Table 1. Effects of pheromone treatment

We constructed prototrophic strains of *S. cerevisiae* with the *tps1* or the *tps2* mutations. Y440 is the parental strain prototrophic for all amino acids except leucine (*MATa leu2-3,112*). The mutant gene was synthesized by PCR and used to replace the wild-type copy of *TPS1* or *TPS2* in the chromosome (PCR analysis to confirm presence of the deletions; heat-shock sensitivity phenotype conferred by *tps1* and *tps2* deletions was confirmed). Y2150 is isogenic to Y440 except for *tps1::LEU2*; Y2151 is isogenic to Y440 except for *tps2::LEU2*; and Y2152 is isogenic to Y2150 except for being capable of growing on glucose. The cells were grown at 30 °C in 2 % peptone, 1 % yeast extract and 2 % galactose. For G_1 arrest determination, fixed cells were sonicated briefly to disrupt any clumps. Cells were diluted and counted in the haemocytometer by microscopic examination. G_1 arrest was determined by the ratio of shmoo cells to total cell number. For the pheromone assay, strains were grown for approximately 16 h to an OD_{600} of 0.6. α factor was added to a final concentration of 10 $\mu\text{g ml}^{-1}$. Aliquots were taken each hour and cells were fixed with 20 % formaldehyde; cell morphology was assessed by direct microscopic inspection.

Strains	Budding index at 0 h	Shmoo index at:		
		1 h	4 h	8 h
Y440	0.69±0.02	0.12±0.02	0.99±0.01	0.80±0.02
Y440 Leu ⁺	0.69±0.03	0.24±0.12	0.99±0.01	0.78±0.02
Y2150	0.67±0.04	0.23±0.14	0.99±0.01	0.38±0.03
Y2152	0.66±0.02	0.13±0.02	0.99±0.01	0.34±0.04
Y2151	0.68±0.02	0.21±0.08	0.99±0.01	0.80±0.01

operates with a delay often involving a number of modifications of cell surface receptors. As trehalose 6-phosphate is known to regulate at least one membrane-linked enzyme (hexokinase II), it might also interfere with the cytosolic portion of membrane receptors. After MATa cells are exposed to pheromone, the α factor receptor is rapidly hyperphosphorylated on its cytosolic tail (9), causing its desensitization. A kinase responsible for this phosphorylation has not been identified. It would be informative to analyse the number of α factor receptors in the membrane of a *tps1* mutant after pheromone treatment, compared to wild-type cells.

Conformational changes in repressor or inducer proteins could be induced by interactions with small molecules, and such changes are crucial to a transcriptional regulatory system that could rapidly respond to different environmental conditions. A number of gene products involved in recovery are known to be induced by the pheromone signal transduction pathway itself, so that adaptation arises from built-in feedback loops. The immediate target of the activated G protein that is critical for subsequent signal propagation is not known, nor is it known if the effector acts by modulating the level of any classical second messenger. However, five protein kinases have been identified as required for signal transmission, and the activity of at least three of these enzymes is increased when the pathway is stimulated. The pleiotropic effects of the *tps1* mutation may interfere with the phosphorylation/dephosphorylation process. In order to test this hypothesis it would be necessary to verify the levels of phosphorylation in the *tps1* mutant. There is evidence to suggest a role for Ca^{2+} /calmodulin-dependent phosphoprotein phosphatase in the recovery process (3) and an influx of Ca^{2+} occurs late during the mating process (7). Therefore,

another possibility for the role of trehalose 6-phosphate in the recovery process could be as a Ca^{2+} channel regulator. Without trehalose 6-phosphate and in the presence of α factor, an increase in Ca^{2+} influx would occur, leading to a faster desensitization.

The molecular basis of *TPS1* function has not been revealed, but it is evident that it has extremely wide-ranging effects. The complex phenotype associated with *tps1* mutations leads to the speculation that the trehalose-6-phosphate synthase complex may be a broadly acting modulator or regulator of signalling processes in yeast cells.

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**Patricia M. B. Fernandes
and Anita D. Panek***

Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, 21949-900, RJ, Brazil.

*For correspondence. Tel/fax: +55 21 290 7248. e-mail: anita@acd.ufrj.br

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Recombination is unaffected by mutation of *Escherichia coli* mfd

Escherichia coli Mfd protein targets excision repair proteins to damaged DNA in the transcribed strand, thus ensuring preferential repair of coding sequences (5). Recent studies have shown that *Bacillus subtilis* has a 1177-residue Mfd homologue that has similar *in vitro* properties (1). Mutation of *B. subtilis* Mfd causes a deficiency in homologous recombination. We set out to test whether mutation of the *mfd* gene in *E. coli* had a similar deleterious effect on recombination. Moreover, since Mfd displays significant homology to the recombination protein RecG (5), a potential overlap in activities was examined by analysing the phenotype of *mfd recG* double mutants.

Neither *mfd* null (UNC361045) nor deletion (UNCNOMFD) mutants (6, 7) exhibited significant deficiencies in repair (both UV and ionizing radiation) or conjugational recombination (Table 1). The results suggest that Mfd in *E. coli* is not involved in recombination. However, it was possible that another protein such as RecG could be substituting for its function. RecG mediates the branch migration of Holliday junctions during recombination (4). A $\Delta recG::\text{Km}$ mutation was therefore transduced into UNC361045. The resulting *mfd recG* construct (GS1860) is slightly more UV-sensitive than a single *recG* mutant (Table 1). However, this is to be expected from a combination of mutations from different repair pathways. Such modest increases in UV-sensitivity have also been observed with *mfd recA* (5) and *mfd ruv* strains (see below). Conjugational recombination and double-strand break recombination repair (γ -radiation survival data) in GS1860 were not significantly different from the *recG* single

Table 1. Effect of *mfd* mutation on recombination and DNA repair

To measure DNA repair, cells were grown in LB medium (containing 0.5 g NaCl l⁻¹) to an OD₆₅₀ of 0.4. Appropriate dilutions in 56/2 salts media were applied to agar plates and allowed to dry before exposing to UV light or a caesium-137 γ source. The UV dose was 60 J m⁻² and the γ -radiation dose was at 44 krad. Plates were incubated at 37 °C for 16 h and surviving colonies were scored. Conjugal recombination was measured by recovery of Pro⁺ transconjugants after 40 min mating using HfrC (KL226). Recombination and viability measurements were performed as described by Lloyd & Buckman (3). The absolute values for AB1157 viability and recombination were 2.5×10^8 and 1.0×10^7 cells ml⁻¹, respectively. All values are the mean of at least two independent experiments.

Strain (genotype)	Viability	UV	γ	Recombination
AB1157 (wild-type)	1.00	0.47	0.51	1.00
UNC361045 (<i>mfd</i>)	0.99	0.30	0.46	0.88
N3793 (<i>recG</i>)	0.82	0.025	0.024	0.16
GS1860 (<i>mfd recG</i>)	0.80	0.0089	0.026	0.24

mutant (Table 1). Essentially identical results for recombination and DNA repair were obtained with a combination of $\Delta mfd::Km$ and *recG*162 alleles (data not shown). These observations indicate that there is no functional overlap between Mfd and RecG with respect to homologous recombination.

Mutations in *recG* do show a functional overlap with *ruv* mutants which are defective in the RuvABC Holliday junction resolution system (2). *mfd* was therefore tested for interaction with *ruv*. Double mutants were constructed by transducing the $\Delta mfd::Km$ allele into N2057 (*ruvAB60::Tn10*).

Recombination and repair of ionizing radiation damage were no different from the single *ruvAB* mutant, although there was a 50–60 % reduction in survivors following exposure to UV light at doses between 20 and 60 J m⁻² (data not shown).

The experiments described here provide persuasive evidence that Mfd does not participate in homologous recombination in *E. coli*. This contrasts with the situation reported in *B. subtilis* and may indicate that the mechanism of recombination is different between these organisms or that the *B. subtilis* Mfd has additional functions that *E. coli* Mfd lacks.

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Gary J. Sharples* and Lisa M. Corbett

Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK.

*For correspondence. Tel: +44 115 970 9404.

Fax: +44 115 970 9906.

e-mail: gary.sharples@nottingham.ac.uk

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