Adaptation and major chromosomal changes in populations of
Saccharomyces cerevisiae

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Summary. Thirteen independent populations of Saccharomyces cerevisiae (nine haploid and four diploid) were maintained in continuous culture for up to approximately 1000 generations, with growth limited by the concentration of organic phosphates in medium buffered at pH 6. Analysis of clones isolated from these populations showed that a number (17) of large-scale chromosomal-length variants and rearrangements were present in the populations at their termination. Nine of the 16 yeast chromosomes were involved in such changes. Few of the changes could be explained by copy-number increases in the structural loci for acid phosphatase. Several considerations concerning the nature and frequency of the chromosome-length variants observed lead us to conclude that they are selectively advantageous.

Key words: Chromosome length variants – Adaptation – Yeast – Continuous culture

Introduction

The genomes of prokaryotes appear to be much more labile (Krawiec and Riley 1990) than those of higher eukaryotes (e.g., O'Brien, Seuanez and Womack 1988). Duplications, sometimes involving large sections of the genome, occur at a high frequency in Salmonella and other prokaryotes (Krawiec and Riley 1990; Petes and Hill 1988), and it has been suggested that large-scale genomic changes represent an important mechanism of adaptation in such organisms (Anderson and Roth 1977; Sonti and Roth 1989). Genome rearrangements caused by inversions and translocations are also seen frequently in prokaryote populations (Krawiec and Riley 1990) and may be important agents of adaptive change (Ornston et al. 1990). Less is known of the evolutionary role that deletions may play, though comparison of the genomes of Escherichia coli and Salmonella typhimurium suggest that large deletions may have been significant factors in the evolutionary divergence of these two species (Riley 1984).

Surprisingly little is known of the role that large-scale genomic rearrangements play in evolution and adaptation in yeast populations, though strain-specific differences in chromosome size are known to exist (Link and Olson 1991). There is also some evidence that industrial populations of baker's yeast undergo radical changes in their genomic structure (including changes in ploidy level and chromosome loss (S. Fogel personal communication). In this paper we describe large-scale chromosomal rearrangements that have occurred in populations of haploid and diploid strains of Saccharomyces cerevisiae evolving in organic-phosphate-limited environments for approximately 700–1 000 generations. We show that: (1) adaptation is frequently accompanied by large-scale duplications of the genome, (2) large deletions also occur but are less frequent and (3) that some populations have developed polymorphisms for chromosome length.

Materials and methods

Strains. The populations were initiated with either the haploid strain CP1AB-1A, its diploid parent strain CP1AB (Paquin and Adams 1982), or with strain PO43-283B. This last strain contains a duplication of the right arm of chromosome II, including the acid-phosphatase loci PH03 and PH05, translocated to chromosome XVI (S. Puskas-Rozsa and J. Adams, unpublished results), and therefore carries two copies of PH05-1, the wild-type allele of PH05. The left terminus of the duplicated region of chromosome II was mapped to a position between PH05 and TSM134, no more than 4 cM to the left of PH05 (Hansche et al. 1978). Strain PO43-283B was constructed, by conventional genetic procedures, from strains S07 (a mating type) and S12 (a mating type) which carry pho5, a null allele of PH05 on chromosome II, and also an allele of PH05 conferring high acid-phosphatase activity on the duplicated region (Hansche et al. 1978). Strains S07 and S12 (Hansche et al. 1978) were crossed and the resulting diploid, A225, sporulated. Rare recombinant events between chromosome II and the translocated region result in the production of ascospores carrying the null allele (pho5) on both chromosome II and the translocated region. Such recombinants were identified from the analysis of
dissected ascii which showed a 3:1 segregation ratio for acid-phosphatase activity (+ : -). Presence or absence of inducible acid-phosphatase activity was determined by plate assay as described by Hansche et al. (1978). An acid-phosphatase negative strain carrying the duplication (A225-1A), identified in this way, was then crossed to strain CPIAB-1A, and the resulting diploid sporulated. A similar analysis of segregation ratios for acid-phosphatase activity allowed the identification of strain PO4-238B, carrying the wild-type allele for PHO5, derived from CPIAB-1A, on both chromosome II and on the duplicated/translated region of chromosome II.

**Media, growth and sampling.** Batch cultures were grown at 30 °C in a gyratory shaker at 150–200 gyrations/min. Continuous cultures were operated as chemostats and maintained at 30 °C in culture vessels between 150 and 350 ml in volume. Dilution rates in the continuous cultures were 0.17 h⁻¹, equivalent to a cell generation time of approximately 4.1 h. The defined organic-phosphate-limited medium buffered at pH6, described by Adams and Hansche (1974), was used with the following modifications; the major salts (that is, the five salts added in concentrations ≥1 mM) were used at 1.5 x the concentration described, glucose concentration was 0.8% (w/v), and organic phosphate concentration was 0.2 mM of PO₄. The organic phosphates employed were phosphoglyceric acid (PGA), β-glycerol phosphate (βGP), glucose-1-phosphate (G1P), glucose-6-phosphate (G6P) and uridine-5-monophosphate (UMP), all obtained from the Sigma Chemical Co., St. Louis, MO. Glucose, vitamins and the organic phosphates were filter-sterilized using a "Sterivex GS" filter (Millipore Corp., Bedford, MA), and added to the medium after autoclaving. Preliminary experiments demonstrated that the cultures were indeed growing under phosphate limitation, as doubling the concentration of any of the organic phosphates resulted in a doubling of the equilibrium culture population density. The concentration of organic phosphate was lowered in three of the long-term populations, at generations 415 (population J), 299 (population K), and 693 (population E), maintaining cell density within the range 3 x 10⁸−9 x 10⁹, in order to ensure that growth continued to be limited by the concentration of organic phosphate. Cell number was estimated using an electronic particle counter (Celloscope 111, Particle Data Inc. Elmhurst, Ill.; or Coulter Counter ZM, Hialeah, Fla), after sonication with a Braunsonic 1510 sonicator and a needle probe to separate clumped cells. Continuous cultures were sampled approximately every 25 generations and an aliquot stored in 40% glycerol at −70 °C for later analysis. YEPD medium contained 1% yeast extract, 2% bacto-peptone, and 2% glucose. For solid medium 1.5% agar was added.

**Preparation of chromosomal DNA for pulse-field gel analysis.** Stationary-phase cultures (10 ml) were pelleted and washed twice in 50 mM EDTA (pH 7.5). Pellets were resuspended in 2 ml of 50 mM EDTA (pH 7.5) and heated to 37 °C in a heating block. A 1% solution of Seakem agarose (FMC Bioproducts, Rockland, ME) in 0.125 M EDTA (pH 7.5) was heated to boiling and cooled to 45 °C. Then 38 μl of zymolase solution (1 M sorbitol, 100 mM sodium citrate, 60 mM EDTA, 0.1% zymolase, 0.8% β-mercaptoethanol; final pH 5.6) and 2.5 ml of agarose solution were added to 2 ml of the cell suspension. The agarose-cell mixture was shaken, dispensed into plexiglass slots (1 mm × 28 mm × 4 mm) to facilitate the formation of the agarose plugs, and allowed to cool. The solidified plugs were extruded from the slots, covered with buffer (0.5 M EDTA, 0.15% Tris-HCl, 7% β-mercaptoethanol; pH 7.5), and incubated at 37 °C overnight. The overlay was then replaced with NDS buffer (0.5 M EDTA, pH 8.0, 0.15% Tris-HCl, 1% lauryl sarcosine; 0.1% protease K added immediately before use), and the samples incubated at 50 °C overnight. NDS buffer was removed and replaced with 50 mM EDTA, pH 8.0. Samples were incubated at 50 °C for 2–3 days with four changes of EDTA. Whole chromosomes embedded in agarose were stained in 50 mM EDTA at 4 °C for up to 1 month (Carle and Olson 1985; Vollrath and Davis 1987).

**Chromosome separations.** Prepared chromosome samples were cut to fit the wells of a 1% agarose (Boehringer Mannheim (Indianapolis, IN) low EEO agarose) gel in 1 x TBE buffer (Maniatis et al., 1982). Chromosomes were separated by the CHEF method of pulse-field gel electrophoresis (Chu et al. 1986) in 0.5 x TBE buffer. The running buffer was recirculated through 4 °C coolant, resulting in a running temperature of the gel of about 8 °C. Various combinations of voltage and switching times were used to optimize separation of the different size ranges. The sizes of the smaller chromosomal length variants were estimated by linear interpolation using bacteriophage lambda concatemers ("ladders") as size markers. Sizes of the larger chromosomal length variants were estimated by linear interpolation using the chromosome bands of CPIAB-1A as size markers. Estimates of the sizes of these chromosomes were obtained from Mortimer and Schild (1985).

**DNA manipulations and hybridization procedures.** Details of the procedures for preparation of total yeast DNA and probes, and of the procedure for DNA-DNA hybridization have been described previously (Adams and Oeller 1986). Identities of the chromosome-specific hybridization probes are described by Carle and Olson (1985). For one chromosome length variant observed in population J, chromosome assignment could not be made using the chromosome-specific probes. In this case, the chromosome was excised from the gel and the DNA purified using "GeneClean" (Bio 101 Inc., La Jolla, Calif.), following the protocols recommended by the manufacturer. The DNA was then radioactively labelled by the random priming method, using a kit supplied by Boehringer-Mannheim, and used as probe. The ladders were prepared as described previously (Carle and Olson 1984).

**Estimation of copy-number changes in the structural loci for acid phosphatase.** The DNA to be analysed was digested either with EcoRI and Clal or with PstI restriction endonucleases (Boehringer Mannheim), using protocols suggested by the manufacturer, and the fragments separated by electrophoresis on 0.7% agarose "submarine" gels (Maniatis et al. 1982). Double digestion with EcoRI/Clal results in a single band containing both the PHO5 and PHO3 genes, and two more bands each composed of homologous sequences from both PH010 and PH011, while digestion with PstI allows the four structural genes for acid phosphatase to be resolved into four distinct bands detectable by DNA-DNA hybridization. The acid-phosphatase genes were derived from pAP18 and pAP20, described by Andersen et al. (1983). Due to the homology shared by these genes (Hinnen et al. 1987), it is possible to detect all four loci with any one of the probes under slightly reduced stringency conditions. pAP18 is composed of pBR322 with a 5 kb EcoRI insert containing the l 401 bp open reading frame of PH011, plus approximately 1.4 kb and 2.1 kb from outside the S' and 3' ends of the gene respectively. pAP20 is composed of pBR322 with an 8 kb EcoRI insert containing both of the l 401 bp open reading frames from the directly oriented, tightly linked PH05 and PH03 genes. Probes were the entire pAP18 plasmid when probing for the PH011 and PH010 genes, and a 3.5 kb Clal fragment from pAP20 when probing for PH05 and PH03. The 3.5 kb Clal fragment from pAP20 was obtained by elution from agarose gels using Schleicher and Schuell (Keene, NH) NA45 DEAE cellulose paper. This 3.5 kb Clal fragment contains both of the PH05 and PH03 open reading frames, a 451 bp intergenic region separating the two genes, and a total of 319 bp from the extreme ends of the tandem pair. Upon initial examination of the autoradiographs by eye it was apparent that the terminal clones from several of the populations had experienced an increase in copy number of some of the four acid-phosphatase loci. Since each lane of the gels was loaded with approximately the same amount of DNA, between-lane comparisons of hybridization intensities were sufficient to indicate the qualitative differences in copy number. Differences apparent from observation of the conventional gels were also confirmed by visual and/or densitometric analysis of hybridization spectra of pulse-field gels hybridized with the same probes. The relative copy number of each of the acid-phosphatase loci was estimated by first scanning individual lanes of the autoradiographs with a laser densitometer (LKB Ultrascan XL, LKB Produkter AB, Bromma, Sweden). Then, one of the