Meiotic double-strand breaks in *Schizosaccharomyces pombe*

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Meiotic DNA double-strand breaks (DSBs) are associated with recombination hot spots in the yeast *Saccharomyces cerevisiae* and are believed to initiate the process of recombination. Until now, meiosis-induced breaks have not been shown to occur regularly in other organisms. Here we show, by pulsed-field gel electrophoresis of DNA, that meiotic DSBs occur transiently in all three chromosomes of the fission yeast *Schizosaccharomyces pombe*. In a repair defective mutant, carrying a mutation in the *RecA* homolog gene *rhp51*, meiotic DSBs accumulate. In contrast to expectation from the genetic map of *S. pombe*, however, many chromosomal DNA molecules remain unbroken during meiosis.

**Key words** Meiosis · DNA double-strand breaks · DNA repair · Fission yeast

**Introduction**

Meiosis reduces the genetic complement of eukaryotic cells from diploid to haploid. During meiosis the cells undergo one round of DNA replication followed by two successive nuclear divisions, resulting in the formation of four haploid spores. In the first meiotic division the homologous chromosomes segregate from each other and in the second division the sister chromatids dissociate. Recombination and the resulting chiasmata ensure the proper segregation of homologues at the first meiotic division in most eukaryotic organisms.

In the budding yeast, *Saccharomyces cerevisiae*, there is compelling evidence that double-strand breaks (DSBs) initiate meiotic recombination (Sun et al. 1989; Cao et al. 1990; Game 1992; Zenvirth et al. 1992; Goldway et al. 1993; Roeder 1995). This evidence includes the physical occurrence of meiosis-specific double-strand breaks and the correlation between the location of these breaks and hot spots for recombination. The broken DNA molecules are further processed by resection of the two 5′ ends of each break, to create 3′ overhangs (Sun et al. 1991). Later, “joint molecules” were isolated from meiotic cells (Collins and Newlon 1994; Schwacha and Kleckner 1994), which are believed to be transition structures between the stage of resected ends and the fully recombinant molecules. Analysis of whole meiotic chromosomes by pulsed-field gel electrophoresis has demonstrated that DSBs are widespread in the yeast genome (Game 1992; Zenvirth et al. 1992). There are numerous “preferred sites” of meiotic DSB breaks on each chromosome, which have been mapped for the three short chromosomes of *S. cerevisiae* (Klein et al. 1996a; Baudat and Nicolas 1997). In a given “preferred site”, meiotic DSBs do not occur at a unique DNA sequence, but are found to vary in their exact location among individual molecules, over a region of 150 to 270 bp (de Massy et al. 1995; Liu et al. 1995; Xu and Kleckner 1995; Xu and Petes 1996). These regions show meiosis-induced sensitivity to micrococcal nuclease (Ohta et al. 1994), thus resembling an open chromatin structure. Many of the genes involved in the formation and repair of meiotic DSBs have been identified in *S. cerevisiae*. The genes *RAD50*, *SPO11*, *XRS2*, *MRE11*, *MEI4*, *MER1*, *MER2*, *MRE2*, *REC102*, *REC104* and *REC114* have been shown to be involved in DSB formation (see reviews by Smith and Nicolas 1998; Paques and Haber 1999). The *RAD51* (Shinohara et al. 1992) and *DMC1* (Bishop et al. 1992) genes encode proteins that are homologous to the bacterial *RecA* strand-exchange protein, and are involved in the repair of the meiotic DSBs. In cells with *rad51* or *dmc1* mutations, the DSBs are only partially processed, and broken molecules with 3′ overhangs accumulate.

In *rad50S* mutant meiotic cells, unprocessed DSBs are accumulated, with Spo11 protein covalently bound.
to the 5′ ends of the broken DNA (Keeney et al. 1997). Spo11p, a member of the DNA topoisomerase VI group of proteins, was suggested to be the meiotic endonuclease (Bergerat et al. 1997), although it has not yet been demonstrated to have DNA cleavage activity. Putative Spo11 homologues have been identified in archeabacteria, fission yeast (Bergerat et al. 1997; Keeney et al. 1997), nematodes (Dernburg et al. 1998), Drosophila (McKim and Hayashi-Hagihara 1998) and mouse (Keeney et al. 1999). The Schizosaccharomyces pombe homologue of Spo11, rec12, was shown to be induced in meiosis and to be required for meiotic recombination (Lin and Smith 1994). Conservation of the Spo11 protein among eukaryotic organisms suggests that the initiation of meiotic recombination by DNA double-strand breaks may also be conserved. This is also supported by the finding that yeast artificial chromosomes (YACs) comprised mainly of human DNA are also subjected to double-strand breakage at specific sites during yeast meiosis (Klein et al. 1996b). Therefore, the rate of meiotic DSB formation on the YACs appears to reflect the level of meiotic recombination in human meiosis in the chromosomal regions from which the YAC DNA originated. Nevertheless, DNA double-strand breaks have not been reported during meiosis in any organism other than budding yeast.

Here we report the occurrence of DNA DSBs in meiotic cells of the fission yeast S. pombe. In strains in which the repair machinery is functional, the breaks are transient, whereas in cells in which repair of the DSBs is compromised (by a mutation in rhp51, the homologue of the budding yeast RAD51), broken DNA molecules appear to accumulate.

**Materials and methods**

**Strains and growth conditions**

*S. pombe* haploid strain #60 [pat1-114(ts) ade6-216 h+] was provided by Dr. P. Nurse. The double mutant strain pat1-114(rhp51A) was derived from a cross between strain #60 and strain JAC1.51Δ (ade6-704 leu1 ura4 rhp51Δ:ura4+ h+). The resulting haploid strain, DZ21, has the genotype pat1-114(ts) ade6-216 rhp51Δ:ura4+ h+. Yeast were grown in standard liquid or solid Yeast extract media (Gutz et al. 1974) supplemented with 225 mg/l of adenine, histidine, leucine, uracil and lysine hydrochloride (YES).

**Induction of meiosis**

Starters of the pat1-114(ts) haploid strains were grown overnight in 2 ml of YES medium at 23 °C. The starters were diluted 1:500 in YES and grown overnight at 23 °C, to 5–7 × 10⁶ cells/ml. Meiosis was induced by transferring the cells to Malt extract (ME) medium (Gutz et al. 1974) supplemented as YES, but without lysine, and with a temperature shift to 34 °C. In order to shift the cells to the higher temperature as rapidly as possible, ME was prewarmed to 34 °C before re-suspension. Cell samples of 20 ml each were taken immediately upon transfer into ME (0 h) and after various times in ME; the cells were washed, re-suspended in 5 ml of EDTA 0.05 M and kept at 4 °C.

**DNA manipulations**

Chromosomal DNA was extracted from meiotic cultures using the following protocol provided by Drs. J. Hayles and P. Nurse (ICRF, London): cells were washed (twice in CSE (20 mM citrate phosphate pH 5.6, 40 mM EDTA, 1.2 M sorbitol) and re-suspended in 2 ml of CSE with 3 mg of Zymolase-20T. After incubation for 1 h at 37 °C, cells were pelleted and re-suspended in 0.2 ml of TSE (10 mM Tris-HCl pH 7.5, 45 mM EDTA, 0.9 M Sorbitol). An equal volume of 1% LGT (low-gelling agarose) in TSE was added and aliquots were dispensed to a plug mold. Cells were lysed by incubating the plugs in ETS (0.25 M EDTA, 50 mM Tris-HCl pH 7.5, 1% SDS) for 90 min at 55 °C, and then transferred to 1% lauryl sarcosine, 0.5 mM of EDTA, 1 mg/ml of Proteinase K and incubated at 55 °C for 48 h (fresh Proteinase K was added after 24 h). Plugs were washed three times with 50 mM of EDTA and stored at 4 °C. DNA electrophoresis was done in 0.8% agarose and 1x TAE buffer (Sambrook et al. 1989), on a CHEF-DR II apparatus (Bio-Rad), under the following conditions: 2100 s pulses, 57 V, 72 h. Fresh buffer was introduced after 36 h. Alkaline Southern blots using Hybond-N+ nylon membrane (Amerham) was performed according to the manufacturer’s recommendation, and then hybridized to a 32P-labeled probe according to Sambrook et al. (1989). The probe was stripped from the membrane, which was then re-probed, as described earlier (Zervinth et al. 1992). Broken DNA molecules of chromosome III were detected using as a probe a 1.8-kb HindIII fragment from plasmid pUC18uvrA (Ayoub et al. 1999) consisting of the gene ura4. A 6.3-kb HindIII fragment from plasmid pBSSK-12, consisting of the gene mat2 (N. Ayoub and A. Cohen, personal communication), was used as a probe for the detection of broken molecules of chromosome II.

**Results and discussion**

DSBs occur transiently in pat1-114(ts) haploid cells that undergo meiosis.

The original observations of meiotic DSBs in *S. cerevisiae* (Sun et al. 1989; Cao et al. 1990; Goldway et al. 1993) were facilitated by several experimental features. First, cells went through meiosis in a synchronized fashion, so that one could hope to detect even rare and transient events. Second, Southern analysis of meiotic DNA preparations focused on recombination hot spots, where one could expect to find a relatively frequent occurrence of the molecular events that initiate recombination. Thus the regions near ARG4 (Sun et al. 1989), HIS4: LEU2 (Cao et al. 1990) and THR4 (Goldway et al. 1993) were examined. Third, mutants were available, notably rad50S (Cao et al. 1990), in which repair of the breaks was not expected to occur, and therefore DSBs were expected to accumulate, resulting in stronger signals in the molecular analysis.

In *S. pombe*, highly synchronized meiosis can be obtained in haploid strains containing the temperature-sensitive mutation pat1-114(ts). This mutation enables haploid cells to undergo meiosis when cells are shifted from the permissive (23 °C) to the restrictive temperature (34 °C). An attempt to identify meiotic DSBs in pat1-114(ts) mutant cells was already reported by Bähler et al. (1991), who examined the region near the recombination hot spot of ade6-M26, a well known meiotic