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Transposon mutagenesis reveals novel loci affecting tolerance to salt stress and growth at low temperature

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Abstract Using transposon mutagenesis in the haploid Saccharomyces cerevisiae strain W303-1A we have identified genes required for growth in high salt medium, survival of a hypo-osmotic shock and growth at 15 °C. Screening 25,000 transposon insertions revealed a total of 61 insertions that caused salt-sensitivity; and those insertions affected 31 genes. Only 12 of those genes were previously known to be required for salt-tolerance. Among the 61 insertions, three caused general osmo-sensitivity. We identified one single insertion mutant in the already-known gene, FPS1, required for survival of hypo-osmotic shock. A total of 31 insertions caused failure to grow at low temperature. Those identified ten different genes, three of which had previously been reported to affect cold-tolerance. Four genes were identified in both the salt and the cold-sensitivity screen. We found several unusual insertion mutations: (1) insertions in or close to essential genes, (2) insertion in an intergenic region and (3) insertions causing stress-sensitivity in W303-1A, while the deletion mutant in BY4741 did not show such a phenotype. Surprisingly, our mutant set and that reported in the large-scale transposon insertion project (TRIPLES, http://yacammed.yale.edu/triples/triples.htm) only marginally overlap. We discuss some of the features of transposon mutagenesis in light of the availability of the complete set of yeast deletion mutants and we discuss the possible roles of the genes we identified.

Keywords Functional genome analysis · Stress responses · Low temperature stress · Saccharomyces cerevisiae

Introduction

The availability of the complete genome sequence of Saccharomyces cerevisiae has enabled novel approaches to study gene function. Recently, two projects have resulted in large sets of yeast mutants. Using a PCR approach, an international consortium has generated a largely complete set of deletions of all of the annotated 6,138 yeast genes; and the set is available to the research community, both as haploids and as homo- and heterozygous diploids (Winzeler et al. 1999; Delneri et al. 2001; Vidan and Snyder 2001). In an alternative approach, Snyder and colleagues employed random transposon mutagenesis in Escherichia coli of genomic yeast libraries and subsequently transformed yeast cells with that library (Ross-Macdonald et al. 1997, 1999b). The transposon integrates into the yeast genome via homologous recombination, generating an insertion mutation (Ross-Macdonald et al. 1997). The use of appropriate transposons allows for the simultaneous analysis of gene expression, protein localisation and mutant phenotypes. The approach has been used to study the properties of more than 11,000 strains carrying insertions with a known location in the yeast genome (Ross-Macdonald et al. 1999a). In addition, screens based on transposon insertions for genes involved in pseudohyphal growth, cell surface assembly and zinc homeostasis have been reported (Lussier et al. 1997; Mösch and Fink 1997; Yuan 2000).

Transposon mutagenesis is not based on the computer-assisted annotation of yeast genes and hence may target previously unrecognised loci. In fact, the large-scale analysis of transposon insertions has already led to...
the identification of 300 previously unrecognised yeast genes (Ross-Macdonald et al. 1999a). Dujon and colleagues partially sequenced the genomes of 13 related yeast species and, by sequence comparison, provided evidence that many annotated ORFs are unlikely to be real genes (Blandin et al. 2000). The same analysis identified about 50 new genes and corrected the size of 26 known genes. These observations suggest that the annotation of the yeast genome is far from complete and, while the total number of yeast genes may well be around 6,000, the identity of about 10% of those might change with further analysis.

Transposon insertion mutagenesis has potential additional advantages. In contrast to complete gene deletion, the insertion of a transposon may lead to partial phenotypes and hence to the identification of novel functions. In addition, as demonstrated in previous work, it may allow the identification of different functional domains in a given protein (Ross-Macdonald et al. 1999a). In this work, we generated mutants by transposon insertion and subsequently compared phenotypes to deletion mutants generated in the systematic gene deletion project. We have used transposon insertion solely as a convenient mutagen that allows easy identification of the gene affected.

The initial incentive of this analysis was to identify genes that are required for the survival of a hypo-osmotic shock, i.e. the sudden shift of cells from high to low medium osmolarity. Only mutations in one single gene have so far been reported to cause such a phenotype (Luyten et al. 1995; Tamás et al. 1999). FPS1 encodes a glycerol facilitator required for the rapid export of the osmolyte, glycerol, upon hypo-osmotic shock (Luyten et al. 1995; Tamás et al. 1999). Sensitivity to hypo-osmotic shock is clearly different from the sensitivity to low medium osmolarity and the requirement of an osmotic stabiliser for growth, which is observed for mutants with defects in cell wall assembly (Torres et al. 1991; Klis 1994). However, those mutants were eliminated from our screen, since yeast transformants were selected in low osmolarity medium.

In order to study the survival of a hypo-osmotic shock we first had to shift cells to high osmolarity. We also used this step to select mutants that were unable to grow at elevated salt concentrations. In addition, since relatively little is known about the genetic basis for growth at low temperature, a very common experience in yeast life, we also searched for mutants unable to grow normally at 15 °C.

Altogether we screened 25,000 viable transposon insertion mutants. We found 76 insertions conferring at least one of the above phenotypes and identifying 38 different genes. We described phenotypes for several genes for which previously no functional information was available and we identified novel phenotypes for already-known genes. Surprisingly, the overlap between our mutant set and the TRIPLES collection (Ross-Macdonald et al. 1999a; searched in March 2001 at TRIPLES: http://ygac.med.yale.edu/triples/basic_search.asp) is minimal. In addition, in many instances we found phenotypic differences between the transposon insertion and the deletion mutant.

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**Materials and methods**

Strains and culture conditions

The *S. cerevisiae* strains used in this study are listed in Table 1. Yeast cells were routinely grown at 30 °C in either complete (YPD) or minimal synthetic (SD) media. YPD medium contained 1% yeast extract, 2% bacto-peptone and 2% glucose (Merck). SD medium contained 0.17% yeast nitrogen base without amino acids (Bio101), 0.5% ammonium sulphate (Merck), 2% glucose and appropriate amino acids (30 μg/ml; Bio101). Solid medium contained 2% agar (Merck). SD media were adjusted to pH 6.

The *E. coli* strain used in this study was DH5α (BRL, Bethesda, Md.). It was grown at 37 °C in LB medium (1% yeast extract, 2% bacto-peptone, 2% sodium chloride (Merck)) or on plates (LB medium plus 2% agar), with or without ampicillin (50 μg/ml; Merck).

Generation of mutants using a transposon insertion yeast genomic library

Yeast genomic libraries with random transposon insertions were kindly provided by Drs. Petra Ross-Macdonald and Michael Snyder (Yale University; see http://ygac.med.yale.edu/ for details).

Briefly, the library contained genomic DNA fragments cloned into vector pHSS6. This library was then mutagenised, using transposon TsN::LEU2::lacZ (Seifert et al. 1986; Burns et al. 1994). The mutagenesis treatment was repeated in 15 independent experiments, resulting in 15 different pools, which we used in independent yeast transformations.

The mutated yeast DNA was released from vector DNA by digestion with *NotI* (Seifert et al. 1986; Burns et al. 1994). Typically, 100–200 ng of purified DNA were transformed into W303-1A, using the lithium acetate transformation procedure (Gietz et al. 1995). Transformants were selected on SD medium lacking leucine. This procedure typically yielded about 2,500 transformants per experiment.

Screening and analysis of transposon insertion mutants

Subsequently, leucine-positive transformants were transferred to microtitre plates with 100 μl of YPD and grown overnight at 30 °C (Fig. 1). Then, 5 μl of each culture was transferred to two large (14.4 cm) YPD plates and one large YPD plate containing 1.4 M NaCl. One YPD plate was incubated at 30 °C and served as a control (see Table 2 for details on growth conditions). The other YPD plate was incubated at 15 °C and growth was scored after 5 days. The NaCl plate was incubated at 30 °C and growth was only scored after 3 days. Subsequently, this plate was replica plated onto a fresh YPD plate, incubated at 30 °C overnight and then scored for inability to resume growth after hypo-osmotic shock. Mutants that scored positive in this screen were further analysed by growth assays, using serial dilutions and spotting onto agar plates. Cells were pregrown in liquid medium overnight, the cell density was normalised to an optical density at 600 nm (OD<sub>600</sub>) of 0.25 and cells were allowed to resume growth until an OD<sub>600</sub> of 0.5. Then, 10 μl of tenfold serial dilutions were spotted onto appropriate solid media and incubated for 1–5 days at conditions outlined in Table 2.

Southern blot analysis of transposon insertions

Mutant yeast genomic DNA was digested with *EcoRI*, separated in a 1%-agarose gel, transferred onto a Hybond-N+ nylon