letters to nature

HO induction

Strains were grown in YP-lactate (1% Bacto yeast extract, 2% Bacto peptone, 3% lactic acid, pH 5.5) and HO induction was done as described previously⁴.

5'-3' strand resection at a DSB

Resection was measured as a rate of HO-cut band disappearance. In some cases the resection of sequences distant from the break was also measured. Purified genomic DNA, digested with *Eco*RI (Fig. 3) or *Styl* (Fig. 4), was separated on a 1% agarose gel, transferred to Hybond N⁺ and probed with ³²P-labelled DNA from *MAT*a and from sequences 20 kb proximal to the DSB to establish the rate of resection. Hybridization to *HIS4* or *LEU2* (both more than 100 kb away) was used to normalize the amount of DNA at each time point. The rate of resection at each time point was plotted as the percentage of the density of the initial cut band. The density of the HO-cut band at t = 1 h was set to 100%.

NHEJ efficiency

NHEJ was examined in a donorless *cdc28-as1* strain. 1-NMPP1 inhibitor was added 30 min before *HO* induction. Re-cutting of *MAT*a by HO was prevented by filtering cells out of galactose-containing medium 30 min after DSB induction and diluting cells into YP-dextrose. (1% Bacto yeast extract, 2% Bacto peptone, 2% dextrose, pH 5.5). The efficiency of NHEJ was determined as the intensity of the *MAT*a-containing restriction fragment 3 h after *HO* induction, normalized to the amount of DNA.

Analysis of homologous recombination

MAT switching and other homologous recombination events were analysed on Southern blots⁴. For *MAT* switching, genomic DNA was digested with *StyI* and *BgIII* (Fig. 2a) or with *StyI* (Fig. 2b–d) and probed with a *MAT*-distal probe. For allelic recombination, DNA was digested with *BgIII* and *PvuI* and probed with the *MAT*-distal probe to check the efficiency of HO induction and with a *MAT*-proximal probe to check the appearance of the product. Initial new DNA synthesis after strand invasion was determined by polymerase chain reaction as described⁴, normalized to the amount of *ARG5,6* DNA.

Chromatin immunoprecipitation

Chromatin immunoprecipitation was performed as described previously^{4.6}. Antibodies against Mre11, Rad51 and RPA were provided by J. H. J. Petrini, A. Shinohara and S. Brill, respectively.

Western blots

Protein extracts were performed as described³. Antibodies used for western blots were Rad53 polyclonal antibody JD47 (a gift from J. Diffley), Mre11 polyclonal antibody (produced by the IFOM antibody facility) and monoclonal antibodies 9E10 (anti-Myc epitope), 12CA5 (anti-HA (haemagglutinin) epitope) and 6D2 (ref. 11) (anti-B subunit).

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erratum

High-resolution structure of a retroviral capsid hexameric amino-terminal domain

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In this Letter, the following sentence should have appeared after the author's email address: 'Coordinates and structure factors have been deposited in the Protein Data Bank under accession number 1U7K'.