Overlapping Roles of the Spindle Assembly and DNA Damage Checkpoints in the Cell-Cycle Response to Altered Chromosomes in *Saccharomyces cerevisiae*

Peter M. Garber and Jasper Rine[1]

Department of Molecular and Cell Biology, University of California, Berkeley, California 94720

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ABSTRACT

The *MAD2*-dependent spindle checkpoint blocks anaphase until all chromosomes have achieved successful bipolar attachment to the mitotic spindle. The DNA damage and DNA replication checkpoints block anaphase in response to DNA lesions that may include single-stranded DNA and stalled replication forks. Many of the same conditions that activate the DNA damage and DNA replication checkpoints also activated the spindle checkpoint. The *mad2*Δ mutation partially relieved the arrest responses of cells to mutations affecting the replication proteins Mcm3p and Pol1p. Thus a previously unrecognized aspect of spindle checkpoint function may be to protect cells from defects in DNA replication. Furthermore, in cells lacking either the DNA damage or the DNA replication checkpoints, the spindle checkpoint contributed to the arrest responses of cells to the DNA-damaging agent methyl methanesulfonate, the replication inhibitor hydroxyurea, and mutations affecting Mcm2p and Orc2p. Thus the spindle checkpoint was sensitive to a wider range of chromosomal perturbations than previously recognized. Finally, the DNA replication checkpoint did not contribute to the arrests of cells in response to mutations affecting ORC, Mcm proteins, or DNA polymerase δ. Thus the specificity of this checkpoint may be more limited than previously recognized.

THE ability to accurately transmit genetic material to daughter cells is essential to all life. Eukaryotic organisms have evolved mechanisms called checkpoints that increase the fidelity of genetic transmission. Checkpoints enhance fidelity by delaying cell-cycle progression in cells with defects in chromosomes or in the machinery that segregates chromosomes. Cancer cells display reduced fidelity of genetic transmission and frequently have mutations in checkpoint genes (Li and Benezra 1996; Cahill *et al.*1998; Lengauer *et al.* 1998). Thus checkpoint failure contributes to cancer. Since the defects that checkpoints respond to are likely to be defects that destabilize the genome, identification of those defects should increase our understanding of genetic instability and also our understanding of how checkpoints prevent cancer.

A variety of conditions that disrupt chromosomes and/or chromosome segregation cause *Saccharomyces cerevisiae* cells to arrest prior to anaphase through one or more of three different checkpoints. These checkpoints differ in the types of agents that elicit their response and also in the genes that are required for their function. A checkpoint termed the DNA damage checkpoint arrests cells that have been treated with DNA-damaging agents. This checkpoint requires *RAD9*, *RAD17*, *RAD24*, *RAD53*, *DDC1*, *DDC2*, *MEC1*, and *MEC3* for full function (reviewed by Foiani *et al.* 2000). A second checkpoint, variously called the replication, S-phase, or S-M checkpoint, arrests cells in which replication has been blocked by deoxyribonucleotide depletion. This checkpoint overlaps with the DNA damage checkpoint in its requirement for *RAD53*, *MEC1*, and *DDC2*, but unlike the DNA damage checkpoint, does not require *RAD9*, *RAD17*, *RAD24*, *MEC3*, or *DDC1* (reviewed by Lowndes and Murguia 2000). A third checkpoint, termed the spindle assembly checkpoint, arrests cells in which replicated chromatids fail to achieve bipolar spindle attachment. This checkpoint requires *MAD1*, *MAD2*, *MAD3*, *BUB1*, *BUB3*, *NDC10*, and *MPS1* for full function (reviewed by Hoyt 2001).

In this study, we quantify the roles of these three checkpoints in the preanaphase arrests that occur in cells that have lost the function of various essential replication proteins. Initially, in an attempt to gain insight into the function of the eukaryotic DNA replication initiator, the origin recognition complex (ORC), we quantified the roles of these three checkpoints in the preanaphase arrests of cells that had lost ORC function. We were surprised to find that, although the DNA damage and spindle assembly checkpoints contributed to the arrests of *orc* cells, the DNA replication checkpoint did not. To determine whether this pattern of checkpoint responses was unique to *orc* mutants, we conducted similar analyses of cells harboring conditional mutations affecting Mcm proteins, DNA Polα, and DNA Polδ. We found that the spindle assembly and DNA damage checkpoints jointly mediated arrest responses

[1]*Corresponding author:* Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720. E-mail: jrine@uclink4.berkeley.edu

to a variety of replication mutations and that the spindle assembly checkpoint was capable of mediating arrest responses to a DNA-damaging agent and a DNA replication inhibitor.

MATERIALS AND METHODS

Yeast strains: *mcm2-1*, *mcm3-1*, *cdc2-1*, and *pol1-17* alleles from other strain backgrounds were backcrossed to W303 a minimum of four times. The *orc2-1* strain was constructed by gene replacement of *ORC2* in W303. The resulting collection of replication mutants was crossed to *rad9*Δ, *rad24*Δ, *mad2*, and *mec1*Δ checkpoint mutant strains isogenic to W303 to create the replication mutant strains used in this study (Table 1). The *mad1*Δ*::KanMX* allele, derived from the Research Genetics (Birmingham, AL) *MAT***a** deletion collection, was backcrossed once to W303 before crossing to a W303-isogenic *rad9*Δ *rad24*Δ strain to create JRY7309–JRY7320. Standard genetic procedures were as described (Rose *et al.* 1989).

Growth, synchronization, methyl methanesulfonate treatment, hydroxyurea treatment, and 4′,6-diamidino-2-phenylindole staining: YPD (rich medium) was used in all experiments. Temperature-sensitive replication mutants were maintained at 23°. The restrictive temperature used in all temperature-shift experiments was 37°. Strains lacking replication mutations were grown at 25–28°. α-Factor was used at 2.5–5 μg/ml to synchronize *MAT***a** cells in G1. α-Factor-arrested cells were washed twice in prewarmed YPD before being released into prewarmed YPD containing 5 μg/ml Pronase (Calbiochem 53702) protease. Methyl methanesulfonate (MMS, M-4016; Sigma, St. Louis) was added to YPD medium at 0.033%, except as noted. Hydroxyurea (HU) was added to YPD medium at 200 mm. 4′,6-Diamidino-2-pheynylindole (DAPI) staining of fixed cells was as described (Rose *et al.* 1989), and cell-cycle arrest or progression was determined by calculating the percentage of large-budded uninucleate cells by fluorescence microscopy of >200 cells. All cultures were coded during scoring so that the scorer was blind to the genotype of the culture being scored.

Viability in MMS: The number of colony-forming units (CFU) per microliter in cultures of wild-type, *mad2*Δ, *rad9*Δ *rad24*Δ, and *mad2*Δ *rad9*Δ *rad24*Δ cells was determined both before and at various times after the addition of 0.033% MMS by plating cells on non-MMS-containing medium and counting colonies after 3 days of growth at 25°. At each time point after MMS addition, the viability of each culture was expressed as the relation (CFU per microliter at that time point)/(CFU per microliter before MMS addition). To determine the significance of the effect of *mad2*Δ on the viability of the *rad9*Δ *rad24*Δ strains, the viabilities of both the *rad9*Δ *rad24*Δ strains and the *mad2*Δ *rad9*Δ *rad24*Δ strains were normalized to the mean viability of the *rad9*Δ *rad24*Δ strains at that time point. This normalization permitted compiling the viability of these strains at all time points, which was then expressed as (viability of strains X)/(viability of *rad9*Δ *rad24*Δ strains) ± 95% confidence limits.

Growth rate: Six (wild-type, *mad2*Δ) or seven (*rad9*Δ *rad24*Δ, *mad2*Δ *rad9*Δ *rad24*Δ) log-phase cultures of strains of the indicated genotypes were diluted to OD_{600} ~0.06 and grown for 3.5–5 hr at 25°. The ratios of the final ODs to the initial ODs were used to compute the doubling time of each culture, which was then normalized to the mean doubling time of the wild-type cultures. The means of these normalized doubling times ±SD are shown.

RESULTS

To test the roles of the DNA damage, DNA replication, and spindle assembly checkpoints in the arrest responses of cells with replication defects, budding yeast strains harboring temperature-sensitive mutations in genes encoding one of five different replication proteins were studied. Three of these mutations affect proteins involved in replication initiation: *orc2-1* (affecting ORC subunit 2); *mcm2-1* [affecting minichromosome maintenance (MCM) protein 2]; and *mcm3-1* (affecting MCM protein 3) (Yan *et al.* 1991; Foss *et al.* 1993). These proteins are components of the preinitiation complex, which assembles at replication origins, rendering them competent to initiate DNA replication. Intriguingly, mutations in each of these proteins cause cells to arrest prior to anaphase with a genome that is either fully replicated or nearly so (Gibson *et al.* 1990; Yan *et al.* 1991; Bell *et al.* 1993; Pflumm and Botchan 2001). Since it was not clear why mutations affecting the preinitiation complex should cause cells to arrest prior to anaphase, knowledge of which checkpoints, if any, were responsible for this phenotype was a first step toward understanding it.

The other two mutations affect proteins involved in replication elongation. The *cdc2-1* mutation affects DNA polymerase δ, the major replicative DNA polymerase (Boulet *et al.* 1989), and causes cells to arrest in mid-S-phase (Budd and Campbell 1993; P. Garber and J. Rine, unpublished results). The *pol1-17* mutation affects DNA polymerase α (Budd and Campbell 1987), responsible for priming DNA synthesis, and also causes cells to arrest in mid-S-phase (Budd *et al.* 1989). Whereas it was unclear whether unreplicated DNA would be present during the late S/G2 arrests of the initiation mutants, the mid-S arrest of these mutants ensured that significantly underreplicated chromosomes would be present for potential detection by the various checkpoints.

In a current model of the DNA-responsive checkpoint pathways in *S. cerevisiae*, *MEC1* is essential to the checkpoint responses to both DNA damage and stalled replication. In contrast, *RAD9* and *RAD24* are essential only to the checkpoint response to DNA damage and are not required for the response to stalled replication (Figure 6a). In preliminary experiments with the replication mutants, we found that combined *rad9*Δ and *rad24*Δ mutations relieved the cell-cycle arrests of all of the mutants other than *pol1-17* to the same degree as did the *mec1*Δ mutation. Therefore the *RAD9*- and *RAD24*-independent, *MEC1*-dependent replication checkpoint pathway did not make a significant contribution to the arrests of these mutants. Since use of the *mec1*Δ mutation prevents distinguishing between the contributions of the DNA replication and DNA damage checkpoints, we used the combined *rad9*Δ and *rad24*Δ mutations and left *MEC1* intact in the majority of these experiments.

The spindle checkpoint protein Mad2p arrested cells in response to replication mutations: Activation of the DNA damage or the spindle checkpoint causes budding yeast cells to arrest with a large bud and an undivided nucleus. This type of arrest can be quantified by fluorescence microscopic determination of the percentage of large-budded uninucleate cells in a population. After creating yeast strains that harbored the replication mutations as well as mutations in the DNA damage checkpoint (*rad9*Δ *rad24*Δ), the spindle checkpoint (*mad2*Δ), or both, we incubated cultures of these strains at the restrictive temperature and then determined the percentage of large-budded uninucleate cells in each.

In strains in which both the DNA damage and DNA replication checkpoints were intact, *mad2*Δ significantly reduced the arrests of *mcm3-1* and *pol1-17* strains (Figure 1; checkpoint+ *vs.* *mad2*Δ). Thus, the full arrest response to these mutations required Mad2p. The effect of the *mad2*Δ mutation on *mcm2-1*, *cdc2-1*, and *orc2-1* strains was highly variable; thus whether the arrest responses to these mutations required Mad2p was not resolved by this experiment. However, Mad2p did contribute to the residual arrests of *mcm2-1* and *orc2-1* strains lacking the DNA damage checkpoint (Figure 1; compare the *rad9*Δ *rad24*Δ double mutant to the *rad9*Δ *rad24*Δ *mad2*Δ triple mutant). Furthermore, Mad2p, in conjunction with Rad9p and Rad24p, was required for the full arrest response to the *cdc2-1* mutation (Figure 1; compare *cdc2-1* with *cdc2-1 mad2*Δ *rad9*Δ *rad24*Δ). Thus, Mad2p, and therefore presumably also the spindle checkpoint, detectably responded to the altered chromosomes generated in each of the replication mutants studied. These included mutations affecting both the initiation and elongation of replication and mutations causing cells to arrest either in mid-S-phase or in late S-G2.The combined *mad2*Δ*rad9*Δ*rad24*Δ mutations eliminated the accumulation of large-budded uninucleate cells in *mcm2-1*, *mcm3-1*, *orc2-1*, and *cdc2-1* cultures held at the restrictive temperature (Figure 1; *mad2*Δ *rad9*Δ *rad24*Δ *vs.* no treatment). Thus, in the absence of these checkpoints, none of these replication mutations created a block to anaphase progression. Moreover, although *MEC1* was intact in these strains, they failed to arrest. Therefore, inactivation of these replication proteins failed to detectably activate the *MEC1*-dependent, *RAD9 RAD24*-independent replication checkpoint.

The spindle checkpoint arrested cells in response to methyl methanesulfonate and hydroxyurea: These results with replication mutants suggested that the spindle checkpoint may be sensitive to a wide range of DNA perturbations. To explore this range further, the ability of Mad2p to arrest cells treated with the DNA-damaging agent MMS was tested. No effect of *mad2*Δ was observed on the arrests of strains with an intact DNA damage checkpoint (Figure 2a), consistent with previous reports that *MAD2* is not required for normal DNA damage responses (Hoyt *et al.* 1991; Hardwick *et al.* 1999). However, *rad9*Δ *rad24*Δ mutations only partially relieved the MMS-induced arrest. MMS-treated *rad9*Δ *rad24*Δ strains accumulated 45% large-budded uninucleate cells compared to the 10–14% observed in the untreated strains (Figure 2a, *rad9*Δ *rad24*Δ *vs.* no treatment). The *mad2*Δ mutation significantly reduced this residual accumulation to ~26% (Figure 2a, *rad9*Δ *rad24*Δ *vs.* *mad2*Δ *rad9*Δ *rad24*Δ). Thus, Mad2p was able to arrest a portion of MMS-treated cells and did so when the DNA damage checkpoint was not present.

Although Mad2p is not known to have a function outside of its role in the spindle checkpoint, we considered the possibility that the Mad2p-dependent arrest responses in our experiments reflected a spindle checkpoint-independent function of Mad2p. To test this possibility, we determined whether inactivation of a different component of the spindle checkpoint, Mad1p, would also relieve cell-cycle arrest responses to DNA damage. Similarly to *mad2*Δ, the *mad1*Δ mutation significantly reduced the arrest response of *rad9*Δ *rad24*Δ cells to MMS (Figure 2b). Thus, two different spindle checkpoint genes each promoted cell-cycle arrest in response to DNA damage. The simplest interpretation of this finding is that the spindle checkpoint itself promotes cell-cycle arrest in response to DNA damage.

The cell-cycle arrest defect of *rad9*Δ *rad24*Δ cells relative to *mad2*Δ cells treated with MMS indicated that the spindle checkpoint was less efficient at mediating this response than was the DNA damage checkpoint. One explanation for this difference could be that the DNA damage checkpoint recognizes most MMS-induced lesions whereas the spindle checkpoint recognizes only a subset of them. For example, only a subset of the MMS-induced lesions might interfere with centromere function and hence activate the spindle checkpoint. If this model were correct, then it should be possible to reduce the concentration of MMS to a level at which most or all cells experience a lesion that activates the damage checkpoint, while only a subset of cells experience a lesion that activates the spindle checkpoint. Therefore, reduced MMS concentrations were evaluated for their effects on the arrests of *mad2*Δ and *rad9*Δ *rad24*Δ strains. A fourfold reduction in MMS concentration had no detectable effect on the arrest of the *mad2*Δ strains, presumably reflecting the ability of the DNA damage checkpoint to respond to low levels of MMS-induced damage. In contrast, the lower MMS concentration reduced the arrest of the *rad9*Δ *rad24*Δ strains from 45 to 25% (Figure 2c). These data suggested that only a subset of MMS-induced lesions could activate the spindle checkpoint.

To explore further the spindle checkpoint's ability to respond to DNA perturbations, the ability of *mad2*Δ to relieve the arrest response to an agent that stalls DNA replication was tested. HU stalls DNA replication by inhibiting ribonucleotide reductase, thereby depleting

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source[a]
W303-1a	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 can1-100*	R. Rothstein
JRY7194	W303 *MAT*α *rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7195	W303 *MAT*α *rad9*Δ*::HIS3 rad24*Δ*::TRP1 ADE2 lys2*Δ	
JRY7196	W303 *MAT***a** *rad9*Δ*::HIS3 rad24*Δ*::TRP1 ADE2*	
JRY7197	W303 *MAT*α *mec1*Δ*::TRP1 sml1*Δ*::HIS3*	
JRY7198	W303 *MAT***a** *mec1*Δ*::TRP1 sml1*Δ*::HIS3 ADE2 lys2*Δ	
JRY7199	W303 *MAT***a** *mec1*Δ*::TRP1 sml1*Δ*::HIS3*	
JRY7200	W303 *MAT***a** *mad2*Δ*::URA3 ADE2 lys2*Δ	
JRY7201	W303 *MAT***a** *mad2*Δ*::URA3*	
JRY7202	W303 *MAT*α *mad2*Δ*::URA3*	
JRY7203	W303 *MAT*α *mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7204	W303 *MAT***a** *mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7205	W303 *MAT***a** *mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7206	W303 *MAT***a** *mcm2-1 ADE2 lys2*Δ	
JRY7207	W303 *MAT***a** *mcm2-1 ADE2*	
JRY7208	W303 *MAT*α *mcm2-1*	
JRY7209	W303 *MAT*α *mcm2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7210	W303 *MAT***a** *mcm2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7211	W303 *MAT***a** *mcm2-1 rad9*Δ*::HIS3 rad24::TRP1 ADE2 lys2*Δ	
JRY7212	W303 *MAT***a** *mcm2-1 mec1*Δ*::TRP1 sml1*Δ*::HIS3 ADE2 lys2*Δ	
JRY7213	W303 *MAT*α *mcm2-1 mec1*Δ*::TRP1 sml1*Δ*::HIS3 lys2*Δ	
JRY7214	W303 *MAT***a** *mcm2-1 mad2*Δ*::URA3 ADE2*	
JRY7215	W303 *MAT*α *mcm2-1 mad2*Δ*::URA3 lys2*Δ	
JRY7216	W303 *MAT***a** *mcm2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7217	W303 *MAT***a** *mcm2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24::TRP1*	
JRY7218	W303 *MAT*α *mcm3-1 lys2*Δ	
JRY7219	W303 *MAT***a** *mcm3-1 lys2*Δ	
JRY7220	W303 *MAT***a** *mcm3-1*	
JRY7221	W303 *MAT*α *mcm3-1 mad2*Δ*::URA3*	
JRY7222	W303 *MAT*α *mcm3-1 mad2*Δ*::URA3*	
JRY7223	W303 *MAT***a** *mcm3-1 mad2*Δ*::URA3*	
JRY7224	W303 *MAT*α *mcm3-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7225	W303 *MAT***a** *mcm3-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7226	W303 *MAT***a** *mcm3-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7227	W303 *MAT*α *mcm3-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7228	W303 *MAT*α *orc2-1 lys2*Δ	
JRY7229	W303 *MAT***a** *orc2-1 lys2*Δ	
JRY7230	W303 *MAT***a** *orc2-1 ADE2 lys2*Δ	
JRY7231	W303 *MAT*α *orc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 ADE2 lys2*Δ	
JRY7232	W303 *MAT***a** *orc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7233	W303 *MAT*α *orc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7234	W303 *MAT***a** *orc2-1 mad2*Δ*::URA3 ADE2 lys2*Δ	
JRY7235	W303 *MAT*α *orc2-1 mad2*Δ*::URA3 ADE2 lys2*Δ	
JRY7236	W303 *MAT***a** *orc2-1 mad2*Δ*::URA3 ADE2 lys2*Δ	
JRY7237	W303 *MAT***a** *orc2-1 mec1*Δ*::TRP1 sml1-1*	
JRY7238	W303 *MAT*α *orc2-1 mec1*Δ*::TRP1 sml1-1 lys2*Δ	
JRY7239	W303 *MAT***a** *orc2-1 mec1*Δ*::TRP1 sml1*Δ*::HIS3 ADE2 lys2*Δ	
JRY7240	W303 *MAT***a** *orc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7241	W303 *MAT***a** *orc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 ADE2 lys2*Δ	
JRY7242	W303 *MAT*α *orc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7243	W303 *MAT***a** *cdc2-1*	
JRY7244	W303 *MAT***a** *cdc2-1*	
JRY7245	W303 *MAT*α *cdc2-1*	
JRY7246	W303 *MAT***a** *cdc2-1 mad2*Δ*::URA3*	
JRY7247	W303 *MAT*α *cdc2-1 mad2*Δ*::URA3 lys2*Δ	
JRY7248	W303 *MAT***a** *cdc2-1 mad2*Δ*::URA3*	
JRY7249	W303 *MAT***a** *cdc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	

(*continued*)

TABLE 1

(Continued)

Strain	Genotype	Source[a]
JRY7250	W303 *MAT*α *cdc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7251	W303 *MAT***a** *cdc2-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7252	W303 *MAT***a** *cdc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7253	W303 *MAT*α *cdc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7254	W303 *MAT***a** *cdc2-1 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7256	W303 *MAT***a** *pol1-17 lys2*Δ	
JRY7257	W303 *MAT***a** *pol1-17 lys2*Δ	
JRY7258	W303 *MAT***a** *pol1-17 mec1*Δ*::TRP1 sml1*Δ*::HIS3*	
JRY7259	W303 *MAT*α *pol1-17 mec1*Δ*::TRP1 sml1*Δ*::HIS3*	
JRY7260	W303 *MAT***a** *pol1-17 mec1*Δ*::TRP1 sml1*Δ*::HIS3*	
JRY7261	W303 *MAT***a** *pol1-17 mad2*Δ*::URA3*	
JRY7262	W303 *MAT*α *pol1-17 mad2*Δ*::URA3*	
JRY7263	W303 *MAT***a** *pol1-17 mad2*Δ*::URA3 lys2*Δ	
JRY7264	W303 *MAT*α *pol1-17 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7265	W303 *MAT*α *pol1-17 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7267	W303 *MAT*α *pol1-17 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7268	W303 *MAT***a** *pol1-17 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1 lys2*Δ	
JRY7269	W303 *MAT*α *pol1-17 mad2*Δ*::URA3 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7270	W303 *MAT***a** *mcm2-1 rad24*Δ*::TRP1 lys2*Δ	
JRY7271	W303 *MAT***a** *mcm2-1 mad2*Δ*::URA3 rad24*Δ*::TRP1 ADE2*	
JRY7272	W303 *MAT*α *rad9*Δ*::HIS3 rad24*Δ*::TRP1 ADE2 lys2*Δ	
JRY7274	W303 *MAT***a** *mec1*Δ*::TRP1 sml1-1*	
JRY7275	W303 *MAT***a** *mec1*Δ*::TRP1 sml1*Δ*::HIS3 mad2*Δ*::URA3*	
JRY7309	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1*	
JRY7310	*MAT*α *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1lys2*Δ	
JRY7311	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1*	
JRY7312	*MAT*α *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7313	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7314	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1*	
JRY7315	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 mad1*Δ*::KanMX*	
JRY7316	*MAT*α *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 mad1*Δ*::KanMX*	
JRY7317	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 mad1*Δ*::KanMX*	
JRY7318	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 mad1*Δ*::KanMX*	
JRY7319	*MAT*α *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 mad1*Δ*::KanMX*	
JRY7320	*MAT***a** *leu2-3,112 his3-11 ura3-1 ade2-1 trp1-1 rad9*Δ*::HIS3 rad24*Δ*::TRP1 mad1*Δ*::KanMX*	

All strains are congenic with W303-1a, with additional mutations as noted.
[a] Except as noted, all strains were produced for this study.

cells of deoxyribonucleotides. This condition activates the replication checkpoint, which is independent of *RAD9* and *RAD24* but requires *MEC1*. Therefore we tested the ability of *mad2*Δ to relieve arrest in hydroxyurea-treated *mec1*Δ cells, which lack the replication checkpoint. After 3.5 hr in 200 mM HU, ~80% of wild-type cells or cells lacking the DNA damage checkpoint arrested (Figure 2d). The *mec1*Δ cells arrested less well, yet still exhibited more cell-cycle arrest than has been reported by others (Figure 2d, wild type *vs. mec1*Δ; Weinert *et al.* 1994; Desany *et al.* 1998). At later time points *mec1*Δ relieved a larger portion of the HU-induced arrest (Figure 5 and data not shown), possibly explaining this discrepancy. *mad2*Δ significantly reduced the residual arrest observed in HU-treated *mec1*Δ cells (Figure 2d, *mec1*Δ *vs. mec1*Δ *mad2*Δ). Since HU treatment stalls replication efficiently and creates only small amounts of DNA damage, this result indicated that incompletely replicated chromosomes *per se* may activate the spindle checkpoint.

The checkpoints acted in the first cell cycle following damage: In the preceding experiments, cell-cycle arrest was quantified in cultures that were growing asynchronously at the time of insult. When cells fail to accumulate at the arrest point in such an experiment, they may do so either by passing through the arrest point (a checkpoint defect) or by failing to ever arrive at the arrest point (a viability defect). Experiments on synchronized cell populations allowed us to distinguish between these possibilities. This question was relevant to the mechanism of spindle-checkpoint-mediated arrest. Mitosis in the presence of damaged or partially replicated chromosomes can lead to aneuploidy and chromosome breakage. Since aneuploidy and small linear chromosomes can

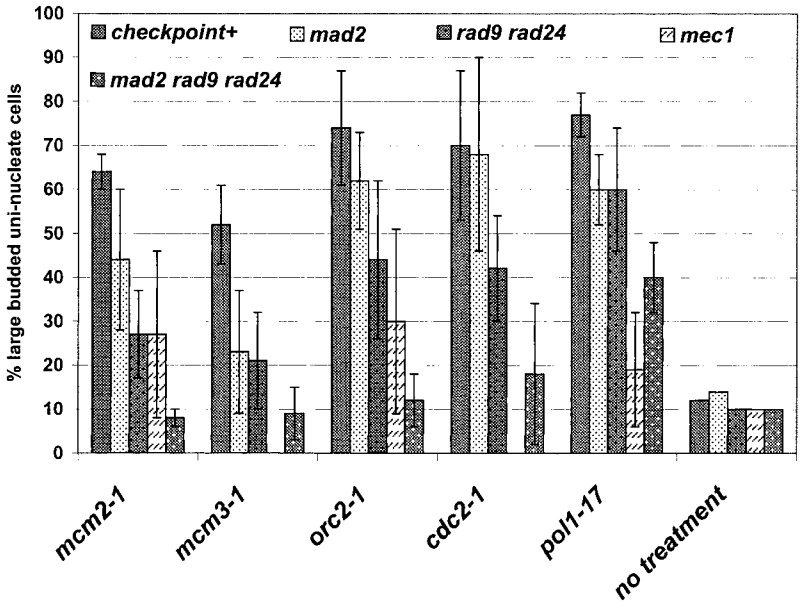

FIGURE 1.—*MAD2*-mediated arrest responses to a variety of replication mutations. Cultures of strains of the indicated genotypes were incubated at the restrictive temperature of 37° for 3.5 hr and then harvested, fixed, and stained with DAPI. The percentage of large-budded uninucleate cells in each culture was then determined by fluorescence microscopic examination of >200 cells. Each bar represents the mean of 3–11 such trials, and the error bars represent the 95% confidence limits of that mean. Excepting *mcm3-1 rad9*Δ *rad24*Δ, each genotype was represented by a minimum of two, and usually three or more, independently derived strains. "No treatment" refers to control strains lacking replication mutations but containing the indicated checkpoint mutations.

activate the spindle checkpoint in *S. cerevisiae* (WELLS and MURRAY 1996), it was possible that the spindle checkpoint responses to MMS, HU, or replication mutations required a prior mitosis in the presence of these insults. To test these ideas, we synchronized cells in G1 with α-factor prior to the time of insult and then quantified the number of cells both arriving at and passing through the arrest point.

In the first synchronized-cell experiment, the *mcm2-1* mutation was used to activate the checkpoints. *RAD9* was left intact in the strains used in this experiment since *rad9*Δ in combination with other mutations caused low viability that prevented synchronization. The *mcm2-1*, *mcm2-1 mad2*Δ, *mcm2-1 rad24*Δ, and *mcm2-1 mad2*Δ *rad24*Δ strains were synchronized in G1 at the permissive temperature and then released from the G1 block into restrictive-temperature medium. Under these conditions, bud emergence and bud growth occurred with similar kinetics in the *mcm2-1* and *mcm2-1 mad2*Δ strains, but were slightly slower and/or less synchronous in the *mcm2-1 rad24*Δ and *mcm2-1 rad24*Δ *mad2*Δ strains (Figure 3, a and b). By 100 min postrelease, 80% of the *mcm2-1* cells with both checkpoints intact accumulated at the large-budded uninucleate stage (Figure 3c, *mcm2-1*). By contrast, only 12% of *mcm2-1* cells lacking both the DNA damage and spindle checkpoints accumulated at this stage (Figure 3c, *mcm2-1 mad2*Δ *rad24*Δ). Subsequent increases in the proportions of binucleate and unbudded cells in this strain demonstrated that these cells were passing through the arrest point rather than failing to arrive at it (Figure 3d, *mcm2-1 mad2*Δ *rad24*Δ; Figure 3a, *mcm2-1 mad2*Δ *rad24*Δ). Thus, the preanaphase arrest of *mcm2-1* cells was due solely to the responses of the DNA damage and spindle assembly checkpoints to the *mcm2-1* mutation.

Furthermore, 55% of the *mcm2-1 mad2*Δ cells accumulated at the arrest point, indicating that the DNA damage checkpoint arrested this proportion of cells in the first cycle following inactivation of Mcm2p. Similarly, 45% of the *mcm2-1 rad24*Δ cells accumulated at the arrest point, indicating that the spindle checkpoint arrested this proportion of cells in the first cycle following inactivation of Mcm2p. Thus, both DNA damage checkpoint- and spindle checkpoint-mediated arrest occurred in the first cycle after inactivation of Mcm2p.

To assess checkpoint responses in the first cell cycle after exogenously induced DNA damage, the experiment was repeated using MMS rather than *mcm2-1* to activate the checkpoints. Wild-type, *rad9*Δ *rad24*Δ, *mad2*Δ, and *mad2*Δ *rad9*Δ *rad24*Δ cells were synchronized in G1 and then released into medium containing MMS. In this experiment, bud emergence and growth occurred with similar kinetics in all the strains (Figure 4, a and b). In cells with both checkpoints intact (WT in Figure 4) and in cells with just the DNA damage checkpoint intact (*mad2*Δ), >80% of the population arrested (Figure 4c). In cells with only the spindle checkpoint intact (*rad9*Δ *rad24*Δ), 55% of the population arrested (Figure 4c). However, in cells with neither checkpoint intact (*mad2*Δ *rad9*Δ *rad24*Δ), only 14% of the population arrested (Figure 4c). Cell-cycle progression in the triple mutant cells was confirmed by subsequent increases in unbudded cells, small-budded cells, and large-budded binucleate cells (Figure 4, a, b, and d). Thus both DNA damage checkpoint activation and spindle checkpoint activation occurred in the first cycle after treatment with MMS.

To explore further the role of *MAD2* in the arrest of *mec1*Δ cells in HU, we monitored the cell-cycle progression of wild-type, *mec1*Δ, and *mec1*Δ *mad2*Δ cells released from the α-factor block into HU-containing medium. In this experiment, all three strains behaved similarly up until 120 min after release into HU-containing medium, accumulating from 50 to 70% large-budded uninucleate

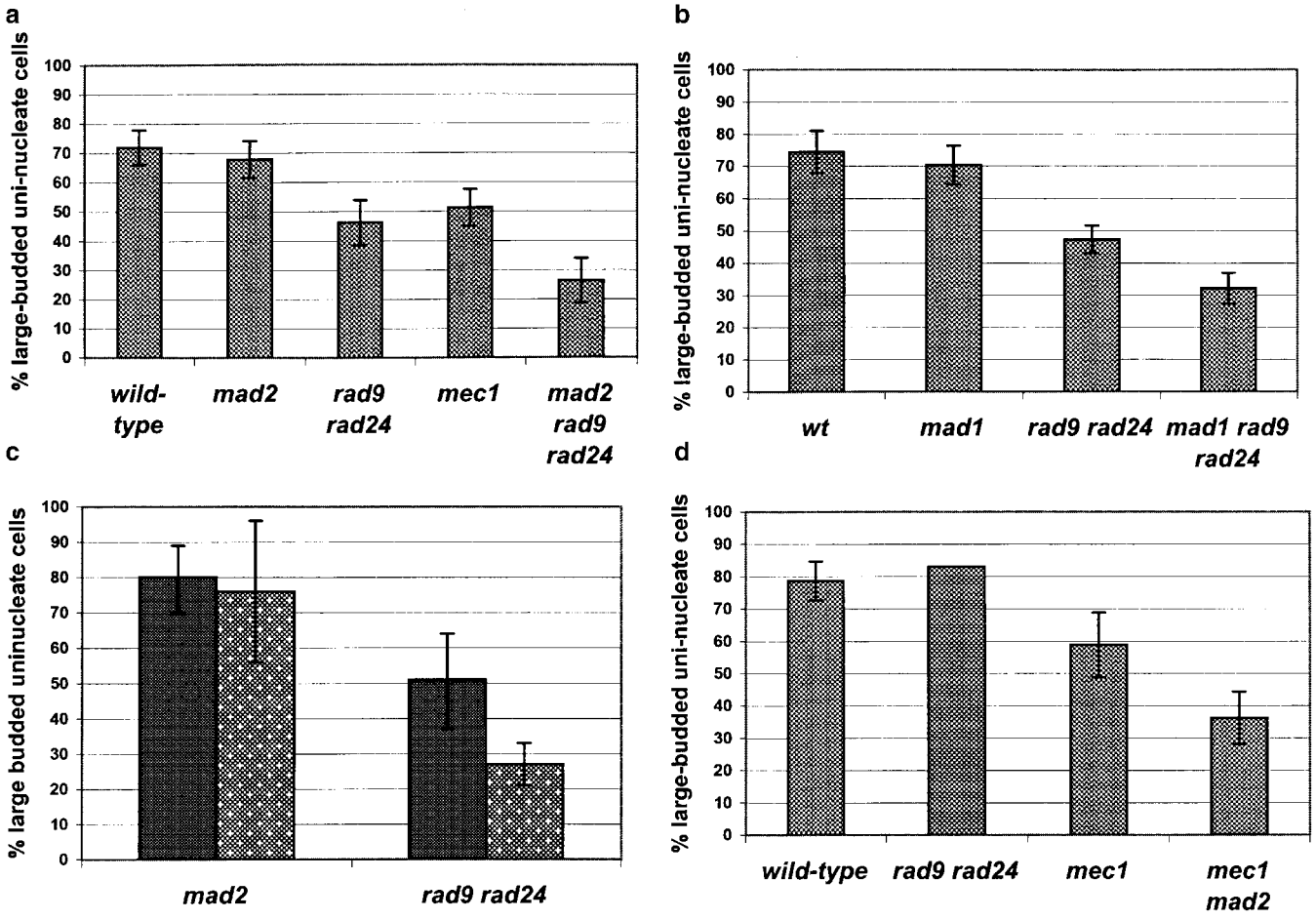

FIGURE 2.—The spindle-checkpoint-arrested cells lacking the DNA damage or DNA replication checkpoints in response to MMS and HU. The percentage of large-budded uninucleate cells in each culture was determined as in Figure 1. (a) Cultures of strains with the indicated checkpoint mutations were treated with 0.033% MMS for 3.5 hr prior to fixation. From 7 to 11 cultures of each genotype were tested, and the mean and 95% confidence limits of that mean are shown. (b) Experiments were performed as in a. Three strains of each genotype were tested either two (*mad1*Δ) or three (wt, *rad9*Δ *rad24*Δ, *mad1*Δ *rad9*Δ *rad24*Δ) times each, and the mean and 95% confidence limits are shown. (c) Three cultures each of a *mad2*Δ strain and a *rad9*Δ *rad24*Δ strain were incubated with either 0.033% MMS (shaded) or 0.008% MMS (stippled) for 3.5 hr. Means and 95% confidence limits are shown. (d) Three cultures each of wild-type, *mec1*Δ, and *mec1*Δ *mad2*Δ strains were incubated with 200 mM hydroxyurea for 3.5 hr prior to analysis. Means ±95% confidence limits are shown.

cells. Thus, the initial cell-cycle arrest response to HU occurred as efficiently in the *mec1*Δ *mad2*Δ strain as in the *mec1*Δ strain (Figure 5c). However, by 150 min, the percentage of large-budded uninucleate cells in the wild-type strain continued to increase, whereas this percentage remained constant in the *mec1*Δ strain, and in the *mec1*Δ *mad2*Δ strain it declined. The rise in the percentage of binucleate cells during this time indicated that the loss of uninucleate cells was due to nuclear division (Figure 5d). Thus the spindle checkpoint was able to block nuclear division in a portion of HU-treated *mec1*Δ cells. Since the initial arrest response to HU occurred as efficiently in the *mec1*Δ *mad2*Δ strain as it did in the *mec1*Δ strain, these results suggested a role for *MAD2* in maintaining the anaphase block in HU-treated cells rather than in establishing the block.

The spindle checkpoint contributed to the growth rate and DNA damage resistance of *rad9*Δ *rad24*Δ mutants: Cell-cycle delay in the presence of DNA damage often preserves cell viability, and indeed many checkpoint mutants have been identified by their sensitivity to DNA-damaging agents. Therefore we tested whether the *mad2*Δ mutation affected survival of MMS treatment. In tests for growth on solid MMS-containing medium, the *mad2*Δ mutation did not significantly affect the survival of either wild-type cells or *rad9*Δ *rad24*Δ cells (data not shown). Thus spindle checkpoint function did not enhance survival of cells during chronic exposure to MMS. However, to test whether spindle checkpoint function could rescue cells from acute exposure to MMS, we treated wild-type, *mad2*Δ, *rad9 rad24*Δ, and *mad2*Δ *rad9*Δ *rad24*Δ cells in liquid culture with MMS, removing cells at various times to test viability. This analysis revealed that the viability of the *mad2*Δ *rad9*Δ *rad24*Δ strains (39 ± 12% relative to *rad9*Δ *rad24*Δ viability) was significantly lower than that of the *rad9*Δ *rad24*Δ strains

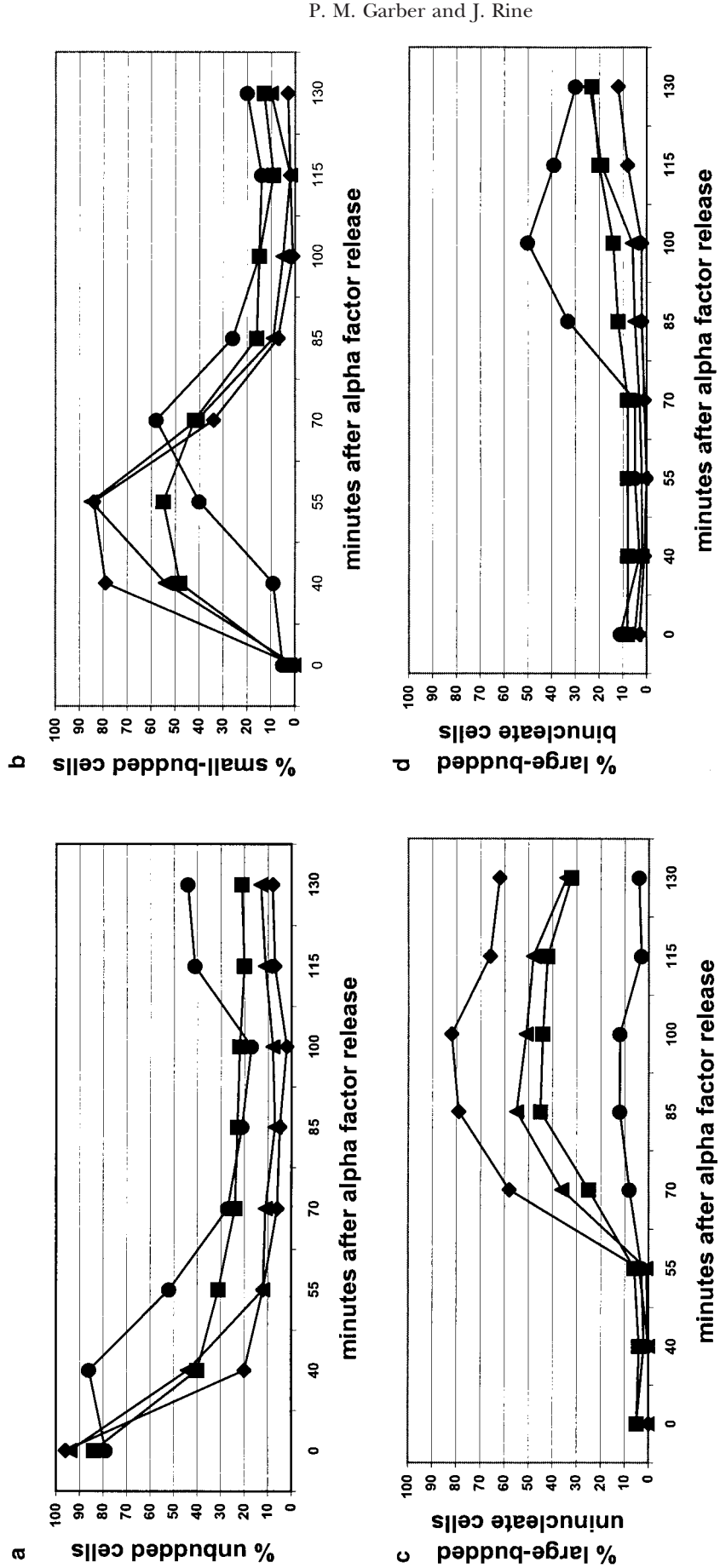

FIGURE 3.—The spindle-checkpoint-arrested cells in the first cell cycle after inactivation of Mcm2p. Cultures of (◆) *mcm2-1* (JRY7208), (▲) *mcm2-1 mad2*Δ (JRY7214), (■) *mcm2-1 rad24*Δ (JRY7270), and (●) *mcm2-1 mad2*Δ *rad24*Δ (JRY7271) cells were arrested in G1 with α-factor and then shifted to 37° for 45 min prior to release into α-factor-free 37° medium. At the indicated time points, aliquots were removed and fixed for DAPI staining. At each time point, >200 cells of each strain were scored as unbudded (a), small-budded (b), large-budded uninucleate (c), large budded with dividing nucleus (not shown), or large-budded binucleate (d).

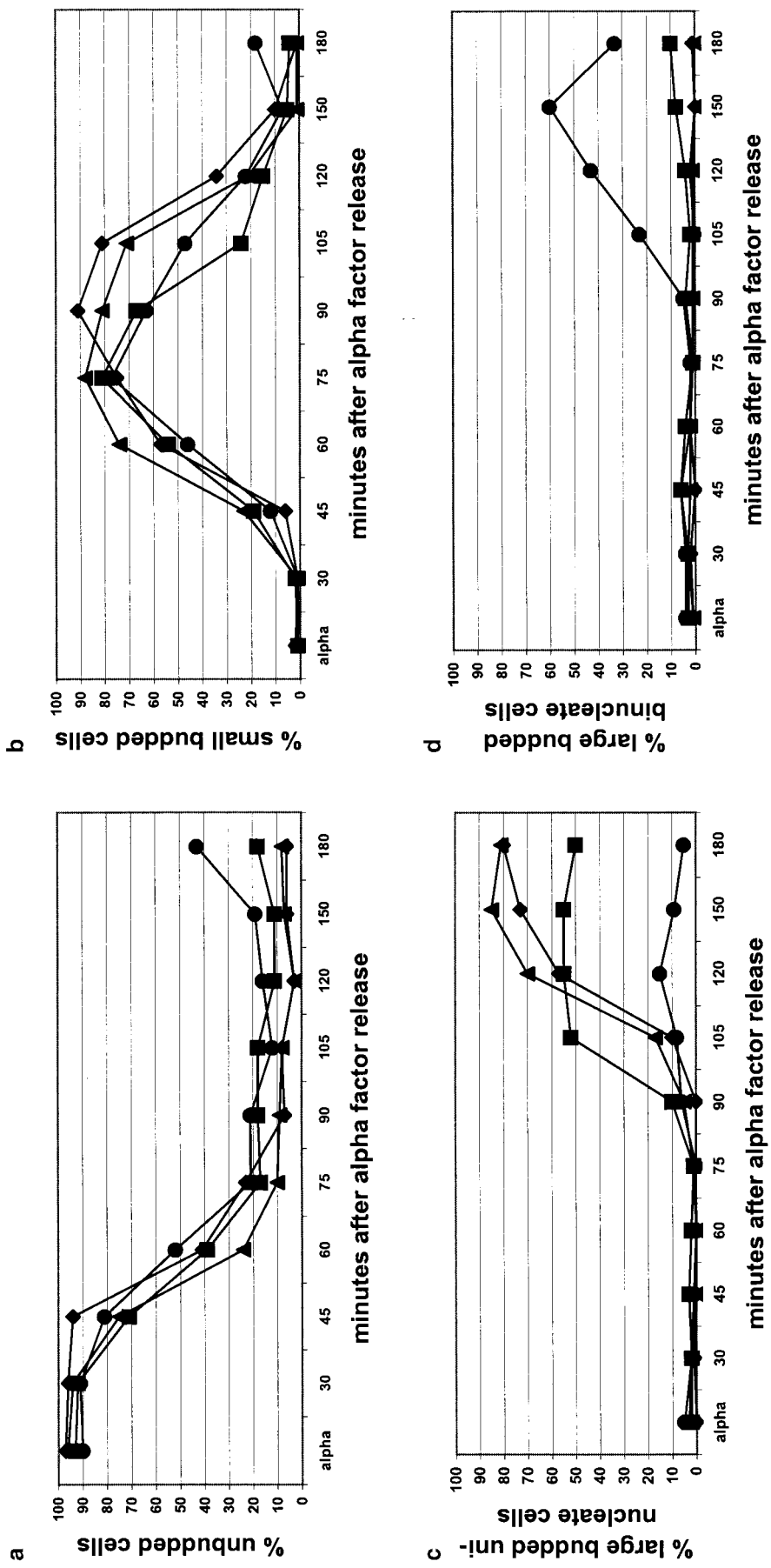

FIGURE 4.—The spindle-checkpoint-arrested cells in the first cell cycle after MMS exposure. Cultures of (◆) wild-type (JRY2334), (▲) *mad2*Δ (JRY7201), (■) *rad9*Δ *rad24*Δ (JRY7196), and (●) *mad2*Δ *rad9*Δ *rad24*Δ (JRY7204) cells were arrested in G1 with α-factor and then released into α-factor-free medium containing 0.033% MMS. At the indicated time points, aliquots were removed and fixed for DAPI staining. At each time point, >200 cells of each strain were scored as unbudded (a), small-budded (b), large-budded uninucleate (c), large-budded with dividing nucleus (not shown), or large-budded binucleate (d).

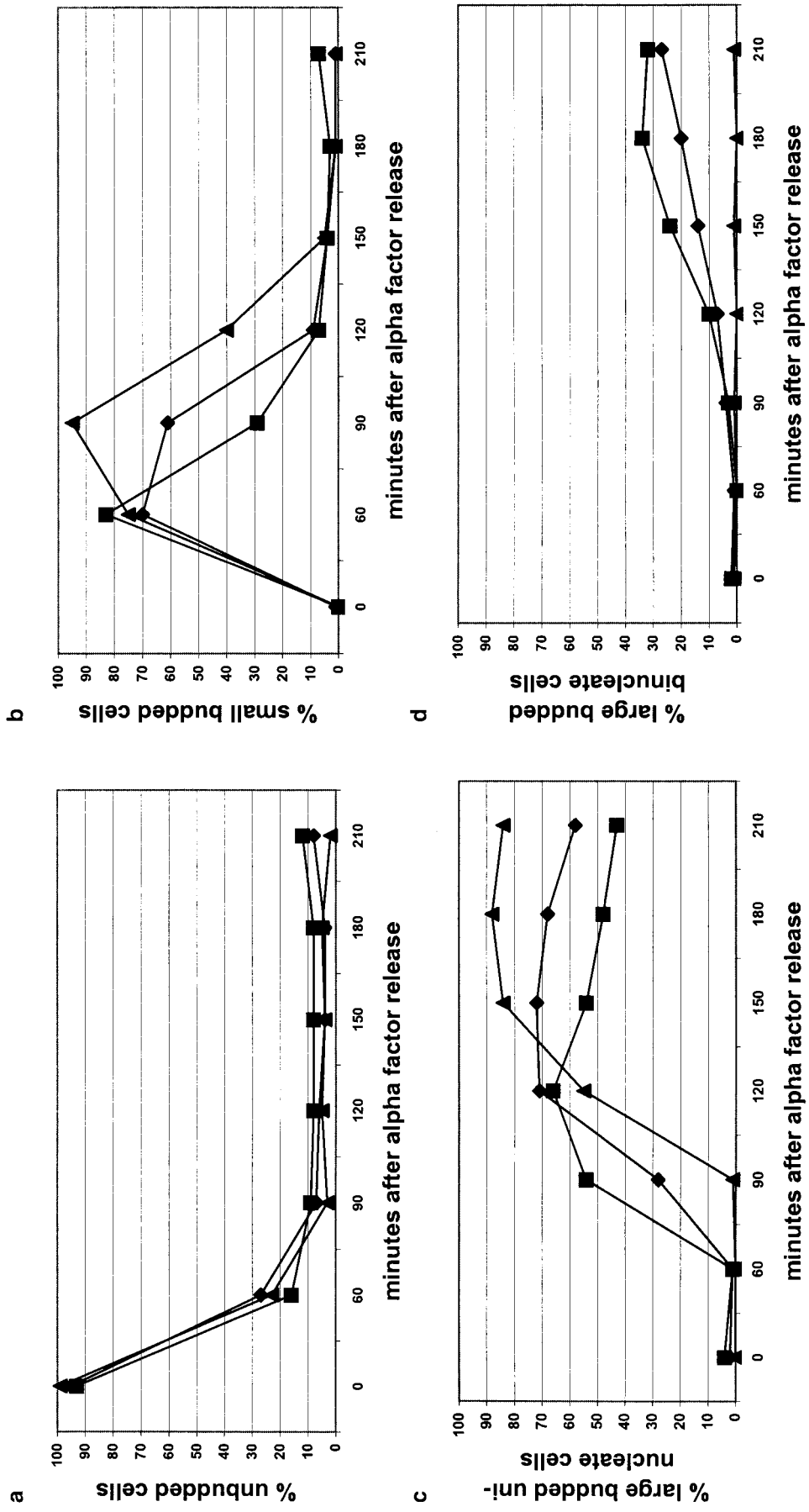

FIGURE 5.—The spindle-checkpoint-arrested cells in the first cell cycle after HU treatment. Cultures of (▲) wild-type (JRY2334), (◆) *mec1Δ* (JRY7274), and (■) *mec1Δ mad2Δ* (JRY7275) cells were arrested in α-factor and then released into α-factor-free medium containing 200 mM HU. At the indicated time points aliquots were removed and fixed for DAPI staining. At each time point, >200 cells of each strain were scored as unbudded (a), small-budded (b), large-budded uninucleate (c), large-budded with dividing nucleus (not shown), or large-budded binucleate (d).

(100 ± 12%) at all times after MMS treatment. Thus, spindle checkpoint function did contribute to the viability of *rad9*Δ *rad24*Δ cells during short-term exposure to MMS.

Relative to wild-type yeast strains, the *mad2*Δ *rad9*Δ *rad24*Δ strains formed small colonies. To quantify this effect, we determined the doubling times of wild-type, *mad2*Δ, *rad9*Δ *rad24*Δ, and *mad2*Δ *rad9*Δ *rad24*Δ strains. The growth of the *mad2*Δ strain was indistinguishable from wild type (99 ± 3.6% of wild-type doubling time). The *rad9*Δ *rad24*Δ strain grew more slowly (107 ± 3.3% of wild-type doubling time), and the *mad2*Δ mutation enhanced this defect (117 ± 3.4% of wild-type doubling time). Since the cell-cycle data presented above indicated that the checkpoints that these genes control respond to aberrant replication, the slower doubling times of these strains likely reflected a requirement for checkpoint response to aberrant replication events during normal cell divisions.

DISCUSSION

We investigated the relative contributions of the DNA damage, DNA replication, and spindle assembly checkpoints to the preanaphase arrest responses that occur in *S. cerevisiae* cells exposed to a variety of chromosome-perturbing conditions. These conditions included mutations affecting the origin recognition complex, Mcm proteins, DNA polymerase α, and DNA polymerase δ, as well as nucleotide depletion and exposure to a DNA-damaging agent. Several findings arose from this work. First, the spindle checkpoint was able to contribute to the arrest responses to all of the conditions tested. Second, spindle checkpoint function was essential for cells to achieve a full arrest response to mutations affecting Mcm3p and Pol1p. Third, the *RAD9*-independent, *MEC1*-dependent replication checkpoint made a detectable contribution to the arrests of *pol1-17* mutants and HU-treated cells, but not to any of the other conditions tested.

Identification of the checkpoints that become activated under a certain condition should offer insight into the molecular defects associated with that condition. In the case of the spindle checkpoint, the activating molecular defect has been well characterized. A large body of evidence indicates that kinetochores not under tension from the mitotic spindle cause this checkpoint to become activated (Rieder *et al.* 1995; Chen *et al.* 1996; Li and Nicklas 1997). Some of this evidence derives from studies of *S. cerevisiae*, in which unreplicated chromosomes and chromosomes with defects in sister chromatid cohesion both activate the spindle checkpoint (Castano *et al.* 1996; Skibbens *et al.* 1999; Hannah *et al.* 2001; Mayer *et al.* 2001; Stern and Murray 2001). Similarly, incomplete DNA replication in Drosophila embryos results in a *BUB1*-dependent mitotic arrest (Garner *et al.* 2001). Since some of our experimental conditions caused cells to arrest in early S-phase (the DNA polymerase mutations, hydroxyurea treatment, and MMS treatment), mono-oriented kinetochores on unreplicated centromeres were likely sources of spindle checkpoint activation in these cells. However, the *orc* and *mcm* mutations caused cells to arrest in late S-phase or G2. Since unreplicated centromeres were less likely to be present in these cells, spindle checkpoint activation in these mutants may signal defects in sister chromatid cohesion or may reflect heretofore unsuspected roles of ORC and MCM proteins in promoting the bipolar attachment of chromosomes to the mitotic spindle.

The lesions recognized by the DNA damage and DNA replication checkpoints have not been determined as precisely as that of the spindle checkpoint. It has been proposed that single-stranded DNA (ssDNA) is the lesion recognized by the DNA damage checkpoint. Correlations between the presence of ssDNA and DNA damage checkpoint activation support this model (Lydall and Weinert 1995; Lee *et al.* 1998). However, since ssDNA is an expected intermediate in the processing of most types of DNA damage, this correlation is compatible with other models as well. Therefore, we interpret the DNA damage checkpoint activation in our experiments to signal the presence of some DNA lesion that may be an intermediate in DNA damage processing that may or may not be ssDNA.

The molecular defect responsible for activation of the *MEC1*-dependent, *RAD9*-independent DNA replication checkpoint is widely thought to be ongoing or stalled replication forks (reviewed by Lowndes and Murguia 2000). However, our results, in conjunction with data from other labs, suggest that this view may need refinement. Specifically, the preanaphase arrest responses to a variety of conditions in which replication is stalled or ongoing require *RAD9*; thus, these conditions all fail to significantly activate the *RAD9*-independent replication checkpoint. For example, the preanaphase arrest responses to the *cdc2-1* and *cdc2-2* mutations, which inactivate DNA Polδ, require *RAD9* (Weinert and Hartwell 1993; P. Garber and J. Rine, unpublished results). Similarly, although two-dimensional gel analyses indicate that arrested *orc2-1*, *orc5-1*, and *mcm2-1* mutant cells contain replication forks, these mutants require *RAD9* for preanaphase arrest (Liang *et al.* 1995; Lei *et al.* 1997). Furthermore, yeast cells harboring an origin-deficient artificial chromosome require *RAD9* to stably maintain the chromosome (van Brabant *et al.* 2001). Thus, neither the stalled forks in *orc* and *mcm* mutants nor the ongoing replication forks of the artificial chromosome significantly activate the *RAD9*-independent, *MEC1*-dependent replication checkpoint.

In our experiments and in those of others, the replication checkpoint contributed to the arrests of deoxyribonucleotide-depleted cells and cells lacking Polα DNA polymerase activity, but not to the other conditions tested (Weinert and Hartwell 1993; Weinert *et al.*

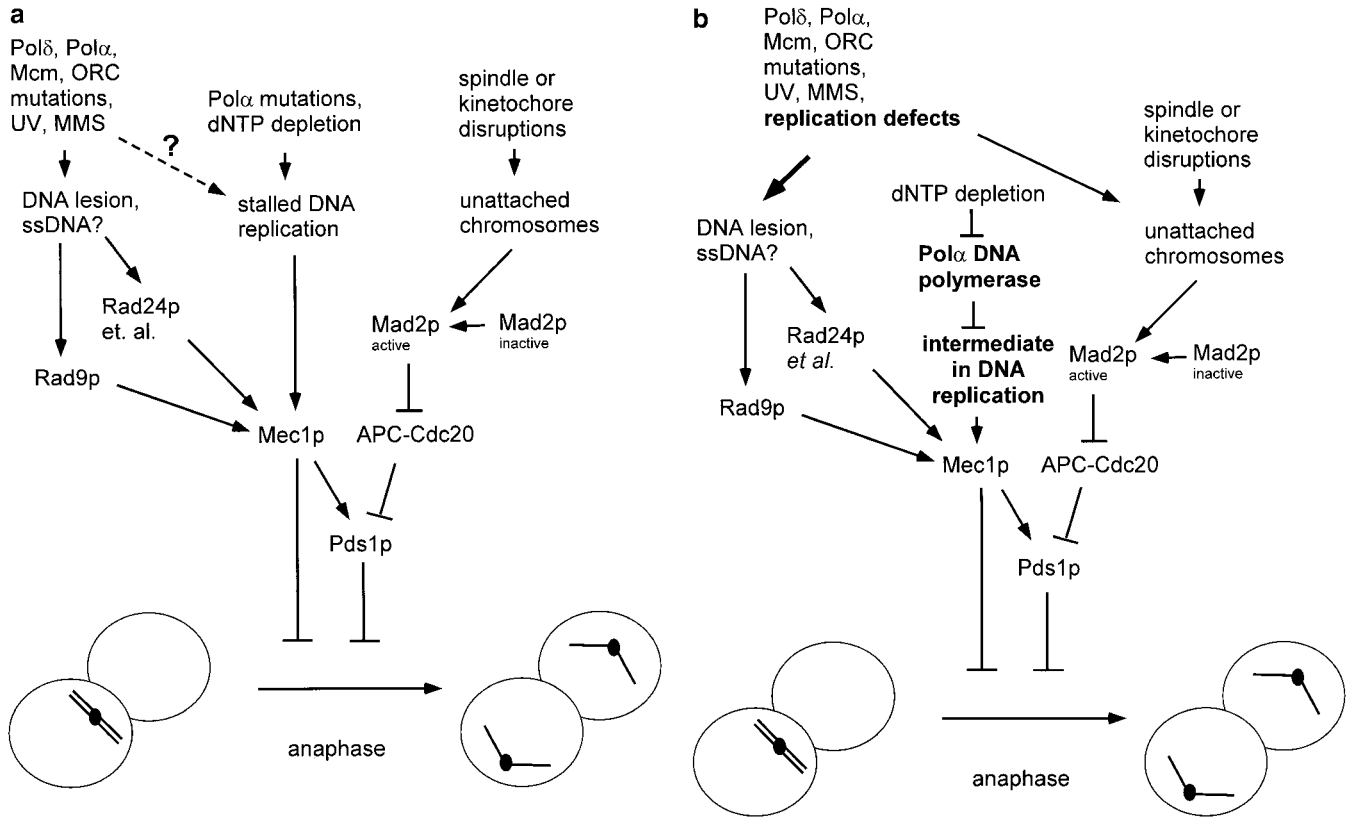

FIGURE 6.—(a) A model of checkpoint pathways involved in responding to stalled replication and DNA damage. The *MEC1* pathway is the cell's most sensitive DNA-responsive checkpoint. The response to DNA-damaging agents such as MMS and ionizing radiation requires *RAD9*, the *RAD24* epistasis group, *MEC1*, and additional downstream protein kinases. In contrast, the response to the replication inhibitor hydroxyurea requires *MEC1*, but does not require *RAD9* or the *RAD24* epistasis group. The response to mitotic spindle disruptors requires *MAD2* and occurs independently of *MEC1*. Both the *MEC1*-dependent checkpoint and the *MAD2*-dependent checkpoint block anaphase by stabilizing the anaphase inhibitor Pds1p. *RAD9* and the *RAD24* group activate this pathway in response to DNA damage and DNA structures resulting from stalled or aberrant DNA replication. (b) Enhancements to the model derived from results presented here. The MEC1-dependent pathway's requirement for *RAD9* and the *RAD24* group was abrogated only in *pol*α mutants and hydroxyurea-treated cells. The hydroxyurea effect may be mediated by the impact of lowered deoxynucleotide pools on Polα. The *MAD2*-dependent spindle checkpoint was less sensitive than the *MEC1*-dependent pathway to most chromosome perturbations, but was equally or more sensitive than the *MEC1*-dependent pathway to certain aberrant chromosome structures such as those found in *pol1-17* and *mcm3-1* mutants.

1994; P. GARBER and J. RINE, unpublished results; Figures 2 and 3c). Since stalled replication structures are likely to be present in *orc*, *mcm*, and polδ mutants as well as in polα mutants and HU-treated cells, we propose that the replication checkpoint, rather than recognizing stalled replication structures *per se*, recognizes a DNA lesion that is specific to cells lacking Polα DNA polymerase activity and cells lacking deoxyribonucleotides. When considering what lesion might be common to these two conditions, we note that polα mutants and HU-treated cells are each compromised for Polα DNA polymerase activity. Thus, one possibility is that the replication checkpoint recognizes an intermediate in DNA replication that persists only when Polα's DNA polymerase is not active. Studies in *Xenopus laevis* extracts indicate that RNA primers activate the replication checkpoint. Hence, we suggest that the unextended RNA primers that might accumulate in polα mutants activate the replication checkpoint in *S. cerevisiae*. A model that takes into account this more limited role of the replication checkpoint in responding to replication problems is presented in Figure 6b.

Although the spindle checkpoint was capable of arresting cells in response to all of the chromosome-perturbing conditions we employed, the responses to *mcm2-1*, *orc2-1*, HU, and MMS did not require spindle checkpoint function as long as the *MEC1*-dependent checkpoints were intact. Thus the *MEC1*-dependent checkpoints responded readily to these conditions and mediated a maximal arrest response to them whether or not the spindle checkpoint was present. By contrast, the *MEC1*-dependent checkpoints responded less readily to the *mcm3-1* and *pol1-17* mutations, and under these conditions the spindle checkpoint contributed to arrest even when the *MEC1*-dependent checkpoints were intact. Thus chromosome perturbations vary in the degree

to which they activate each of these checkpoints. On one end of the spectrum lie chromosome perturbations such as the presence of multiple linear minichromosomes, which activate the spindle checkpoint without apparently activating the DNA damage checkpoint (Wells and Murray 1996). On the other end of the spectrum lie chromosome perturbations such as low levels of MMS and the *cdc13-1* mutation, which activate the DNA damage checkpoint while causing little or no spindle checkpoint activation (Hardwick *et al.* 1999; P. Garber and J. Rine, unpublished results; Figure 2). The *mcm3-1* and *pol1-17* mutations fall between these two extremes. The ability to thus classify the checkpoint responses to a particular mutation may lend insight into the underlying molecular phenotype of the mutants.

The majority of cancers display a chromosome instability phenotype (Lengauer *et al.* 1998). While some cancer cells are defective for spindle checkpoint function and/or the expression of spindle checkpoint genes (Li and Benezra 1996; Cahill *et al.* 1998), the molecular basis for chromosome instability in most cancers remains unknown (Lengauer *et al.* 1998). Therefore, discovery of the lesions responsible for chromosome instability remains a critical step in understanding tumorigenesis. The ability of a variety of replication perturbations to activate the spindle checkpoint suggests that the proper attachment of chromosomes to the mitotic spindle can be disrupted by defects in replication. If so, then replication defects could contribute directly to the segregation defects underlying much of the chromosome instability in cancer cells. Furthermore, the response of the spindle checkpoint to the same types of perturbations as the DNA damage checkpoint suggests that mutations affecting these two pathways would synergistically contribute to genome instability. Hence, tumor cells with spindle checkpoint mutations may be more sensitive to chemotherapeutic agents that damage DNA than are cells with functional spindle checkpoints.

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