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The nucleotide sequence of chromosome I from *Saccharomyces cerevisiae*

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ABSTRACT **Chromosome I from the yeast *Saccharomyces cerevisiae* contains a DNA molecule of ≈231 kbp and is the smallest naturally occurring functional eukaryotic nuclear chromosome so far characterized. The nucleotide sequence of this chromosome has been determined as part of an international collaboration to sequence the entire yeast genome. The chromosome contains 89 open reading frames and 4 tRNA genes. The central 165 kbp of the chromosome resembles other large sequenced regions of the yeast genome in both its high density and distribution of genes. In contrast, the remaining sequences flanking this DNA that comprise the two ends of the chromosome and make up more than 25% of the DNA molecule have a much lower gene density, are largely not transcribed, contain no genes essential for vegetative growth, and contain several apparent pseudogenes and a 15-kbp redundant sequence. These terminally repetitive regions consist of a telomeric repeat called W′, flanked by DNA closely related to the yeast *FLO1* gene. The low gene density, presence of pseudogenes, and lack of expression are consistent with the idea that these terminal regions represent the yeast equivalent of heterochromatin. The occurrence of such a high proportion of DNA with so little information suggests that its presence gives this chromosome the critical length required for proper function.**

The yeast *Saccharomyces cerevisiae* has been the focus of intensive study as a model eukaryote. As part of this effort, an international program is under way to determine the nucleotide sequence of the 16 chromosomes that constitute its 13.5-Mbp nuclear genome. This endeavor will provide both a complete eukaryotic gene set and a reference set of experimentally amenable genes for comparison with those of other organisms. Currently, four yeast chromosomes have been sequenced (1–4); all have a high gene density, and a majority of the genes found are newly sequenced and of unknown function. Chromosome I is the smallest *S. cerevisiae* chromosome. It contains a DNA molecule that is only 231 kbp, making it the smallest known fully functional nuclear chromosome. This chromosome has been studied intensively, and mutants are available for a large number of its genes (5–7). Here we report the nucleotide sequence of chromosome I and describe several unusual features of its gene organization and chromosome structure as well as many newly discovered genes.**

MATERIALS AND METHODS

DNA Sources. Four sources of chromosome I DNA, all from S288C-derived yeast strains, were used to generate the templates for DNA sequencing. These were the library of Riles *et al.* (8), a cosmid from the collection of Dujon (9), chromosome walking (10), and PCR amplified fragments of genomic DNA. DNA fragments, except those generated by PCR which were used directly, were subcloned into the Bluescript KS(+) plasmid from Stratagene prior to sequencing. All DNA sequencing was performed using double-stranded DNA templates.

DNA Sequencing. Two methods were used for sequencing DNA templates: manual sequencing and machine-based sequencing with an Applied Biosystems sequencing machine (model 373A). Our manual sequencing used unidirectional nested deletions and was carried out as described (11, 12). For machine-based sequencing, three sets of templates were used: unidirectional nested deletions, PCR amplified chromosomal DNA, and, for the region spanning *YAL062* to *CDC24*, cosmid DNA was shotgun cloned into Bluescript KS(+). In summary, the procedure for the Applied Biosystems machine (model 373A) used dye-labeled dideoxynucleotide terminators and a cycle sequencing kit (Prism Ready reaction dye terminator kit; Perkin–Elmer) and the protocol provided by the supplier. This method allowed us to process all four sequencing reactions in a single reaction tube. The cycle amplification reactions were performed with a Perkin–Elmer DNA thermal cycler (model 9600) in 0.2-ml microcentrifuge tubes. Unincorporated dye terminators were removed by one or more extractions with an equal volume of phenol. A detailed description of the method used for shotgun sequencing will be published elsewhere (R.K.S. and H.B., unpublished data).

Template Preparation. Promega Wizard miniprep kits and the method supplied by Promega were used to prepare the Bluescript based double-stranded DNA templates. Essentially, the method involves growing 3-ml overnight cultures of *Escherichia coli* strain XL-1 Blue (Stratagene) harboring the plasmid of interest in T broth and then preparing a cleared lysate. The plasmid DNA is then extracted by using a silica bead resin. PCR fragments were prepared for DNA sequencing by two precipitations with 0.6 vol of isopropanol.

Sequence Collection and Contig Assembly. DNA sequences generated manually were transferred to a Sun work station after assembly into short (<10 kbp) contigs using the ASSEMGEL program in PC Gene (IntelliGenetics). After resolution of the cycle sequencing reaction products on an Applied Biosystems model 374A automated DNA sequencing machine, the automatically read sequence results were transferred to a Sun work station and assembled into contigs by using the Staden DNA assembly program (13).

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Abbreviation: ORF, open reading frame.

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**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U12980, L20125, L05146, L22015, and L28920).

Computer Analysis and Open Reading Frame (ORF) Assignments. DNA sequences were compiled and analyzed as described above and in ref. 11. ORFs were given a YAR or YAL number (Y, yeast; A, Chromosome I; R, right arm; L, left arm), which increases with distance from the centromere (1). YAR and YAL designations were assigned to any ORFs equal to, or longer than, 100 codons unless they significantly overlapped some known or probable gene. Some ORFs of <100 codons have been included when there was additional evidence, such as an RNA transcript or significant similarity to a known protein (14, 15), to support their existence.

RESULTS AND DISCUSSION

Sequencing of chromosome I was carried out using partially overlapping λ and cosmid clones (5, 8, 9). Telomeres were not sequenced. However, based on physical mapping of terminal restriction fragments, the sequence of 226,903 bp extends to within 2.5 kbp of the end of the left telomere (Y.S., unpub-

Table 1. Chromosome I genes with known function or homology

Position	Gene	Locus	Gene product function or homology	BLAST score	Acc
Accession no.					
U12980					
6426	*YAL067C*		Ycr28p homolog	405	sp P25621
8975	*YAL065C*		Flo1p fragment homolog, pseudogene	1050	sp X78160
25514	*YAL063C*		FLO1 homolog	4489	gp X78160
29118	*YAL062W*		NADP-glutamate dehydrogenase homolog	1962	sp P07262
30999	*YAL061W*		Alcohol/sorbitol dehydrogenase homolog	208	sp Q06004
32706	*YAL060W*		Alcohol/sorbitol dehydrogenase homolog	187	gp L35343
35015	*YAL058W*	*CNE1*	Mammalian calnexin homolog		sp P27825
42573	*YAL054C*	*ACS1*	Acetyl CoA synthetase		sp Q01574
46115	*YAL051W*		Zinc finger protein, HAP1 homolog	113	sp P25611
55349	*YAL045C*		*E. coli bolA* homolog	91	sp P15298
56036	*YAL044C*	*FUN40*	Glycine cleavage H protein homolog	250	sp P23884
58605	*YAL043C*	*PTA1*	tRNA processing, essential		sp Q01329
58869	*YAL042W*	*FUN9*	Novel product, essential		
60745	*YAL041W*	*CDC24*	Polarity establishment, essential		sp P11433
65071	*YAL040C*	*CLN3*	Cyclin		sp P13365
67075	*YAL039C*	*CYC3*	Cytochrome *c* heme lyase		sp P06182
69342	*YAL038W*	*PYK1*	Pyruvate kinase, essential		sp P00549
73701	*YAL036C*	*FUN11*	GTP-binding protein homolog	662	sp P32234
74367	*YAL035W*	*FUN12*	*Bacillus subtilis* IF2 homolog, essential	143	sp P17889
80245	*YAL033W*	*FUN53*	Novel product, essential		sp P28005
82012	*YAL032C*	*FUN20*	*Drosophila* protein Bx42 homolog, essential	112	gp X64536
84825	*YAL030W*	*SNC1*	Synaptobrevin homolog		sp P31109
89809	*YAL029C*	*MYO4*	Yeast MYO2 homolog		sp P32492
97235	*YAL026C*	*DRS2*	ATPase membrane protein, cation transporting		pir S30768
98683	*YAL025C*	*MAK16*	Casein kinase homolog, essential		sp P10962
Accession no.					
L20125					
103411	*YAL024C*	*LTE1*	CDC25 homolog, putative GTP exchange protein		sp P07866
Accession no.					
L05146					
106087	*YAL023C*	*PMT2*	Protein mannosyl transferase	308	sp P33775
110895	*YAL021C*	*CCR4*	Transcription factor, catabolite repression		pir S36713
112151	*YAL020C*	*ATS1*	Human RCC1 and yeast PRP20 homolog	100	gp D25215
112455	*YAL019W*	*FUN30*	Yeast SNF2 homolog, UV sensitivity	543	pir S31583
117761	*YAL017W*	*FUN31*	Serine/threonine kinase, PIM1/SNF1 homolog	241	pir PQ0207
122415	*YAL016W*	*TPD3*	Regulatory subunit type 2A phosphatase		pir S36718
125638	*YAL015C*	*FUN33*	Endonuclease III homolog	116	pdb 1ABK
126805	*YAL013W*	*DEP1*	Regulation of phospholipid metabolism		sp P31385
128335	*YAL012W*	*CYS3*	Cystathionine γ-lyase		sp P31373
133089	*YAL010C*	*MDM10*	Mitochondrial outer membrane protein		gb X80874
Accession no.					
L22015					
133389	*YAL009W*	*SPO7*	Early sporulation		sp P18410
136688	*YAL006W*	*TRN1*	Pro-tRNA		
138966	*YAL005C*	*SSA1*	HSP70 heat shock protein		sp P10591
139699	*YAL003W*	*EFB1*	Elongation factor 1β, essential		sp P32471
148700	*YAL001C*	*TFC3*	Transcription factor IIIC, essential		sp P34111
156152	*YAR007C*	*RPA1*	DNA replication factor, essential		sp P22336
163800	*YAR013W*	*TGA1*	Ala-tRNA		
166902	*YAR015W*	*ADE1*	*N*-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide synthase		pir S20122
169230	*YAR018C*	*KIN3*	Protein kinase		gp P22209
172665	*YAR019C*	*CDC15*	Essential late cell cycle protein kinase		pir S15038

Table 1. (*Continued*)

Position	Gene	Locus	Gene product function or homology	BLAST score	Acc
Accession no. L28920					
174554	*YAR020C*		Ycr104p related fragment, pseudogene	89	sp P32612
177350	*YAR023C*		Ycr7p homolog, membrane protein	237	sp P25354
178668	*YAR024W*	*SUP56*	Leu-tRNA		
180130	*YAR025C*		Ser-tRNA		
181296	*YAR027W*		Ycr7p homolog, membrane protein	592	sp P25354
182418	*YAR028W*		Ycr7p homolog, membrane protein	220	sp P25354
183847	*YAR029W*		Ycr7p homolog		
184362	*YAR031W*		Ycr7p homolog, membrane protein	440	sp P25354
185633	*YAR033W*		Ycr7p homolog, membrane protein	220	sp P25354
187719	*YAR035W*	*YAT1*	Carnitine *N*-acetyltransferase		sp P80235
191131	*YAR044W*	*OSH1*	Oxysterol binding protein, KES1, HES1 homolog		sp P35845
200925	*YAR050W*	*FLO1*	Putative cell wall protein		
216207	*YAR062W*		Flo1p fragment homolog, pseudogene	863	gp U00029
223118	*YAR071W*	*PHO11*	Acid phosphatase		sp P35842
225400	*YAR073W*		Inosine 5′ monophosphate dehydrogenase homolog	866	pir S41064

Genes are listed starting at the left end and continuing to the right end. Information listed in the columns is described below. GenBank accession numbers in boldface are for that entry, followed by the bp position of the ORF or tRNA. ORFs and tRNAs are listed using standard yeast genome sequencing nomenclature, with the name of the gene or element, Y (yeast), A (first chromosome), and L or R (left or right arm) followed by a gene number, which starts at the centromere for each arm, and a W or C for top or bottom strand, respectively (see Fig. 1). The names and functions of previously identified genes are listed next to the ORF. Genes that are essential for growth at 30°C on rich glucose-containing medium based on gene disruption experiments are indicated, as are apparent pseudogenes. Similarities and BLAST alignment scores (14, 15) for the most similar known protein and data base accession numbers (Acc) are shown. Data base accession numbers for previously characterized genes are also noted. sp, Swiss-Prot; gp, GenPept; pir, Protein Identification Resource; pdb, Protein Data Bank. In addition to the GenBank submissions above, which encompass the entire chromosome, an annotated version of the chromosome I sequence will be available as an AScDB data base entry through the *Saccharomyces* genome data base [e-mail: yeast-curator@genome.stanford.edu].

lished data) and to within 1.6 kbp of the end of the right telomere (10). The order of *Eco*RI and *Hin*dIII sites predicted from the final sequence agrees with that determined independently by others (5, 8, 16), providing a check for correct sequence assembly. We estimate our sequence has an overall accuracy of 99.97% based on several criteria including comparison and conflict resolution with previously sequenced regions, independently sequencing some sections twice, and our procedures for checking for possible errors in ORFs. We will continue to support and revise the sequence of chromosome I through the *Saccharomyces* genome data base (see legend to Table 1) and ask to be informed of errors.

The sequence contains 89 nonoverlapping ORFs of 100 or more codons (Table 1; Fig. 1). The average ORF size is 501 codons: the largest ORF, *FLO1* (*YAR050*), has 1582 codons, and 3 ORFs have introns very near their 5′ ends. In addition, there are four tRNA genes, including two with introns, one *Ty1* element, one solo *sigma* 5′ to a tRNA, and two solo *delta* elements. The *delta* elements are in duplicated regions. Included among the ORFs are four apparent pseudogenes, three of which lie in repeated regions. All are related to known yeast genes but contain stop codons and/or frameshifts in the predicted ORFs. Forty-seven of the ORFs either encode a protein of known enzymatic function, are associated with a mutant phenotype suggesting function, or have some significant similarity to other characterized genes (Table 1). In addition, a total of 27 genes have some similarity to known human genes. Forty-two genes have no known function or similarity to characterized genes; however, eight of these have sequence similarity to genes of unknown function and three of these are from organisms other than yeast (*YAL045*, *YAL036*, and *YAL032*). Twenty-nine ORFs have homologs located elsewhere on the yeast genome; nine of these are on chromosome I, principally in the repeated ends and in a large clustered gene family. This gene family contains a set of six somewhat diverged genes (see Table 1; *YAR023–YAR033* sequence from 177350–186337), all of which share sequence similarity with Ycr7p, a putative membrane protein encoded on yeast chromosome III (1, 17, 18). With the exception of *YAR023*, five of the clustered genes are arranged in tandem. *YAR029* within this cluster has several frameshift mutations leading to a shorter ORF. This sequence is either a smaller member of the gene family or possibly a pseudogene. All five of the tandem cluster of genes (*YAR027*, *YAR028*, *YAR029*, *YAR031*, and *YAR033*) are transcribed during vegetative growth (Fig. 1). Several tandemly repeated gene families have been characterized in higher eukaryotes. Notably the globin (19) and immunoglobulin (20) gene clusters are both composed of related but nonidentical members, with each member showing differential gene expression at some level. This type of gene cluster has not been previously found in smaller eukaryotes, and its existence may suggest that these large tandem repeated diverged gene clusters occurred very early in evolution.

Overall, there is a gene every 2386 bp and an average distance between genes of 945 bp. The top (W) strand contains 52 genes, and the bottom (C) contains 41, with 29 genes occurring in four runs of 7 or 8 genes on one strand. Although the probability of a run of 8 genes on one strand is only 1 in 256, these runs may reflect preferred arrangements to prevent collisions between the transcription and replication complexes (21). The organization of the central 165 kbp of the chromosome is typical of other sequenced yeast chromosomes (1–4) with a high gene density and with most ORFs transcribed during vegetative growth (Table 2). The regions that flank this central region and extend to the telomeres are distinctly different. They have lower ORF and transcript densities (Table 2), several repeat sequences and pseudogenes, and they contain <12 kbp of sequence that is not duplicated elsewhere. The region between 10 and 25 kbp from the right end of chromosome I is duplicated at the equivalent position on the left arm of chromosome I. In addition, the entire 25 kbp at the right end of chromosome I is duplicated on the right arm of chromosome VIII (3, 10). In all three cases, the duplications begin very close to the 5′ end of the coding sequence for the *FLO1*-like genes. These *FLO* genes are involved in cell flocculation and encode large serine/threonine-rich cell wall proteins that have a large,

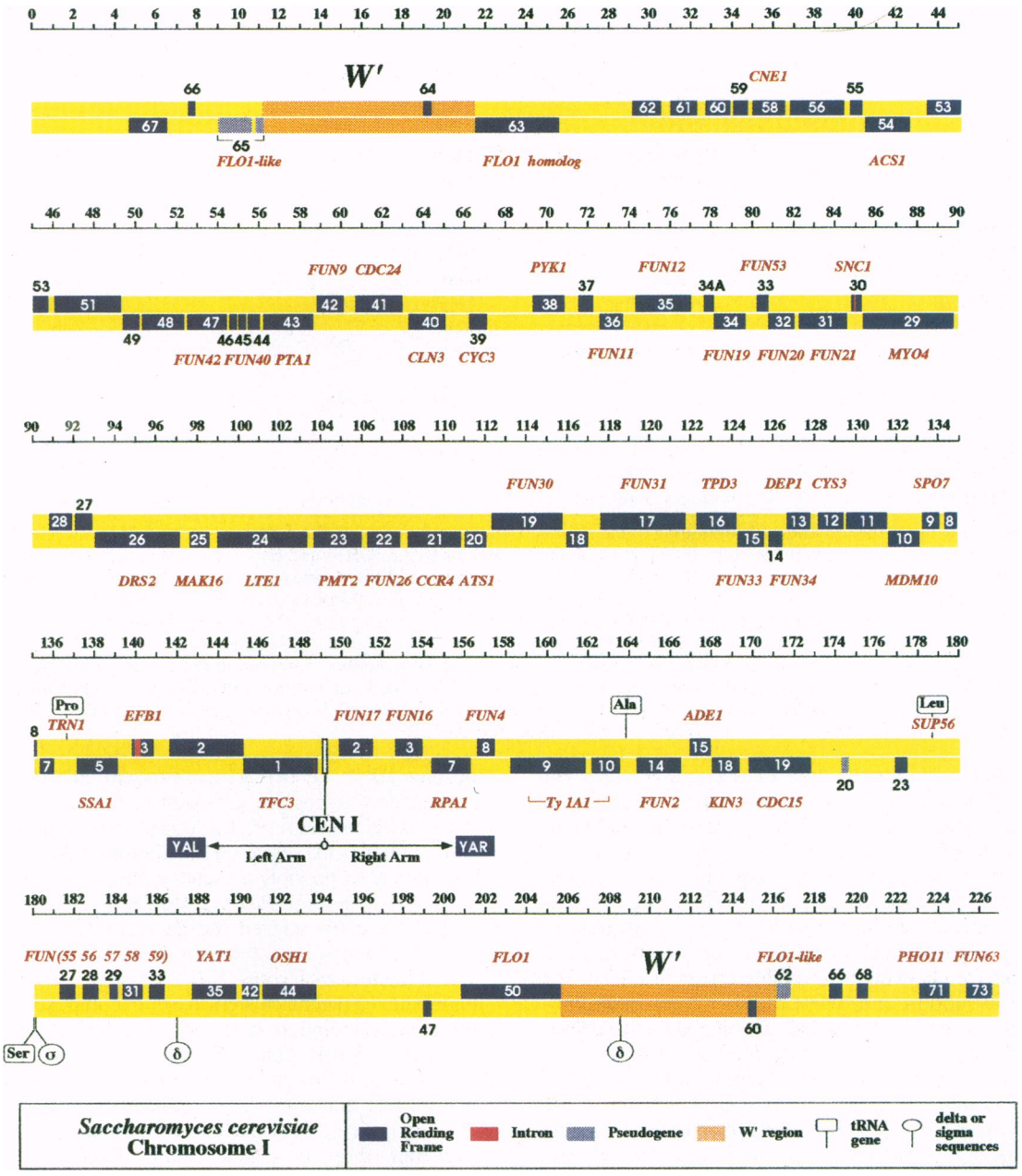

FIG. 1. Chromosome I of *S. cerevisiae*. The two DNA strands of the chromosome determined by nucleotide sequencing are depicted in yellow (left to right) with the upper, or W, strand (5′ to 3′), and the lower, or C, strand (3′ to 5′). The scale is in kbp beginning at the end of the left arm. Superimposed on these strands are positions of ORFs and other genetic entities. ORFs are shown in blue and are numbered outward from the centromere on both the left and right arms as YAL and YAR numbers, respectively. Some numbers are skipped for consistency with earlier GenBank submissions (see Table 1). The names of known genes are indicated by conventional three-letter symbols above the ORF; these are described further in Table 1. ORFs with a published detectable transcript (when grown on yeast extract/peptone/dextrose, a rich glucose-containing medium, at 30°C) are shown with a FUN designation (Function Unknown Now). Introns within genes are shown in red; positions of ORF fragments that may represent pseudogenes are shown stippled in light blue. tRNA genes are boxed with the appropriate amino acid; *delta* and *sigma* elements are shown in ellipses. Positions of the left and right end W′ repeats are also shown stippled in brown. Figure was constructed as described by Dujon *et al.* (2), using the unpublished software of C. Marck.

but variable, number of internal repeats (22, 23). The duplicated sequences also contain a common 10-kbp region apparently devoid of coding sequence or informational content. As this sequence is also on the right end of chromosome VIII, it represents a newly discovered subtelomeric element which we call W′. These W′ repeats terminate in *FLO*-like sequences

Table 2. Spacing of genes on chromosome I

Chromosome position	% coding	Average ORF density (1/bp)	% ORFs transcribed	Average ORF spacing, bp		
				Convergent	Divergent	Tandem
Central (165 kbp)	75	2254	72	285	711	519
Ends (62 kbp)	26	4765	14	2850	1722	3535

that contain frameshift errors in the ORFs, making it possible that they are pseudogenes. These subtelomeric regions thus have many of the features characteristic of heterochromatin.

To further define the chromosome I genes identified by sequencing, vegetatively expressed transcript maps as well as gene disruptions have been made. Transcripts have been detected from 66 genes. Four additional genes may be transcribed during vegetative growth; however, further examination is required to confirm their activity. Thus, a minimum of 74% (66/89) of the ORFs are expressed during vegetative growth. More than half of the genes from chromosome I have now been disrupted, either singly or as part of larger deletions. So far, there are 12 known essential genes defined, most clustered near *CDC24* (7) (Table 1). Large deletions that removed >30 kbp from each end including all the repeated regions did not cause lethality, indicating that these regions are not essential for vegetative growth (A.B.B. and D.B.K., unpublished data). These nonessential repeated regions likely contribute to chromosome I size polymorphisms that have been observed. Indeed, a known polymorphism maps to the region of the *FLO1* duplication on the right arm (24). Although small chromosomes have an increased recombination frequency and segregate with high fidelity at meiosis (25, 26), there may be some advantage to having a critical chromosome length. This idea is supported by studies of chromosome III where 150-kbp chromosome fragments and artificial chromosome constructs were 4 times less stable than authentic copies of the chromosome (27, 28). Thus, the low gene density DNA found at the ends may be acting as "filler" DNA to increase the size and stability of this small chromosome.

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1. Oliver, S. G., Van der Aart, Q. J., Agostini-Carbone, M. L., Aigle, M., Alberghina, L., *et al.* (1992) *Nature (London)* **357,** 38–46.
2. Dujon, B., Alexandraki, D., André, B., Ansorge, W., Baladron, V., *et al.* (1994) *Nature (London)* **369,** 371–378.
3. Johnston, M., Andrews, S., Brinkman, R., Cooper, J., Ding, H., *et al.* (1994) *Science* **265,** 2077–2082.
4. Feldmann, H., Aigle, M., Aljinovic, G., Andre, B., Baclet, M. C., *et al.* (1994) *EMBO J.* **13,** 5795–5809.
5. Kaback, D. B., Oeller, P. W., Steensma, H. Y., Hirschman, J., Ruezinsky, D., Coleman, K. G. & Pringle, J. R. (1984) *Genetics* **108,** 67–90.
6. Harris, S. D., Cheng, J., Pugh, T. A. & Pringle, J. R. (1992) *J. Mol. Biol.* **225,** 53–65.
7. Barton, A. B. & Kaback, D. B. (1994) *J. Bacteriol.* **176,** 1872–1880.
8. Riles, L., Dutchik, J. E., Baktha, A., McCauley, B. K., Thayer, E. C., Leckie, M. P., Braden, V. V., Depke, J. E. & Olson, M. V. (1993) *Genetics* **134,** 81–150.
9. Thierry, A., Gaillon, L., Galibert, F. & Dujon, B. (1994) *Yeast* **11,** 121–135.
10. Steensma, H. Y., Crowley, J. C. & Kaback, D. B. (1987) *Mol. Cell. Biol.* **7,** 410–419.
11. Ouellette, B. F. F., Clark, M. W., Keng, T., Storms, R. K., Zhong, W., Zeng, B., Fortin, N., Delaney, S., Barton, A., Kaback, D. B. & Bussey, H. (1993) *Genome* **36,** 32–42.
12. Clark, M. W., Zhong, W., Keng, T., Storms, R. K., Barton, A., Kaback, D. B. & Bussey, H. (1992) *Yeast* **8,** 133–146.
13. Dear, S. & Staden, R. (1991) *Nucleic Acids Res.* **19,** 3907–3911.
14. Altschul, S. F., Boguski, M. S., Gish, W. & Wooton, J. C. (1994) *Nat. Genet.* **6,** 119–129.
15. Altschul, S. F., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
16. Tanaka, S., Yoshikawa, A. & Isono, K. (1992) *J. Bacteriol.* **174,** 5985–5987.
17. Biteau, N., Fremaux, C., Hebrard, S., Menara, A., Aigle, M. & Crouzet, M. (1992) *Yeast* **8,** 61–70.
18. Goffeau, A., Nakai, K., Slonimski, P. & Risler, J. L. (1993) *FEBS Lett.* **325,** 112–117.
19. Fritsch, E. F., Lawn, R. M. & Maniatis, T. (1980) *Cell* **19,** 959–972.
20. Tonegawa, S. (1983) *Nature (London)* **302,** 575–584.
21. Brewer, B. J. (1988) *Cell* **53,** 679–686.
22. Teunissen, A. W. R. H., Holub, E., Van Der Hucht, J., Van den Burg, J. A. & Steensma, H. Y. (1993) *Yeast* **9,** 1–10.
23. Watari, J., Tanaka, Y., Ogawa, M., Sahara, H., Koshino, S., Onnela, M.-L., Airaksinen, V., Jaatinen, R., Pentillä, M. & Keränen, S. (1994) *Yeast* **10,** 211–225.
24. Ono, B. & Ashino-Arao, Y. (1988) *Curr. Genet.* **14,** 413–418.
25. Kaback, D. B., Steensma, H. Y. & de Jonge, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3694–3698.
26. Kaback, D. B., Guacci, V., Barber, D. & Mahon, J. W. (1992) *Science* **256,** 228–232.
27. Surosky, R. T., Newlon, C. S. & Tye, B.-K. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 414–418.
28. Murray, A. W., Schultes, N. P. & Szostak, J. W. (1986) *Cell* **45,** 529–536.