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A Large-Scale Screen in *S. pombe* Identifies Seven Novel Genes Required for Critical Meiotic Events

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Summary

Meiosis is a specialized form of cell division by which sexually reproducing diploid organisms generate haploid gametes. During a long prophase, telomeres cluster into the bouquet configuration to aid chromosome pairing, and DNA replication is followed by high levels of recombination between homologous chromosomes (homologs). This recombination is important for the reductional segregation of homologs at the first meiotic division; without further replication, a second meiotic division yields haploid nuclei. In the fission yeast *Schizosaccharomyces pombe*, we have deleted 175 meiotically upregulated genes and found seven genes not previously reported to be critical for meiotic events. Three mutants (*rec24*, *rec25*, and *rec27*) had strongly reduced meiosis-specific DNA double-strand breakage and recombination. One mutant (*tht2*) was deficient in karyogamy, and two (*bqt1* and *bqt2*) were deficient in telomere clustering, explaining their defects in recombination and segregation. The *moa1* mutant was delayed in premeiotic S phase progression and nuclear divisions. Further analysis of these mutants will help elucidate the complex machinery governing the special behavior of meiotic chromosomes.

Results and Discussion

A Screen for Novel Meiotic Mutants

To identify genes required for successful meiosis, we took advantage of the recent meiotic transcriptome data of *S. pombe* [1] because previous studies of meiosis have revealed that many critical meiotic events are controlled by genes specifically induced during meiosis. Several laboratories have used microarrays to analyze the genome-wide expression pattern of meiotic cells [1–3] and found that meiotic genes can be grouped into temporal expression classes that correlate with functional meiotic landmarks. In order to identify new genes required for meiosis, several functional genomic approaches have recently been performed in budding yeast, including the systematic deletion of genes upregulated during meiosis [4]. These studies have successfully identified novel meiotic functions that in some cases are evolutionarily conserved. The meiotic transcriptome data of *S. pombe* revealed hundreds of meiotically upregulated genes [1], some of which are conserved at the level of protein sequence in both budding and fission yeast [1–3, 5]. We have therefore deleted a large set of previously uncharacterized genes upregulated during the meiotic program. The same approach has been used in a parallel study [6]; these studies are complementary because only 10% of the genes analyzed are common to both projects.

For our functional screen, we selected 184 *m*eiotically *u*pregulated *g*enes (*mug*) based on microarray expression data [1]. We have focused primarily on two classes of genes: "early genes," whose induction corresponds with premeiotic DNA synthesis, chromosome pairing, and recombination; and "middle genes," whose induction corresponds with chromosome segregation. Among these temporal classes of genes, we have selected for our analysis only those of unknown function (annotated in [7] and at http://www.sanger.ac.uk/Projects/S_pombe/ as sequence orphans, hypothetical proteins, or proteins with conserved domains and unknown function) that are meiotically upregulated by a factor of at least four, in the case of early genes (40 genes selected), or by a factor of at least seven, in the case of middle genes (135 genes selected). The parallel study [6] focused on middle genes. In addition, we selected six genes sharing a common biphasic expression pattern (induced early in meiosis, sharply repressed during MI and MII, and induced again just after MII) and three late-expressed genes (expressed during spore formation) encoding two putative protein kinases and one putative ubiquitin protein ligase. The whole collection of genes and their mutant phenotypes are listed on the website http://telecic.cicancer.org/pombe.

Of the 184 selected genes, the entire open reading frame (ORF) was successfully deleted by a PCR-based gene-targeting strategy in 175 cases [8; http://telecic.cicancer.org/pombe]. Deletions of 167 genes were nonlethal and were generated in a homothallic (h^{90}) haploid strain, which switches mating type, to give rise to cells of both mating types. In nitrogen-limiting medium, these cells mate to form homozygous diploids that enter the meiotic program. After transformation to G418 resistance, determined by *kanMX6*, correct deletion of the genes was checked by colony PCR and, in those mutants displaying meiotic phenotypes, confirmed by Southern blot hybridization.

Novel Mutants Deficient in Nuclear Segregation and Meiotic Recombination

As an initial approach for the detection of meiotic phenotypes, meiotic nuclear divisions and sporulation efficiency were systematically analyzed in the collection of

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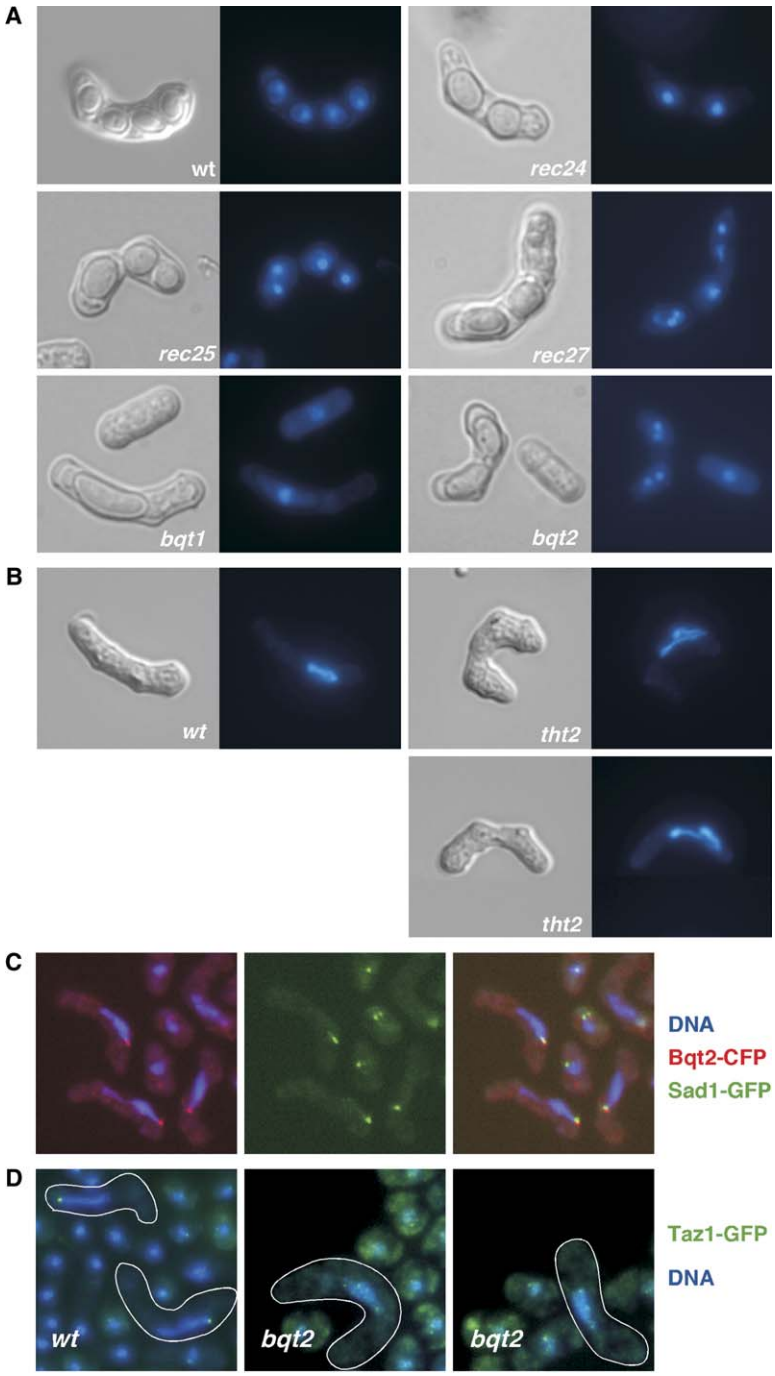

Figure 1. Aberrant Nuclear Behavior in *mug* Mutants

(A) Aberrant nuclear segregation in representative "class a" mutants (Table S1). Wild-type and mutant h^{90} strains were incubated for 2–3 days on malt extract agar medium to induce mating and sporulation, fixed, stained with DAPI, and observed under the fluorescence microscope. *rec24*, *rec25*, *rec27*, *bqt1*, and *bqt2* mutants are shown as examples. The phenotype of these "class a" mutants is a mix of the different nuclear-segregation defects shown in this figure; details of the frequency of abnormal nuclear segregation in each mutant are in Table 1.

(B) Twin horsetail nuclei in the *tht2* mutant. Cells were stained with DAPI during the horsetail stage (at 24 hr on MEA medium) and examined by microscopy. Approximately half of the cells had twin (side-by-side) horsetails at this time point.

(C) Colocalization of Bqt2 and the spindle pole body component Sad1. Cells expressing Bqt2-CFP and Sad1-GFP were examined during the horsetail stage by microscopy. In all of 20 cells examined, the foci colocalized. Left panel, Bqt2-CFP. Middle panel, Sad1-GFP. Right panel, merged images.

(D) Bqt2 is required for telomere clustering. Wild-type and *bqt2* mutant cells expressing Taz1-GFP were examined during the horsetail stage by microscopy. Taz1-GFP appeared as a single focus in 19 of 19 wild-type cells and in 0 of 16 *bqt2* cells.

167 viable *mug* mutants. Strains were allowed to mate and sporulate [9], and zygotic asci were examined microscopically for spore number and morphology, as well as for the number and relative size of DAPI-staining bodies (nuclei). As controls, well-known mutants deficient in critical meiosis-specific events, such as *rec12* (recombination; homolog of *S. cerevisiae SPO11*), *rec8* (sister chromatid cohesion and recombination), and *sgo1* (sister centromere cohesion), were generated from the same h^{90} strain and included for comparison in our phenotypic analysis.

Thirty-three *mug* mutants (20%) showed a meiotic phenotype and fell into three classes (see Table S1 in the Supplemental Data available with this article online). (a) Thirteen *mug* mutants displayed aberrant segregation of nuclei as manifested by abnormal number and size of spores and of DAPI-staining bodies; (b) fifteen *mug* mutants were defective in spore formation (no spores or abnormal spore morphology) but contained four equal-sized nuclei, except for *mug36*, *mug66*, *mug77*, *mug78*, and *mug179*, which contained 15%–20% of asci with more than four DAPI-stained bodies; and (c) five *mug* mutants showed a mixed phenotype (defective nuclear segregation and defective spore formation). Images of asci from representative *mug* mutants belonging to phenotypic "class a" are presented in Figures 1A and 1B.