Supplementary Material

Materials and methods

Plasmids

pFOX2 is a pUC8-based plasmid containing his3+ and ade6-L469 (Osman et al., 2000). PMW700 and pMW701 were made by inserting a blunt-ended MscI-SmaI RTS1 fragment from pBZ142 (Dalgaard and Klar, 2001) into the HincII site between his3+ and ade6-L469 in pFOX2. pMW700 carries RTS1 in orientation 1, whereas pMW701 carries it in orientation 2 (see Figure 2A for definition of orientations). pMW702 and pMW703 contain the same RTS1 fragment inserted at the HincII site within ade6-L469 of pFOX2. The RTS1 element is in orientation 1 in pMW702 and orientation 2 in pMW703.

Strains

The strains used in this study are listed in the Table in Supplementary Material. Wild-type strains with the intrachromosomal recombination substrate containing the RTS1 element were made by the integration of the appropriate plasmid (pMW1767 – 1770), which had been linearised with BlpI, at ade6-M375 in FO1236. Southern blotting and/or colony PCR was used to determine that the linear plasmid had integrated at the correct site.

Probes

Probes A and C are restriction fragments from pFOX2 (EcoNI – XbaI and EcoRV – PstI, respectively). Probes B and D are DNA fragments amplified from genomic DNA by PCR using primers oMW704 (5’-TGAATAATGTGCTGTGAAGC-3’) and oMW705 (5’-
AAGAACCTACTGAGCCTACG-3’) for probe B, and oMW706 (5’-AAAGGCCTCGCTTCTCGAG-3’) and oMW707 (5’-AGCAGCATACGCTAAAATC-3’) for probe D. Probes were labelled with [α-32P]dATP and [α-32P]dTTP by random prime labelling.

**Recombination assays**

Mitotic recombination was assayed by the recovery of Ade+ recombinants from strains containing the intrachromosomal recombination substrate. To measure the effect of CPT and HU on recombinant frequency colonies were resuspended in YES and incubated for 2 hours at 30°C prior to splitting the culture. 10 μM CPT or 10 mM HU was then added to one half of the culture and the other half was left untreated. Incubation was then continued at 30°C prior to washing the cells in water and plating them at indicated times as described (Osman et al., 2000).

**Preparations of S. pombe genomic DNA**

To isolate DNA for running 2-D gels, 1 litre cultures of *S. pombe* were grown overnight at 30°C. The cells were then killed by adding 0.1% sodium azide (Sigma) followed by pouring them onto frozen 40 mM EDTA. Cells were washed in chilled water prior to DNA isolation by CTAB extraction (Lopes et al., 2003). Purified DNA was digested with the appropriate restriction endonuclease(s) for 5 hours at 37°C. NaCl was then added to the digest to a final concentration of 1 M, before loading the sample onto a 0.5 – 1 ml benzoylated naphthoylated DEAE (BND)-cellulose (Sigma) column, pre-equilibrated in NET buffer (1 M NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 8.0)), to enrich for the
replicating DNA. After washing the column with 5 ml NET buffer, the DNA, containing replication intermediates, was eluted with 1.5 ml of pre-warmed (50°C) NET buffer plus 1.8% caffeine. The DNA was then precipitated with isopropanol and resuspended in TE.

The preparation of genomic DNA in agarose plugs was done using a CHEF genomic DNA plug kit (Bio-Rad) according to the manufacturer’s instructions. The plugs were made in 0.75% agarose. DNA in plugs was digested over night at 37°C with 40 – 100 units of restriction enzyme. Plugs were typically cut in half to enable duplicate loadings on the same 1-D gel. The subsequent Southern blot could therefore be cut in half, allowing each half to be probed with a different probe.

**2-D and 1-D gels**

Neutral/neutral 2-D gels were run as described (Brewer and Fangman, 1987). The first dimension was run in a 0.4% agarose gel for ~36 hours at 25 V in 1 x TBE. The second dimension was run at 4°C overnight in a 1% agarose gel at 125 V in 1 x TBE containing 0.3 μg/ml ethidium bromide. The buffer was continuously recirculated in the second dimension. 1-D gels were either 0.7 or 0.8% agarose and were run in 1 x TBE at 50 - 60 V for ~16 hours. Gels were Southern blotted onto Duralon-UV™ membrane (Stratagene) and probed with the indicated ³²P-labelled DNA fragments. Blots were analysed by phosphorimaging using a Fuji FLA3000 and Image Gauge software.
### Supplementary Table. *Schizosaccharomyces pombe* strains

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<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<td>MCW39</td>
<td>h- <em>ura4-D18 leu1-32 his3-D1 ade6-M375</em> int::pUC8/his3+/ade6-L469</td>
<td>Lab strain</td>
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2/ade6-L469

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ade6-M375 int::pUC8/his3+/RTS1 site A orientation
1/ade6-L469

MCW1688  h+ rad22Δura4+ ura4-D18 leu1-32 his3-D1 arg3-D4  This study
ade6-M375 int::pUC8/his3+/RTS1 site A orientation
2/ade6-L469

MCW1695  h+ rhp51Δarg3+ rad22Δura4+ ura4-D18 leu1-32 his3-D1  This study
arg3-D4 ade6-M375 int::pUC8/his3+/RTS1 site A orientation 1/ade6-L469

MCW1696  h+ rhp51Δarg3+ rad22Δura4+ ura4-D18 leu1-32 his3-D1  This study
arg3-D4 ade6-M375 int::pUC8/his3+/RTS1 site A orientation 2/ade6-L469

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MCW1445  h+ rqh1Δlanr ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M375 int::pUC8/his3+/ade6-L469/ RTS1 site B orientation 1

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