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The *Saccharomyces cerevisiae MEC1* Gene, Which Encodes a Homolog of the Human *ATM* Gene Product, Is Required for G_1 Arrest following Radiation Treatment

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The *Saccharomyces cerevisiae* gene *MEC1* represents a structural homolog of the human gene *ATM* mutated in ataxia telangiectasia patients. Like human ataxia telangiectasia cell lines, *mec1* mutants are defective in G_2 and S-phase cell cycle checkpoints in response to radiation treatment. Here we show an additional defect in G_1 arrest following treatment with UV light or gamma rays and map a defective arrest stage at or upstream of START in the yeast cell cycle.

One of the phenotypic hallmarks of cells from patients afflicted with the cancer-prone syndrome ataxia telangiectasia (AT) (10, 12) is a defect in cell cycle arrest at multiple checkpoints responding to radiation damage, such as checkpoints in G_1 (8, 14), G_2 (16), and S phase, the last at the level of both replicon initiation and elongation (8, 15). Several studies (2, 3, 6, 9) but not all (11) have suggested that defective G_1 arrest is correlated with suboptimal up-regulation of the checkpoint determinant p53 in AT cells following treatment with DNA-damaging agents. Normally, enhanced levels of p53 result in the transcriptional induction of the cyclin-dependent kinase (Cdk) inhibitor p21. Indeed, a reduction in cyclin E-associated Cdk activity at the time of G_1 arrest has been detected in gamma ray-treated normal human fibroblasts but not in AT cells (4).

Recently, the AT-complementing gene was cloned and shown to encode a large polypeptide with sequence similarity to phosphatidylinositol-3 kinases (19, 20). Several structural homologs have been isolated from other eukaryotic cells (23). Two genes termed *TEL1* (5, 13) and *MEC1* (= *ESR1* = *SAD3*) (7) have been characterized in the yeast *Saccharomyces cerevisiae*. The *TEL1* gene product shows a higher degree of amino acid sequence similarity to the *ATM* gene product than does that of *MEC1*. *TEL1* is required for maintenance of normal telomere length, but *tel1* mutants are not radiation sensitive or impaired in checkpoint controls (5, 13, 18). *mec1* mutants, on the other hand, are characterized by a defect in radiation-induced G_2/M arrest (22), in S-phase arrest following treatment with hydroxyurea (1, 22), and in the slowing of S phase observed in cells treated with the alkylating agent methylmethane sulfonate (17). We demonstrate here that *mec1* mutants are also defective in radiation-induced G_1 arrest.

mec1 deletion mutants are inviable. We therefore generated viable congenic strains by backcrossing an *mec1* point mutant strain (*esr1-1*) (7) four times with an extensively characterized wild-type strain (SX46A). Wild-type and mutant cells were synchronized in G_1 with the yeast pheromone α-factor, irradiated, and immediately released into fresh medium without α-factor as described previously (21). Samples were withdrawn during postirradiation incubation, and the cellular DNA content was analyzed by flow cytometry (fluorescence-activated cell sorting [FACS] analysis) (21). The G_1 delay found in UV-irradiated wild-type cells was reduced or, at sufficiently low doses, absent in *mec1* cells (Fig. 1). Comparable results were found for gamma-irradiated cells (data not shown). We observed a similar defect for a strain containing a different *mec1* allele (*sad3*) (1) compared with a congenic wild type (data not shown). Furthermore, a plasmid-borne *MEC1* gene complements the defect in G_1 arrest, and thus, variations of the

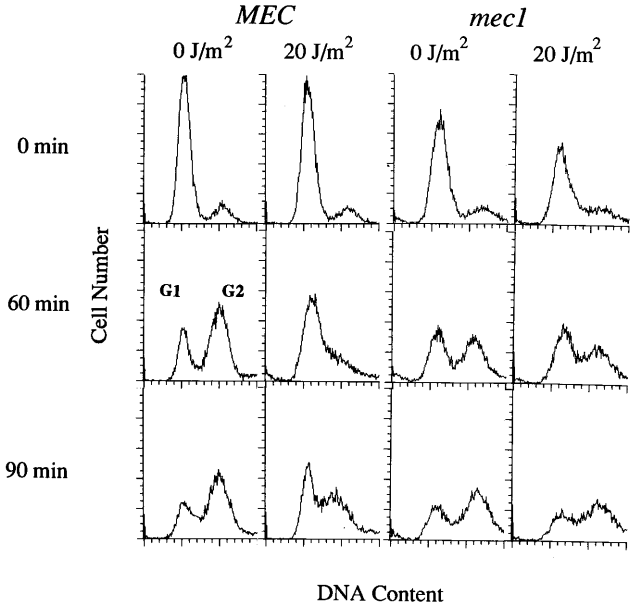

FIG. 1. DNA content of *MEC* and *mec1* cells synchronized in G_1 with α-factor and released from α-factor arrest following irradiation in suspension with UV light (254 nm) at 20 J/m^2 or mock treatment (survival, 91% for *MEC* and 19% for *mec1*). At the times indicated, samples were withdrawn, stained, and flow cytometrically analyzed for DNA content.

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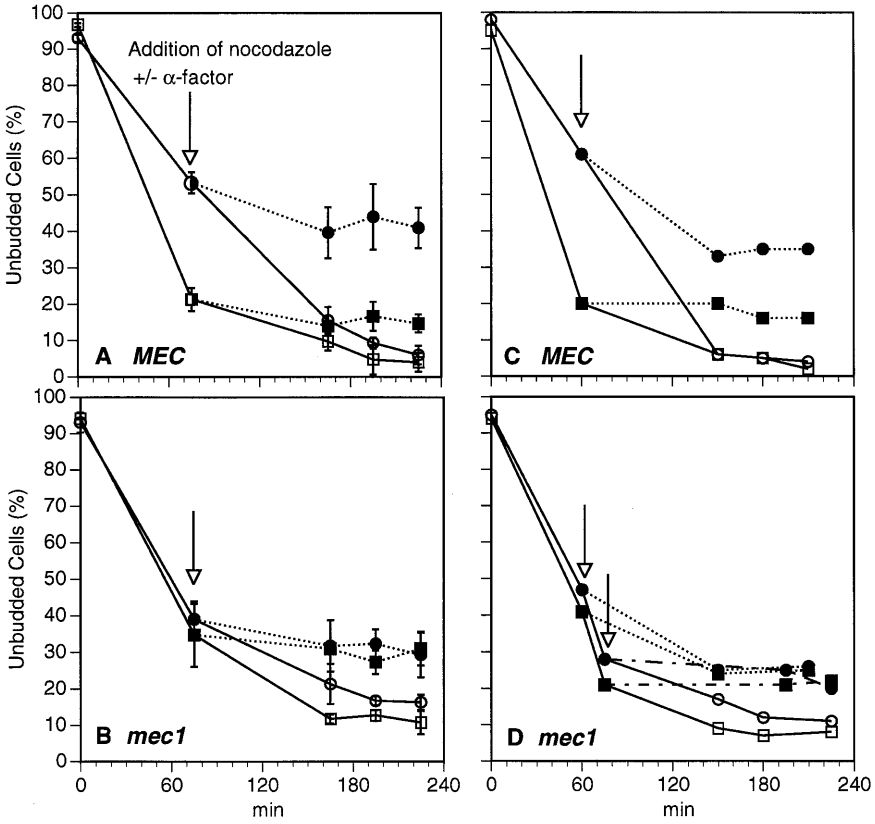

FIG. 2. Percentage of unbudded cells in α-factor-synchronized *MEC* and *mec1* cultures that were irradiated at 0 min with UV at 40 J/m^2 (A and B) (survival, 74% for *MEC* and 3% for *mec1*) or 20 J/m^2 (C and D) (circles) or were not irradiated (squares). As indicated by arrows, nocodazole (10 μg/ml) was added after 75 min (A, B, and D) or 50 min (C and D) of incubation in fresh medium, and incubation was continued. In order to evaluate the fraction of α-factor-responsive cells, α-factor (10 μg/ml) was added to part of the cultures at the time of addition of nocodazole (filled symbols). (A and B) Data represent the averages for three independent experiments; error bars indicate standard errors. (C and D) Data for representative single experiments are shown.

genetic background cannot account for the observed phenotype (data not shown).

We wished to characterize the arrest stage that is defective in *mec1* strains more precisely, since FACS analysis is not sensitive enough to detect a small increase in DNA content and hence cannot distinguish between a defect in cell cycle arrest in early S phase and one in G_1 phase. Sensitivity to α-factor is an important criterion for identifying yeast cells at or upstream of START (roughly equivalent to the mammalian restriction point). We therefore exposed cells at the time of radiation-induced arrest to α-factor again as well as to the tubulin inhibitor nocodazole, to prevent reentry of G_2 cells into G_1 (21). Given a sufficient amount of time, all cells that were no longer susceptible to α-factor arrest at the time of the addition had progressed farther in the cell cycle and remained trapped in G_2. G_1-arrested cells are morphologically unbudded single cells that can be readily distinguished from large-budded G_2 cells by microscopic examination. Hence, this technique permits an estimate of the fraction of cells at or upstream of START in the stage of radiation-induced G_1 arrest.

We added nocodazole after 75 min of incubation following irradiation with UV at 40 J/m^2 (Fig. 2A and B) and evaluated the fraction of unbudded cells following another 90 to 150 min of incubation. During this time period, the irradiated cultures had progressed from their G_1 arrest stage and virtually all cells had entered G_2 in both irradiated and unirradiated portions of the cultures (<10% unbudded cells for the wild type; the values for the *mec1* strain stayed somewhat higher, most likely because of cells in the synchronized cultures that never reenter the cell cycle). When α-factor was added together with nocodazole, the fraction of unbudded cells remained larger in the irradiated wild-type culture than in the unirradiated control, indicating checkpoint arrest at or upstream of START (Fig. 2A) while no such difference was evident in the cultures of the congenic *mec1* strain (Fig. 2B). We varied the UV dose and the time of addition of the drugs (Fig. 2C and D) but could not detect any condition that would reveal a significantly larger fraction of α-factor-sensitive cells in irradiated *mec1* cultures than in unirradiated controls (Fig. 2D). Simultaneous FACS analysis confirmed these results, but some DNA profile abnormalities due to extended nocodazole treatment were evident, especially for the *mec1* strain (data not shown). Comparable results were found for gamma ray-treated cultures (data not shown).

In conclusion, we have demonstrated the dependence of radiation-induced G_1 arrest in yeast cells on the presence of functional Mec1 protein and conclude that the various checkpoint defects in AT cells are indeed mirrored in yeast *mec1* mutants. This defect in G_1 arrest is shared by mutant strains defective in the protein kinase Rad53 (1) that had been placed downstream of Mec1 (18). The role of the *ATM* homolog Mec1 in G_1 checkpoint control in yeast cells may be of special interest since an involvement of the *ATM* gene product in a p53-dependent signal transduction mechanism controlling G_1 checkpoints has been suggested for mammalian cells. It is reasonable to suppose that impairment of this particular func-

tion contributes significantly to genetic instability in AT cells and to the proneness of AT patients to cancer. Our data suggest that the underlying mechanism(s) is evolutionarily conserved and hence might be profitably explored in the yeast *S. cerevisiae*.

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