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Research Article

Structural characterization of chromosome I size variants from a natural yeast strain

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Abstract

Many yeast strains isolated from the wild show karyotype instability during vegetative growth, with rearrangement rates of up to 10^{-2} chromosomal changes per generation. Physical isolation and analysis of several chromosome I size variants of one of these strains revealed that they differed only in their subtelomeric regions, leaving the central 150 Kb unaltered. Fine mapping of these subtelomeric variable regions revealed gross alterations of two very similar *loci*, *FLO1* and *FLO9*. These loci are located on the right and left arms, respectively, of chromosome I and encompass internal repetitive DNA sequences. Furthermore, some chromosome I variants lacking the *FLO1* locus showed evidence of recombination at a DNA region on their right arm that is enriched in repeated sequences, including Ty LTRs. We propose that repetitive sequences in many subtelomeric regions in *S. cerevisiae* play a key role in karyotype hypervariability. As these regions encode several membrane-associated proteins, subtelomeric plasticity may allow rapid adaptive changes of the yeast strain to specific substrates. This pattern of semi-conservative chromosomal rearrangement may have profound implications, both in terms of evolution of wild strains and for biotechnological processes. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: karyotype variability; wine yeast; macroarrays; FLO genes; subtelomeric genes; repetitive sequences

Introduction

Karyotype instability during vegetative growth is a common feature of many wild yeast strains (Adams *et al*., 1992; Bakalinsky and Snow, 1990; Codon and Benitez, 1995; Gasent-Ramírez *et al*., 1999; Longo and Vezinhet, 1993; Miklos *et al*., 1997; Nadal *et al*., 1999; Pérez-Ortín *et al*., 2002a; Pérez-Ortín *et al*., 2002b). Its prevalence in natural yeast strains suggests that it confers some evolutive advantage, e.g. chromosomal rearrangements may be a major source of genetic variability in strains with an essentially asexual life cycle. However, it is difficult to reconcile the potentially devastating effects of gross chromosomal rearrangements, such as chromosome loss and loss of heterozygosity, among others, with their putative advantageous effects.

We have characterized strain DC5, a natural wine yeast strain with high karyotype instability, with rearrangement rates of 10^{-2} chromosomal changes per generation during vegetative growth in non-selective medium (Carro and Piña, 2001). This value is two to three orders of magnitude higher than the standard rate of recessive mutations in haploid *Saccharomyces* strains (10^{-5}) and that of spontaneous chromosome loss and rearrangements in diploid laboratory strains (10^{-4}; Hiraoka *et al*., 2000). Such a high rearrangement rate allowed us to study and to isolate several chromosome size variants to characterize the precise nature of these rearrangements. We focused on size variants from chromosome I, one of the most variable chromosome bands in DC5, because they are easy to

identify in PFGE gels as the bands with the highest mobility.

Gross chromosomal rearrangements, involving the fusion of large regions from both homologous and non-homologous chromosomes, occur both in laboratory and in wild yeast strains with frequencies close to the standard mutation rates, from 10^{-4} to 10^{-5} (Puig *et al*., 2000; Hiraoka *et al*., 2000; Umezu *et al*., 2002), or even lower, 10^{-8} (Chen and Kolodner, 1999). These rare events may give decisive advantages to rearranged clones in certain circumstances, e.g. the increased resistance to sulphite of some wine strains has probably evolved after a crossing over mediated by microhomology between chromosomes VIII and XVI (Pérez-Ortín *et al*., 2002b). However, they cannot account for rapid evolutive adaptation to new media, e.g. we have reported several episodes of rearranged clones displacing the parental DC5 strain out of the culture during vegetative growth in rich media (Carro and Piña, 2001). Here we attempt to identify the type of chromosomal rearrangements responsible for these relatively frequent episodes of adaptation to the medium.

Our results demonstrate that chromosome I size variants differed only in the structure of their right and left arms, with most changes limited to 20–30 Kb of DNA from both telomeres. Clonal variations occurred only in the telomere-proximal region of the left arm. Fine mapping of these ends indicated that repeated sequences played a crucial role in the generation of chromosome I size variants in DC5. This pattern of clonal variation may provide a suitable mechanism of rapid adaptation of *S. cerevisiae* strains to the environment, since these regions encode several gene families related to sugar assimilation and to cell membrane and cell wall functions (Vega-Palas, 2000; Harrison *et al*., 2002).

Materials and methods

Yeast strains

Strain DC5 was isolated and characterized from a collection of wine yeast strains from El Penedès, the main sparkling-wine producing region of Spain (Carro and Piña, 2001; Nadal *et al*., 1999). The laboratory yeast strain W303d (MAT**a**/α, *ura3, leu2, his3, trp1, ade1*) was obtained from the Yeast Stock Center, Berkeley, CA, USA.

Culture medium and conditions

All strains were propagated in YEPS medium (5 g/l yeast extract, 20 g/l sucrose, 10 g/l peptone; Pronadisa, Madrid, Spain) at 30 °C with continuous shaking (250 rpm). YPD plates contained 5 g/l yeast extract, 20 g/l glucose, 10 g/l peptone and 20 g/ Bacto-agar (Pronadisa).

Serial cultures

Single colonies of DC5 were grown in YEPS at 30 °C in 15 ml culture tubes. After 24 h of culture in a roller, when cultures reached near-saturation (A_{600} above 10 in these strains), they were used to inoculate fresh tubes to $A_{600} = 0.05$. The process was repeated until these serial cultures completed 100 doublings, as calculated from the original and final optical densities of each serial culture. Thereafter, a sample of the last tube was spread on a YPD plate (5 g/l yeast extract, 20 g/l sucrose, 10 g/l peptone, 20 g/l Bacto-agar; Pronadisa). At least nine clones were picked, grown in YPD and stored at −80 °C after addition of 50% glycerol. These frozen stocks were used for further analysis.

Karyotype analysis

Yeast cells from late exponential phase cultures were embedded in low melting point agarose (Pronadisa). The resulting plugs were incubated first with lyticase and then with proteinase K (both from Sigma, Saint Louis, MO, USA) to digest yeast wall and yeast proteins, as described elsewhere (Gerring *et al*., 1991). Yeast chromosomes were separated by pulsed field gel electrophoresis (PFGE) in a Hula-Gel apparatus (Hoefer, San Francisco, CA) at 200 V, using a pulse ramp from 60 s to 150 s for 50 h in 0.5× TBE buffer (TBE: 100 mM Tris-hydroxymethylaminomethane, 100 mM borate, 5 mM EDTA, pH 8.4) at 12 °C. Inverted field gel electrophoresis (IFGE) was performed using a switch-back pulse controller (Hoefer) in 0.5× TBE horizontal 1% agarose gels following the manufacturer's instructions for resolution of *S. cerevisiae* chromosomes: 200 V for 24 h at 4 °C with a pulse ramp from 1 s to 30 s with a forward:reverse (F:R) ratio of 3 : 1.

DNA isolation

Yeast genomic DNA was extracted as described elsewhere (Querol *et al*., 1992) with minor modifications. A dense culture was washed in 50 mM

EDTA (pH 7.5) and treated with Lyticase (Sigma, 1 mg/ml) and RNAse A (Sigma, 20 mg/ml) for 1 h at 37 °C. After centrifugation, the cell pellet was resuspended in 800 µl lysis buffer (50 mM Tris-HCl, 10 mM EDTA, 20 g/l SDS, pH 8.0). Upon addition of 150 µl 5 M KOAc/HOAc (pH 4.8), the cells were left on ice for 1 h and spun down in an Eppendorf centrifuge for 15 min. The supernatant was then phenolized three times, extracted once with phenol:chloroform:iso-amylalcohol 25 : 24 : 1, and precipitated with 2 volumes of EtOH (−20 °C, 30 min).

PCR protocols

Polymerase chain reaction (PCR) was performed using 1 unit DyNazyme Ext DNA polymerase (Finnzymes, Espoo, Finland) or Biotools DNA polymerase (Biotools B&M, Madrid, Spain), 0.1 ng DNA (plasmids) or 10 ng genomic DNA, and 10 pmol each primer. After an initial denaturation step for 5 min at 94 °C, primers were annealed for 1 min at 45 °C and extension proceeded for 3 min at 72 °C. After re-denaturation for 1 min at 94 °C, the cycle was repeated 30 times in total.

Southern blot

In situ digestion of agarose plugs was performed as previously described (Carro and Piña, 2001). DNA fragments were separated by IFPGE in 1% agarose, 0.5× TBE-gel electrophoresis, following the manufacturer's guidelines for DNA fragments of 5–250 Kb. The program consisted in two runs, both at 200 V and 4 °C. The first run included 10 min of run-in (no pulse), followed by 12 h of a pulse ramp from 1 s to 20 s with a F:R ratio of 3 : 1. The second run was performed for a further 12 h, with a pulse ramp from 0.8 s to 1.5 s with a F:R ratio of 3 : 1. The gel was then denatured and blotted onto Hybond-N^+ filters (Amersham-Pharmacia, Uppsala, Sweden) in accordance with the manufacturer's instructions. DNA probes for YAL020C, YAL040C, YAL066W, YAR023C, YAR031W and YAR053W were obtained by PCR using the oligonucleotides listed in Table 1 and DNA from W303d as template. DNA fragments were labelled with fluorescein-12-UTP (Roche, Mannheim, Germany) by the random primer protocol (Ready-to-Go, Amersham-Pharmacia). Pre-hybridization was performed in 50% formamide, 0.25 M sodium phosphate buffer, pH 7.2, 7% SDS, 1 mM EDTA and 50 µg/ml salmon sperm DNA (Sigma) at 42 °C for 4 h. Hybridization was performed at 42 °C overnight in the pre-hybridization solution plus the labelled DNA probe. The fluorescein-labelled probe was detected by an alkaline phosphatase-linked antibody (Fluorx-AP, Tropix, Bedford, MA, USA), following the manufacturer's instructions, using CDP-Star (Boehringer, Mannheim, Germany) in 0.1 M diethanolamine, pH 10, 1 mM $MgCl_2$, as chemiluminescent substrate. Chemiluminescence was recorded by exposing Kodak X-OMAT AR (Kodak Ltd., London, UK) films for 2–15 min at room temperature.

DNA sequence analysis

S. cerevisiae genome sequences were obtained and analysed from SDG (**http://genome-www.stanford.edu/Saccharomyces/**).

Minichip design and printing

To characterize isolated chromosome I size variants, we designed a minichip (2 × 6 cm) containing 14 probes from ORFs regularly distributed (about 5–15 apart) along the entire chromosome I (see Figure 3A, for a schematic representation). Probes were generated by PCR amplification from a clone collection. About 50 ng each PCR fragment was printed onto Nylon-N^+ membranes (Amersham Pharmacia Biotech) using the BioGrid arrayer robot (by BioRobotics Ltd.).

Table 1. Primers used in this work

Flo1U	5′-AAGGAACCGTCTATATGTACGCTG-3′
Flo1L	5′-TGATGAAGAAGAATATGTAGGAGA-3′
Flo135	5′-GTTCCATGGCTGAGTTGTAGTCAT-3′
Flo9U	5′-TGAAGACGAATATGTAGACTTTGG-3′
Flo9L	5′-GACTCTGCAATTCTATCAGTCGGT-3′
YAL020C-U	5′-TTGTGTGTATGCGTTTGGGT-3′
YAL020C-L	5′-CGGCTACTTTACCTTTGAGA-3′
YAL040C-U	5′-CAATCTATCGACCTCGACTT-3′
YAL040C-L	5′-CTTGGCCAAAAGATGCTTGA-3′
YAL066W-U	5′-TGCATTGTGAGTTGGTTGCT-3′
YAL066W-L	5′-TATCCAGCCCTGTGTTTTCA-3′
YAR015W-U	5′-TTACGAAGACTGAACTGGACG-3′
YAR015W-L	5′-TGAGACCATTTAGACCCTGT-3′
YAR023C-U	5′-TTGAGGTTTGAAATGTTCGAC-3′
YAR023C-L	5′-CGCAGATTATTTCGAATTTG-3′
YAR031W-U	5′-CGTGACTTTATATAGTGGG-3′
YAR031W-L	5′-GTGGTGTTTTTGGGGCAA-3′
YAR053W-U	5′-AGTGTCTTCATTGCGTCCTT-3′
YAR053W-L	5′-GCAAGAAATGAATAACCACCA-3′

The chromosome I ORF probes included in the minichips were (from left to right arm): YAL066W (1), YAL062W (2), YAL058W (3), YAL047C (4), YAL040C (5), YAL032C (6), YAL025C (7), YAL020C (8), YAL009W (9), YAR002W (10), YAR015W (11), YAR031W (12), YAR053W (13), YAR071W (14). Two more ORFs, YFL016C and YCL043C, from chromosomes VI and III, respectively, were included as chromosome I purification controls. Positive (+, *S. cerevisiae* genomic DNA) and negative (−, *E. coli* genomic DNA) hybridization controls were also included in the minichip. Spots labelled c included only sample solvent without any DNA. The specific position of each ORF on the minichip is shown in Figure 4B. All the ORF DNAs and controls were spotted as duplicates.

Minichip hybridization and analysis

Individual chromosomal bands were extracted from IFGE gels with ELU-Quick (Schleicher & Schuell), and labelled with ^{33}P by the random primer protocol (Ready-to-Go, Pharmacia). The minute amounts of DNA obtained by this protocol (less than 100 ng) prompted us to use the minichip technology, which allowed hybridization with only 1 ml total volume, with a great improvement in sensitivity. Pre-hybridization was performed in 1 ml 5× SSC (sodium saline citrate), 5× Denhardt's and 0.5% sodium dodecyl sulphate at 65 °C for 1 h. Hybridization was performed at 65 °C overnight in the pre-hybridization solution plus the labelled DNA probe. Processed filters were then scanned with a Phosphorimager (BioRad, Hercules, CA). Average values from the two spots for each ORF were used. Raw (R_i) values were corrected for background (negative controls, N; Figure 3) and for positive control values (P) as follows:

$$I_i = \frac{R_i - N}{P - N}$$

Results

Physical structure of chromosome I size variants from DC5

Clonal karyotype variations of the natural yeast wine strain DC5 during vegetative growth implicate both high and low mobility chromosomal bands (Carro and Piña, 2001; see also Figure 3). In many cases, they were particularly evident in the two fast-migrating chromosomal bands, which we tentatively identified as two size variants of chromosome I (Carro and Piña, 2001). In order to confirm this assignment, we hybridized a PFGE-containing karyotype of the laboratory strain W303d and of DC5 with subtelomeric probes for both arms of chromosome I, YAL066W and YAR053W. As seen in Figure 1A, a Southern blot using the left-arm probe (YAL066W) gave the expected two bands for DC5 and a single one for W303d. By contrast, the right-arm probe (YAR053W) showed no hybridization signal in DC5, whereas it gave the two expected bands for W303d, since YAL053W has a closely related sequence in chromosome VIII. This sequence showed a very weak signal in DC5, indicating that also this region is either missing or much altered in DC5 (Figure 1A). We then hybridized the same blot with two centromere-proximal right-arm probes, YAR031W and YAR023C (Figures 1A and 1B). Whereas YAR023C showed the expected two-band pattern for DC5, YAR031W only detected the chromosome I upper band, indicating that the two size variants of chromosome I in DC5 diverged in their right arms. Such a divergence persisted when we analysed different size variants of chromosome I from DC, obtained after 100 doublings in rich media (Carro and Piña, 2001; Figure 2, top). In all cases, YAL066W hybridized to the two bands, whereas YAR031W only hybridized to the lowest-mobility band (Figure 2).

The published sequence for *S. cerevisiae* chromosome I encompasses three *Sma*I cutting sites, and predicts four digestion products of 17679, 53501, 59232 and 99791 bp (Table 2 and Figure 2,

Table 2. Calculated and predicted sizes for *SmaI* digestion products of DC5 chromosome I

Probe(s)	Calculated sizes (Kb)	Predicted sizes (bp)
YAR031W YAR015W YAR023C	75.2	99791
YAL020C	19.3	17679
YAL040C	57.5	53501
YAL066W	68–58 (v) 53–45 (v)	59232

(v), bands with clonal variation.

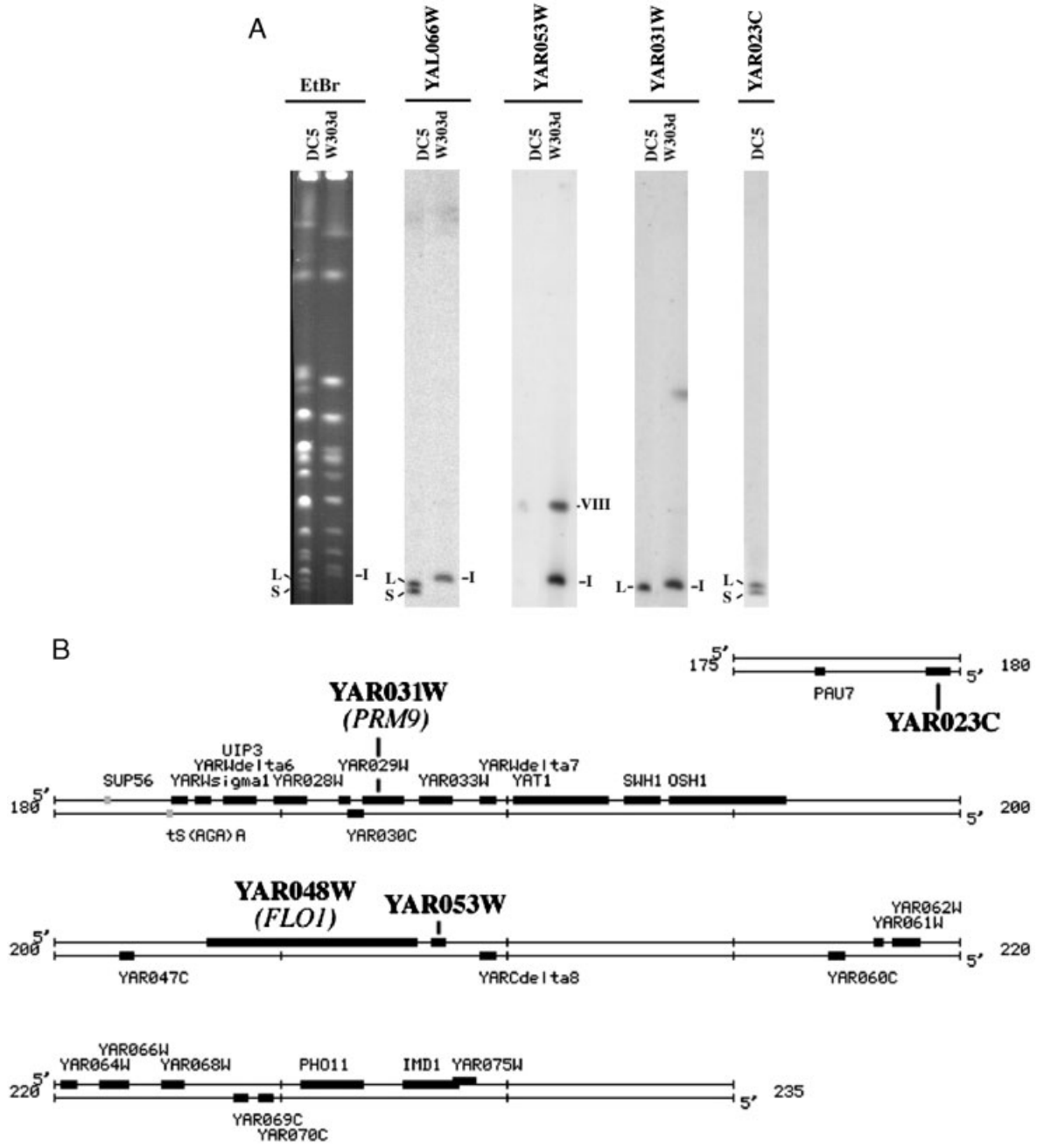


Figure 1. Characterization of chromosome band variants from DC5. (A) PFGE karyotypic profiles of DC5 (left) and of the laboratory strain W303d (right). The left-most panel shows an ethidium bromide-stained gel; the other panels correspond to Southern blots using the chromosome I probes indicated at the top of each panel. Where appropriate, low- and high-mobility chromosome I bands from DC5 are indicated by L and S, respectively. Positions of chromosomes I and VIII from W303d are indicated on the right of the appropriated panels by I and VIII signs, respectively. (B) Structure of the right arm of chromosome I of *Saccharomyces cerevisiae.* The published positions of the probes used in (A) are indicated. Numbers represent distances from the left telomere in kilobases. Data from **http://genome-www.stanford.edu/Saccharomyces/**

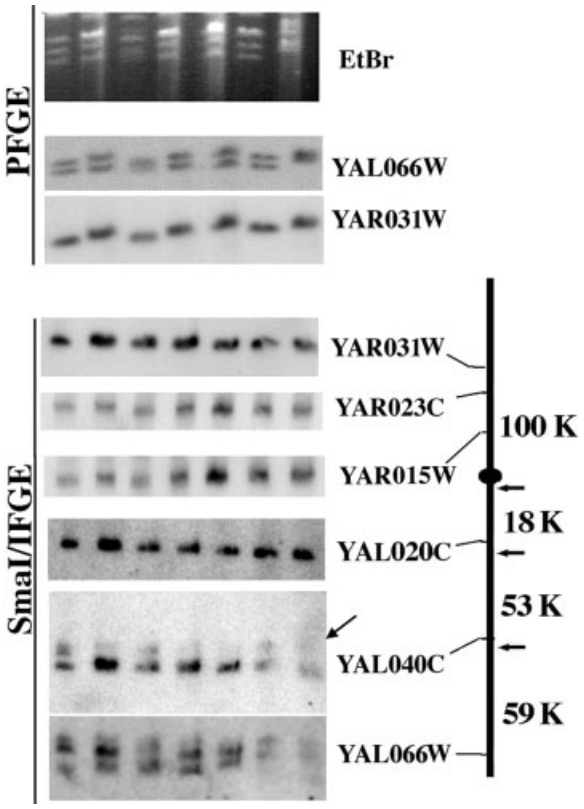


Figure 2. Structural characterization of chromosome I variants from DC5. (Top) PFGE karyotypic profiles of seven clones randomly picked after 100 doublings of a single DC5 clone in YPD. Only the gel portion where chromosome I variants run is shown. Other panels correspond to Southern blots of either the same gel (panels indicated as PFGE on the left) or IFGE analysis of the *Sma*I digestion products from the same agarose plugs (indicated on the left). Small arrow on the right indicates a non-specific band appearing when the probe YAL040C was used; it was not further considered. The diagram on the right shows the expected positions of *Sma*I cutting sites and of the positions of the relevant probes in the published sequence for chromosome I. Sizes of the expected *Sma*I restriction fragments are indicated on the right. In this picture, the right arm of chromosome I is at the top. An oval indicates the position of the centromere

horizontal arrows). Agarose plugs containing chromosomes from individual DC5 clones were digested with *Sma*I and the resulting fragments analysed by IFGE. The gel was then blotted onto a nylon membrane and hybridized with probes specific for the predicted digestion fragments (Figure 2, five bottom panels). Calculated sizes for the bands observed for each probe are shown in Table 2. The physical map of chromosome I from the wild strain DC5 agreed with the predicted published sequence, with the exception of the largest *Sma*I fragment, which was significantly shorter for DC5 (75.2 Kb) than expected from the published sequence (99.8 Kb, Table 2). From the predicted sequence, this fragment should encompass the centromere and the complete right arm of chromosome I (Figure 2). Unexpectedly, this DNA fragment showed a single band when hybridized with either YAR015W or YAR023C (Figure 2). This is at variance with the observed divergence between left arms of the two chromosome I size variants (Figure 1). We concluded that the lack of YAR031W and of the rest of telomere-proximal ORFs was not a consequence of a truncation, but rather of a recombination event that did not implicate a gross change on the total length of the chromosome I left arm. Clonal size differences for chromosome I bands were evident in PFGE gels, either stained with EtBr or hybridized with chromosome I probes (Figure 2, second and third panels from the top). In contrast, *Sma*I restriction products showed essentially no size variations between DC5 clones, except for the pair of bands recognized by the YAL066W probe (Figure 2, bottom panel). As this fragment corresponds to the left arm from chromosome I (Figure 2), these results suggest that chromosome I size variations from DC5 clones corresponded almost exclusively to changes in the length of its left arm.

Analysis of ORFs present in chromosome I variants from DC5

Chromosome I size variants from several DC5 clones were isolated from IFGE. Chromosomal bands corresponding to low- (large, L) and high- (short, S; see Figure 1A) mobility size variants from chromosome I were isolated independently (Figure 3) and used as probes to hybridize a microfilter containing 14 ORFs distributed along chromosome I in laboratory strains (Figure 4A, B). Figure 4C shows a typical result of such an experiment. The left panel corresponds to the single chromosome I band from the laboratory strain W303d, whereas the middle and the right panels correspond to typical L and S size variants of chromosome I from DC5, respectively. These data showed that neither L nor S size variants from DC5 encompassed ORFs YAR053W or YAR071W (labelled 13 and 14 in Figure 4B). In addition, it

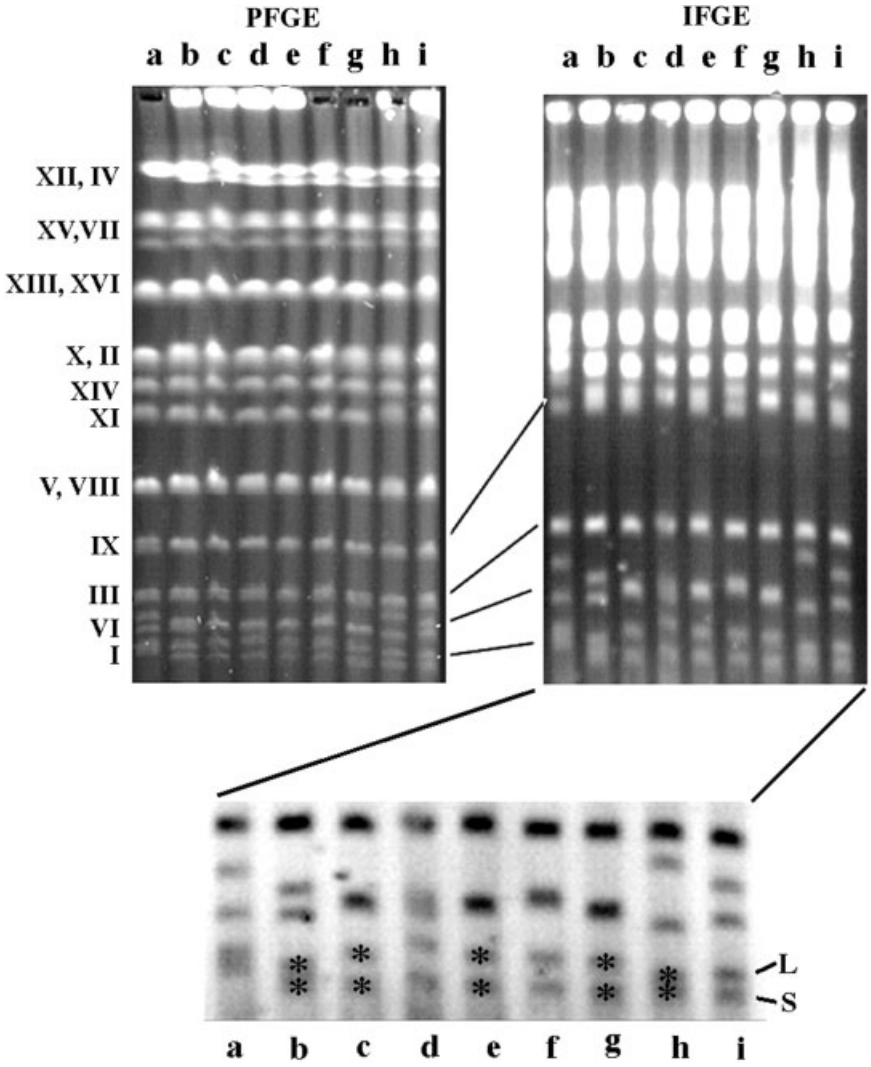
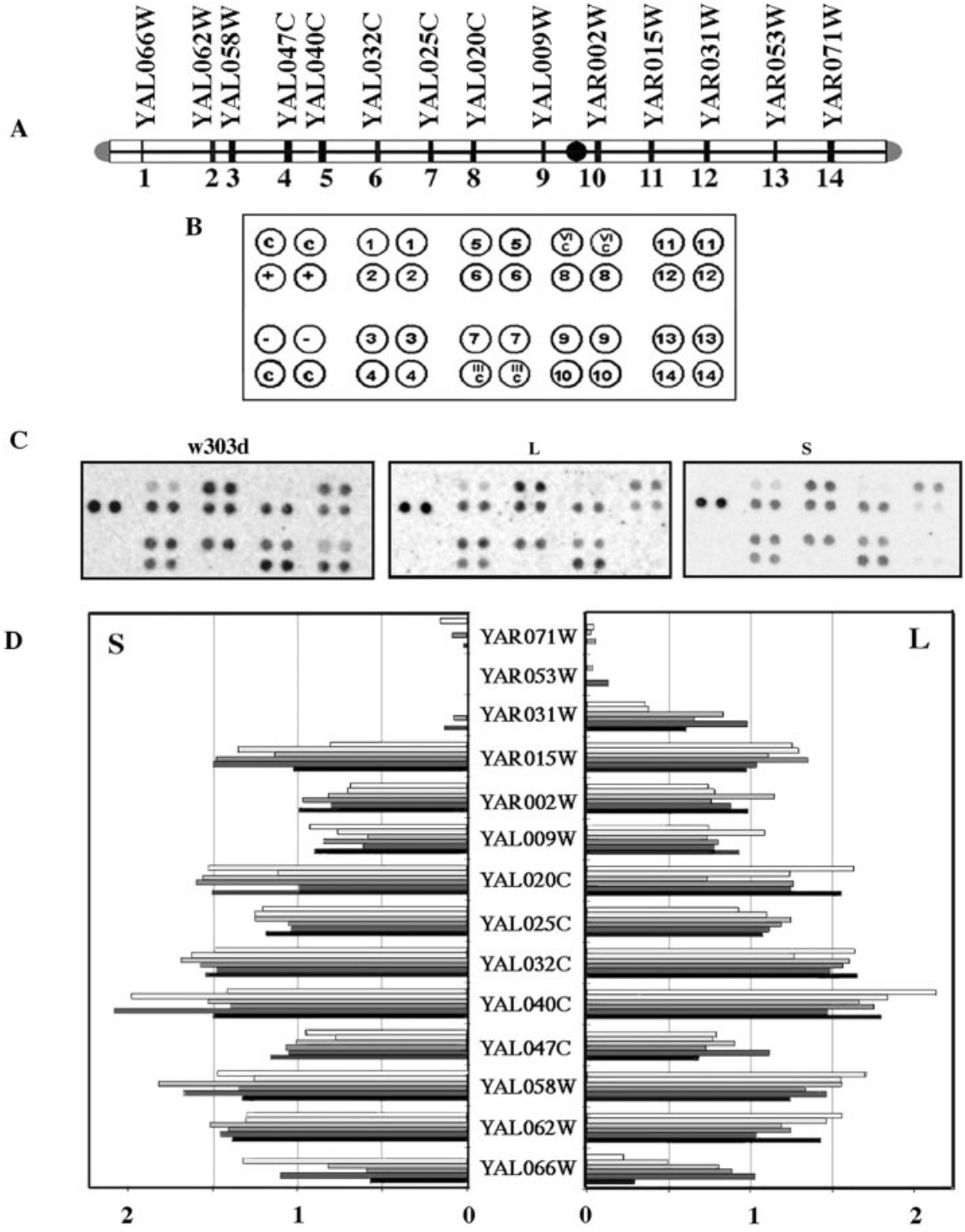


Figure 3. Isolation of chromosome I size variants from DC5. The figure shows a PFGE (left) and a IFGE (right) of clones (labelled a–i) obtained as in Figure 1. Roman numerals indicate putative positions of the different chromosomes from DC5 (see Carro and Piña, 2001). Corresponding bands in both types of gels are linked by lines. The panel on the bottom is a magnification of the relevant region of the IFGE, shown as a negative for better visualizations of chromosomal bands. Bands isolated for further analysis are labelled with asterisks. Positions of large (L) and short (S) forms are indicated on the right

confirmed that the S size variant lacked YAR031W (labelled 12 in Figure 4B). The rest of the ORFs showed essentially the same intensity in the three hybridizations, indicating that the corresponding genes were present in both chromosome I size variants from DC5. This pattern was similar in all DC5 clones we analysed (see below).

Figure 4D shows a quantitation of hybridization intensities for the different ORFs in six independent DC5 clones. Individual clones are distinguished by shades of grey; S and L size variants from the same clones are shown in left and right panels, respectively. Intensity values are given as a ratio of the corresponding values for W303d. All ORFs from YAL062W through YAR015W showed similar intensities among DC5 clones, among S and L size variants from a same individual clone, and between DC5 and W303d. YAR031W was detected with a similar intensity in all L, but not in any S size variants analysed. Finally, YAR053W and YAR071W were absent in all DC5 clones. Intensities for YAL066W showed some



variability among the DC5 clones. However, this heterogeneity may be artifactual, since YAL066W gave a very weak signal in all probes analysed, including the laboratory strain W303d (Figure 4C).

Characterization of DC5 chromosome I arms

The right arm of DC5 chromosome I was peculiar by several criteria. First, it lacks several ORFs as detected by hybridization of PGFE and by ORF hybridization (Figures 1A, 4C, D). In addition, it differentiated L and S variants, although it appeared not to contribute to the clonal size variability occurring during vegetative growth (Figure 2). We then examined DNA sequences in the regions of chromosome I that marked the discontinuity of the co-linearity between L and S size variants and between L variants and W303d (Figure 1B). Only 7.6 Kb of DNA separate YAR023C (present in all genomic DNA analysed) and YAR031W (absent in the S variant) in the published sequence, which comprise a cluster of non-unique DNA sequences, including a Ty1 Δ and Ty3 σ sequences, and two tRNA genes [SUP56 and ts(AGA)A; Figure 1B]. On the other hand, YAR031W (present in DC5 L variants and in W303d) and YAR053W (only in W303d) are separated by more than 20 Kb (Figure 1B), including ORF YAR048W (*FLO1*) among other genes. This gene encompasses 7–10 copies of an internal 135 nucleotide repeat and codifies for a partially repetitive protein (Watari *et al*., 1994). To assess whether these repetitive sequences were still present in DC5, we used a set of three oligonucleotides (Figure 5A). Flo1U (Watson strand) matches a *FLO1*-specific DNA sequence 5′ from the repeats, Flo1L (Crick strand) encompasses a DNA sequence just 3′ from the last repeat, and Flo135 (Crick strand) encompasses a fragment of the 135 nucleotide-repeat sequence (Figure 5A).

The left portion of Figure 5B shows the amplification products for oligonucleotides Flo1U and Flo1L using either genomic DNA from the laboratory strain (W303d), from DC5 (G), or two isolated L bands (L1 and L2). This set of oligonucleotides did not produce any amplification product from DC5 DNA, but it gave a fragment of the expected size with W303d genomic DNA. These results contrast to the ones from a similar experiment using oligonucleotides Flo1U and Flo135 (right part of Figure 5B). In this case, genomic DNA from both W303d and DC5 gave amplification products; however, they differed in the size of the bands obtained. As Flo135 hybridizes to the 135 nucleotide repeat, we expected amplification of a whole set of bands resulting from priming at the different repeats. The laboratory strain W303d showed the expected array of bands separated by roughly 135 bp; the topmost of these bands had a size around 1400 bp (right side of Figure 5B). This is consistent with the presence of seven to eight repeats in the *FLO1* ORF from W303d, in agreement with the published sequence. In contrast, amplification of DC5 DNA with the same oligonucleotides yielded a much simpler set of bands, the largest being 736 bp, which would correspond to only four repeats. In addition, the smallest band obtained with DC5 corresponded to a fragment of 278 bp, shorter than the 380 bp of the corresponding band from W303d (Figure 5B). These results suggest that the right arm of the L

Figure 4. Analysis of the ORF composition of chromosome I size variants from DC5. (A) Relative positions of several probes on chromosome I. Grey semicircles represent telomeres; a black oval indicates the position of the centromere; black segments indicate both position and sizes of the corresponding ORFs. (B) Diagram of the disposition of probes in the minichips used in this study. Figures 1–14 correspond to the ORFs indicated in (A). + and — correspond to positive (*S. cerevisiae* total genomic DNA) and negative (*E. coli* DNA) controls, respectively. c, controls spotted without any DNA added. IIIc and IVc contain DNA from chromosomes III and IV of *S. cerevisiae*, respectively. All dots were spotted as duplicates. (C) Hybridization of minichips with a chromosome I isolated from W303d (left), and L-type (middle) and S-type (right) size variants from DC5, also isolated from a IFGE gel. The pictures were obtained from electronic images from the Phosphorimager. All minichips are orientated as in (B). (D) Quantitative results from minichip hybridization with six pairs (L and S size variants) of chromosome I bands, as in C. Horizontal bars represent intensity values for individual clones (identified by shades of grey) relative to the corresponding values for W303d (I_i/I_{wi}); the 14 ORFs examined are represented co-linearily from the left (bottom) to the right (top) telomeres. Ratios close to 1 (numbers at the bottom) indicate that the hybridization was identical to that obtained in the laboratory strain; values of 0.5–1.5 (vertical lines) were considered within experimental error. The five bottom bars in each set correspond to clones (from bottom to top) b, c, e, g and h from Figure 3; the upper band (white) corresponds to a set of bands isolated in a completely independent experiment. For each individual clone, results from S and L bands are shown on the left and right panels, respectively

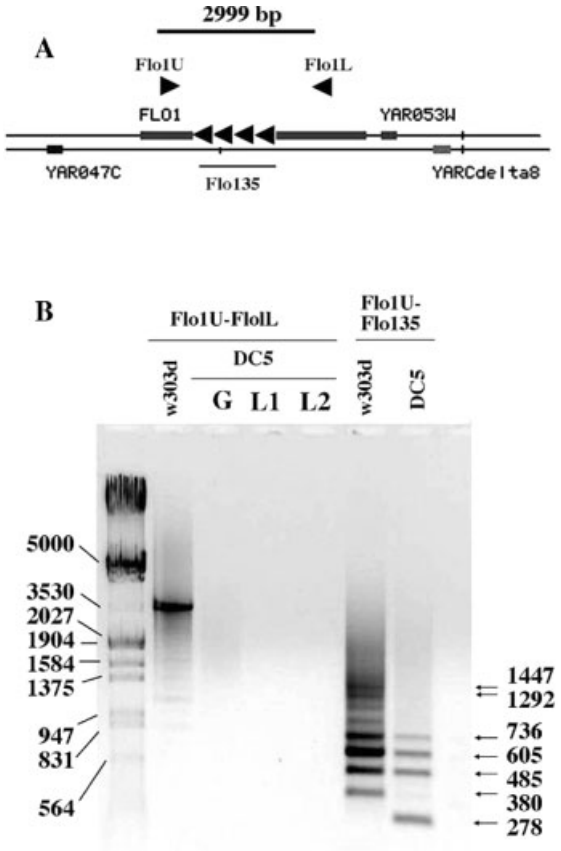


Figure 5. Fine mapping of chromosome I ends from DC5 size variants. (A) Predicted map of the *FLO1* locus and its surroundings in the right arm of chromosome I. Primers used in this study are indicated by arrowheads; their orientations indicate the direction of their predicted extension. The multiple positions of Flo135 priming sites are indicated inside the black box corresponding to the *FLO1* ORF position. For clarity, only four priming sites are indicated, although 7–10 sites are predicted. The centromere would be on the left, the right chromosome I telomere on the right. (B) Amplification products. The left half of the gel corresponds to Flo1U and Flo1L primers, using either W303d or DC5 DNA as templates. Primers and DNAs corresponding to each track are indicated at the top. For DC5, G indicates total DC5 genomic DNA, L1 and L2 correspond to two independently isolated L bands. Size markers correspond to λ DNA digested with *Eco*RI and *Hin*dIII; their sizes in bp are indicated at the left. The right half of the gel corresponds to amplification of genomic DNAs from either W303d or DC5 (on top) using primers Flo1U and Flo135. Arrows and figures correspond to the calculated sizes of the resulting bands, calculated from the electrophoretic mobility of size markers in the left-most track of the gel. For easier recognition of the bands, the gel is represented as a negative of an EtBr-stained gel

size variant was truncated or rearranged precisely at the *FLO1* repeats.

The finding of the peculiar structure of *FLO1* repeats in DC5 prompted us to analyse a similar ORF present in the left arm of chromosome I, *FLO9* (Smit *et al.*, 1992). In this case, the situation is complicated by the presence of *FLO9* sequences at a second locus placed 20 Kb towards the left telomere (Figure 6A). Amplification of W303d DNA with *FLO9*-specific oligonucleotides gave two bands of around 2500 and 850 bp, in agreement with the published sequence (Figure 6A, B). The same oligonucleotides using DC5 genomic DNA as a template gave a completely different pattern, with a set of bands of approximately 1400, 1200 and 1000 bp, in addition to some minor bands (Figure 6B). When isolated chromosome I L bands from DC5 were used as templates, they produced a single 1400 bp band, which was absent in amplifications from S bands (Figure 6C). These data suggest that this locus in DC5 diverges from the published data, and that at least part of the variability present on the left arm of chromosome I from different DC5 clones is due to alterations in the *FLO9* ORF and adjacent *loci*.

Discussion

Mitotic and meiotic karyotype variations in natural and industrial yeast strains are associated with chromosomal translocations due to recombination between homologous sequences interspersed in the yeast genome, such as Ty elements, Δ elements and Y′ elements (Warmington *et al.*, 1987; Codon *et al.*, 1997; Puig *et al.*, 2000; Rachidi *et al.*, 1999; Neuvéglise *et al.*, 2000). This seems to be also the case for chromosomal rearrangements in diploid laboratory strains (Umezu *et al.*, 2002). These events may originate aberrant fusion chromosomes, which would ultimately alter the gene dosage of the cell (Puig *et al.*, 2000; Umezu *et al.*, 2002). Although some of these rearrangement may be beneficial for the cell (Pérez-Ortín *et al.*, 2002b), they may produce an unbearable level of genomic instability in strains with high frequency of these rearrangements, such as DC5.

Despite the high frequency of chromosomal I size variations in DC5, we found a clear structural similarity between these chromosome I size variants and the published sequence for this

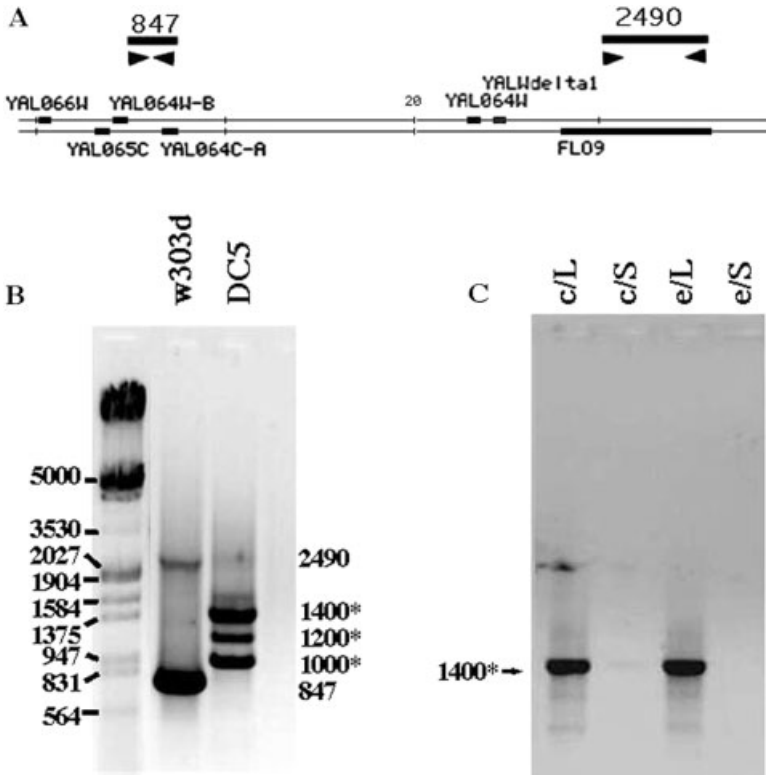


Figure 6. Fine mapping of the YAL066W/*FLO9* intergenic region. (A) Predicted map of the relevant region in the chromosome I left arm. Arrowheads indicated position of the Flo9 primers; expected amplification fragments and their sizes are indicated at the top. In this map, the centromere would be located on the right and the left telomere on the left of the figure. (B) Amplification products of the Flo9 primers with genomic DNA from either W303d or DC5 (top). The left-most track corresponds to the same size marker as in Figure 5. Predicted (plain) or calculated (asterisks) sizes of amplification products are shown on the right. (C) Amplification products form Flo9 primers using L or S size variants from two DC5 clones (c and e clones in Figure 3). Position and approximate size of the single band obtained from L size variants is indicated on the left. The figure shows a negative of the EtBr-stained gel

chromosome. This similarity includes the presence of an identical number of *Sma*I sites placed at very similar distances and a continuous array of at least 11 ORFs (from YAL062W to YAR023C), spanning about 150 Kb of DNA. Our data suggest that this is so because rearrangements leading to these size variants are mostly restricted to the distal 20–30 Kb from both telomeres, leaving the central 150 Kb of chromosome I intact. Subtelomeric regions of *S. cerevisiae* chromosomes (including chromosome I) are characterized by the presence of identical or quasi-identical genes repeated in many chromosomes, such as *FLO*, *PAU* or *COS* genes (Vega-Palas, 2000; **http://www.leicester.ac.uk/genetics/ejl12/ClustersLarge.html**). In addition, they contain a reservoir of disabled ORFs, in many cases repeated in several chromosomes (Harrison *et al*., 2002). All these repeated sequences may serve as targets for ectopic recombination events leading to size variations of wild yeast chromosomes (Codon *et al*., 1997; Nadal *et al*., 1999; Puig *et al*., 2000; Rachidi *et al*., 1999; Neuvéglise *et al*., 2000). In addition, the presence of internally repetitive sequences in chromosome arms, such as *FLO1*/*FLO9* repeats, may also increase the frequency of recombination events in these regions. As the different chromosome I size variants from DC5 diverge precisely in these repetitive regions, we propose that this is the main mechanism responsible for the generation of size variants of chromosome I in DC5.

High karyotype variability in natural yeast strains may represent a beneficial increase of genetic variability for these strains, which have an essentially asexual life cycle. In order to be evolutionarily advantageous, these benefits should compensate for

the risks of increasing levels of genetic instability. Our results suggest that, at least for chromosome I, the most frequent type of chromosomal size variations occur mainly at subtelomeric regions, leaving the main body of the chromosomes intact. These locally restricted rearrangements would rarely be deleterious, since most of the implicated genes have closely related relatives at subtelomeric regions of non-homologous chromosomes. These sibling loci would compensate for the loss of one or more subtelomeric genes (Vega-Palas, 2000; **http://www.leicester.ac.uk/genetics/ejl12/ClustersLarge.html**). If this pattern of clonal recombination applies to the rest of chromosomes, this would explain how wild yeast strains can tolerate high rates of chromosomal size changes without compromising essential cellular functions.

Using an approach similar to the one presented here, Casarégola and colleagues (Neuvéglise *et al*., 2000) described meiotic rearrangements at subtelomeric regions of chromosomes I and III. In this case, the main source of chromosomal length variability was the homologous recombination between Ty elements, together with insertions/transpositions of Ty LTRs and Y′ elements. We consider that a similar mechanism may be related to the origin of the S size variants from DC5, which apparently suffered a recombination and at the same region (between YAR023C and YAR031W) described as the recombination point in several meiotic rearrangements (Neuvéglise *et al*., 2000). However, the origin of the clonal variability we observed during vegetative growth may be different. Although the published *S. cerevisiae* genomic sequence encompasses a Ty1 Δ element in the region we observed clonal length variation with the Flo9 primers, our data suggest that the reason for this variability may be a change on the number of the FLO repeats, which are unrelated to Ty sequences. This hypothesis is reinforced by the observed truncation of the *FLO1* locus on the L size variant of chromosome I in DC5, that we interpret as a recombination event occurred in the middle of the repetitive region of *FLO1*. FLO repeats may be functionally related to Ser-Thr-Pro-rich repeats present in mucins from several organisms, from vertebrates to parasitic protozoans (DiNoia *et al*., 1995). It is suggestive that clonal variations on the length of these repetitive regions help these parasites to escape from the host immune system and to anchor to host cells (DiNoia *et al*., 1995).

Subtelomeric gene families in *S. cerevisiae* are often related to cell membrane and cell wall components, such as lectin-like proteins (the *FLO* family), sugar transporters (the *HXT* family), genes related to cell–cell fusion (the *PRM* family) and some members of *GAL*, *MAL* and *PHO* genes involved in assimilation and utilization of nutrients (Vega-Palas, 2000; Harrison *et al*., 2002; **http://www.leicester.ac.uk/genetics/ejl12/ClustersLarge.html**). It is interesting to note that this kind of arrangement is also found in *Schizosaccharomyces pombe* (Wood *et al*., 2002), in several other fungi (Zolan, 1995), and in several parasitic protozoans, e.g. *Plasmodium falciparum* (Bowman *et al*., 1999), *Trypanosoma brucei* (Fu and Melville, 2002) and *Pneumocystis carinii* (Wada and Nakamura, 1996). As mentioned previously, subtelomeric variability in parasitic protozoans may result on changes on the surface of the cell, facilitating escape from the host immune system. In *S. cerevisiae* (and perhaps in *Sz. pombe*), many of subtelomeric genes are directly involved in biotechnological uses, which have been selected for thousands of years of human biotechnology practices, e.g. the use of alternative carbon sources, such as sucrose, galactose and maltose, or the resistance to toxic substances present in molasses (Ness and Aigle, 1995) may be advantageous to industrial strains. Therefore, rapid changes in the gene composition of these families may increase the chances of acquiring a selective advantage and improve their industrial fitness. We conclude that some level of chromosomal size variation might be a healthy characteristic for industrial yeast strains, and that it may not necessarily imply genetic instability in the strict sense of the term.

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