al. 1994). This view fits with our general understanding of the mitotic spindle as a structure that does not assemble in a fixed order but rather through a series of stochastic processes in which only correctly assembled structures are stable and are able to support correct chromosome partitioning (Hyman and Karsenti 1996).

CHROMOSOME MISSEGREGATION AND DNA DAMAGE

What is the nature of the defect that causes cells lacking a functional mitotic checkpoint to die? One possibility is that problems with chromosome segregation induce DNA damage and that cell cycle arrest and apoptosis are mediated by DNA damage response pathways. Such damage could arise through physical breakage of chromosomes if anaphase occurs in the absence of sister separation or through cleavage by a closing cytokinetic furrow. The most direct evidence that disruption of the mitotic checkpoint might cause DNA damage is that deletion of Drosophila bub1 results in extensive chromosome breakage as well as chromosome loss or gain and eventually apoptosis (Basu et al. 1999). A second piece of evidence linking chromosome missegregation and DNA damage is that treating primary mouse fibroblasts with nocodazole to depolymerize microtubules causes the cells to arrest transiently in mitosis and to then slip out of mitosis and enter a prolonged G1-like arrest (Lanni and Jacks 1998). This G1-like arrest and the subsequent apoptosis are p53-dependent, consistent with the idea that they are induced by DNA damage.

A third link between chromosome segregation and DNA damage is the surprising finding that at least some responses to DNA damage are impaired in cells expressing mutant mitotic checkpoint proteins (Lee et al. 1999). Brca2 mutation, which causes DNA damage to accumulate (see Venkitaraman, this volume), induces cell cycle arrest in cells that can be overcome by introducing a dominant-negative version of Bub1. The molecular mechanisms underlying this bypass are not known, but similar links have been observed in other organisms.

Figure 2. Analysis of chromosome missegregation in Mad2−/− cells. Analysis of chromosome segregation in E6.5-7.5 embryos. Embryos were fixed, embedded, sectioned, stained with Hoechst 33342, and examined by confocal microscopy. Abnormal embryos, with a presumptive Mad2−/− genotype, were distinguished from presumptive wild-type and Mad2+/− embryos by overall size and morphology in H/E-stained sections. (a) Quantitative analysis of chromosome missegregation. The fraction of mitotic cells in anaphase that exhibited a defect in segregation, such as a lagging chromosome (see b) is shown. Each data point is derived from an analysis of at least three embryos. Error bars represent one standard deviation. (b) Spindle morphologies in cells from presumptive Mad2-null embryos at E6.5. A significant fraction of anaphase cells contained one or more chromosomes clearly separated from the bulk of DNA clustered at the poles. These lagging chromosomes are indicated by arrows. (Adapted, with permission, from Dobles et al. 2000 [copyright Cell Press].)

embryonic lethality at E6–E7 (Dobles et al. 2000). Lethality appears to be a consequence of apoptosis induced by an abnormally high rate of chromosome missegregation. It is not known why chromosome missegregation is lethal in higher eukaryotes and not in S. pombe, but a very likely explanation is that only higher cells undergo apoptosis in response to stress. Nevertheless, in both simple and complex eukaryotes, it appears that the mitotic checkpoint is important for ensuring the accuracy of mitosis under normal growth conditions and is not just a rarely utilized fail-safe system.

In cells carrying mutations in mitotic checkpoint genes, chromosome loss and cell death are thought to occur because anaphase begins before all chromosomes have attached to the spindle. In higher eukaryotes, it has not yet been possible to use checkpoint mutants to test whether
yeast, for example, both DNA-damage-dependent and mitotic checkpoints have been shown to stabilize the securin Pds1 (Tinker-Kulberg and Morgan 1999), a protein that must be degraded to initiate anaphase (Cohen-Fix et al. 1996; Yamamoto et al. 1996).

The possibility that chromosome missegregation causes DNA damage and that the spindle checkpoint responds to that damage suggest a self-reinforcing loop in which double-stranded DNA breaks generated by physical shearing of chromosomes cause inappropriate recombination and large-scale rearrangements. Chromosome rearrangements could in turn produce acentric and dicentric chromosomes that cannot bind correctly to the mitotic spindle and therefore suffer additional physical damage (Fig. 3). To begin to examine genetically the connections between DNA damage and mitotic checkpoints, we are crossing Mad2 and p53 null mice. We predict that if p53-dependent DNA damage response pathways are inducing apoptosis, survival of Mad2 null embryos beyond E7 will be observed when p53 is also absent. A precedent for this is the recent demonstration that the embryonic lethality of XRCC4 deletion can be prevented in mice by introducing a knockout of p53 (Gao et al. 2000). As more mouse strains carrying disruptions of DNA damage or spindle checkpoint genes are created, it will be interesting to explore the effects of double mutants on chromosome segregation and the induction of DNA damage.

CHROMOSOME MISSEGREATION AND CANCER

A classical conundrum in cancer biology is that the natural mutation rate in human cells is too low to generate the large number of genetic changes necessary for the development of cancer. The “Nowell Hypothesis” attempts to resolve this paradox by positing genetic instability as a precondition for cancer (Nowell 1986). In this hypothesis, a cell must first acquire a lesion that causes a high rate of mutation before it can become a cancer cell. Although an absolute requirement for genetic instability in tumorigenesis has not been proven, it is clear that mismatch repair defects do contribute to some human cancers. In cells defective for mismatch repair, incorrectly paired bases arising from errors in DNA replication are left uncorrected, causing a large number of mutations to accumulate. Patients with a mutator phenotype caused by mismatch repair defects have a high probability of accumulating mutations in tumor suppresser genes and oncogenes, and thus of developing colorectal and other cancers (Leach et al. 1993; Parsons et al. 1993).
Despite the strong link between mismatch repair defects and colorectal cancer, only about 15% of colorectal tumors carry mismatch repair defects. Cells from the majority of tumors without mismatch repair defects exhibit aneuploidy, raising the possibility that chromosome mis segregation is another potent form of genetic instability (Kinzler and Vogelstein 1996). In at least some cells, aneuploidy appears to arise from ongoing chromosome mis segregation (Lengauer et al. 1997) and not from a one-time catastrophic event (such as a tetraploid mitosis) (Galliou et al. 1996). Several recent results shed light on the connection between chromosome missegregation and cancer. First, mice heterozygous for a knockout of Mad2 have a significantly increased incidence of lung carcinomas relative to wild-type littermates (Michel et al. 2000). We have not yet determined whether the carcinomas in these mice are associated with loss of the wild-type Mad2 allele or reflect Mad2 haplo-insufficiency. However, loss of just one Mad2 allele in HCT116 human cancer cells has been shown to impair checkpoint function (Michel et al. 2000). We hypothesize that mice heterozygous for a disruption of the Mad2 gene have a higher incidence of cancer because a partial loss of checkpoint function increases chromosome loss and generates a mutator phenotype. We are currently testing this idea by examining whether loss of heterozygosity at tumor suppressor loci is increased in Mad2+/− mice relative to wild-type mice.

A second link between the spindle checkpoint and cancer has been uncovered by examining mice with a truncation of Brca2, a tumor suppressor gene implicated in DNA repair (Scully et al. 1997; Sharan et al. 1997). These mice are characterized by a high incidence of thymic lymphoma. In three of four lymphomas examined, a mutation was found in Bub1, and in the fourth, multiple mutations were present in the related kinase BubR1 (Lee et al. 1999). In cells from Bub1 mutant lymphomas, Bub1 is not localized to kinetochores, and in both Bub1 and BubR1 mutants, cells do not arrest in response to nocodazole treatment. These results provide preliminary evidence that loss of checkpoint function is involved in tumorigenesis in at least some cancers in mice.

Sequencing of mitotic checkpoint genes in human tumors has provided a third, albeit rather tentative, link between cancer and mutations in mitotic checkpoint genes. A screen of colorectal tumors has revealed BUB1 mutations in 2 of 18 tumors analyzed (Cahill et al. 1998). In some breast cancer lines, reduced MAD2L1 expression is observed (Li and Benezra 1996; Percy et al. 2000), and in 10% of ovarian cancer cells, MAD2 protein expression is markedly reduced (A. Loeb et al., in prep.). E.M. Petty’s laboratory has also found that approximately 60% of breast and ovarian cancer cell lines lack normal mitotic checkpoint function, as probed by disrupting spindles with antimicrotubule agents (K.A. Myrie et al., unpubl.). However, mutations in known checkpoint genes such as MAD2L1, BUB1, and BUB1/BUBR1 have not been detected in most breast and ovarian cancer lines analyzed to date (Myrie et al. 2000; Percy et al. 2000).

If disruption of the mitotic checkpoint plays a part in the development of human cancer, why has the analysis of cancer cell lines thus far uncovered few checkpoint mutations? Perhaps the correct types of tumors have not been examined. Mismatch repair mutations are commonly found in only a subset of human tumors, including those of the colon and ovary (Jiricny and Nystrom-Lahti 2000). Alternatively, the correct set of genes may not have been examined. Early work on Cdc2 regulators also failed to identify key cancer genes such as the Ink4 locus (Hussussian et al. 1994). A final possibility is that at least some genes already implicated in cancer may have previously undetected roles in the mitotic checkpoint. This latter possibility is supported by our recent discovery that the adenomatous polyposis coli gene product is localized to kinetochores, forms a complex with Bub1/R1-Bub3, and is a potent Bub1 substrate (the only one identified thus far) (K.B. Kaplan et al., in prep.). Adenomatous polyposis coli is mutated at high frequency in colorectal cancers, including cancers exhibiting chromosome instability. Much is known about the role of adenomatous polyposis coli in β-catenin regulation and Wnt signaling (Su et al. 1993; Nathke 1999), but its potential role in mitosis has not previously been examined. We find that embryonic stem (ES) cells with a truncation of adenomatous polyposis coli have highly unstable chromosome number, raising the interesting possibility that at least some of the tumor-promoting effects of adenomatous polyposis coli mutation are caused by chromosome missegregation.

Implicit in the hypothesis that chromosome missegregation has an important role in cancer is the idea that cells can actually survive a loss of checkpoint function and chromosome instability. This appears to contradict our finding that Mad2 is an essential gene in the mouse (Dobles et al. 2000). However, we hypothesize that mutations that impair the induction of apoptosis can suppress the lethality of mitotic checkpoint mutation. Perhaps this explains the apparent restriction of aneuploidy to tumor cells, which have already accumulated anti-apoptotic mutations. We are currently trying to test this idea by crossing Mad2 mutant mice into backgrounds that are apoptosis-defective (see above).

CONCLUSIONS

Our understanding of the spindle assembly checkpoint in animal cells has increased significantly with the isolation of mammalian homologs of genes first identified in budding yeast. However, the molecular analysis of individual genes has not yet advanced sufficiently to provide mechanistic explanations for classical experiments with micromanipulated chromosomes. We do not yet know the precise lesion that is sensed by the mitotic checkpoint nor whether spindle-imposed tension is involved. On an organismic level, we are just starting to investigate the link between chromosome missegregation, aneuploidy, and cancer. The idea that aneuploidy has a role in tumorigenesis has a long history, but we have yet to determine how tumor cells become aneuploid and survive frequent chromosome misassortment. Preliminary data suggest the existence of a complex relationship between pathways that...
detect and respond to DNA damage and pathways that respond to problems in the physical separation of chromosomes.

It is clear that unraveling the mechanism of checkpoint function and the consequences of mitotic errors will require more sophisticated methods and reagents. The only routine method to induce the mitotic checkpoint in animal cells is treatment with antimitotubule drugs such as taxol and nocodazole. However, these drugs accumulate at very different rates in different cells, generally cause wholesale rather than subtle defects in chromosome dynamics, and therefore are of limited value in recapitulating the events that induce checkpoint activation in normally growing cells. Micromanipulation of a single chromosome away from the spindle using a needle (in praying mantid spermatocytes) and overloading the spindle with an excess of minichromosomes (in budding yeast) are two examples of methods previously used to activate the checkpoint without disrupting microtubules (Li and Nicklas 1995; Wells and Murray 1996). Similarly subtle methods are needed to induce checkpoint activation in genetically tractable cells from mice.

Improved readouts for checkpoint function are also required. The mitotic checkpoint regulates the timing of anaphase onset, but most studies of checkpoint genes (including ours) have thus far relied on fixed time point assays. To examine the subtleties of mitotic checkpoint operation, it will be necessary to follow chromosome dynamics in living cells derived from transgenic mice. The use of green fluorescent protein (GFP)-tagged chromosomes has revolutionized the analysis of chromosome segregation in yeast (Straight et al. 1997; He et al. 2000), and we are currently attempting to generate mice carrying a single GFP-tagged chromosome. By introducing the tagged chromosome into various mutant backgrounds, it should be possible to follow the dynamics of checkpoint activation in ES cells and embryonic fibroblasts. We believe that combined genetic and cell biological studies of the mitotic checkpoint in mice are likely to provide new insights into the connections between chromosome missegregation, genetic instability, and tumorigenesis.

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