Analysis of a Circular Derivative of *Saccharomyces cerevisiae* Chromosome *III*: A Physical Map and Identification and Location of *ARS* Elements

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ABSTRACT

DNA was isolated from a circular derivative of chromosome *III* to prepare a library of recombinant plasmids enriched in chromosome *III* sequences. An ordered set of recombinant plasmids and bacteriophages carrying the contiguous 210-kilobase region of chromosome *III* between the *HML* and *MAT* loci was identified, and a complete restriction map was prepared with *Bam*HI and *Eco*RI. Using the high frequency transformation assay and extensive subcloning, 13 *ARS* elements were mapped in the cloned region. Comparison of the physical maps of chromosome *III* from three strains revealed that the chromosomes differ in the number and positions of Ty elements and also show restriction site polymorphisms. A comparison of the physical map with the genetic map shows that meiotic recombination rates vary at least tenfold along the length of the chromosome.

EUKARYOTIC chromosomes, which consist of a single linear DNA duplex complexed with histones and other chromosomal proteins, are faithfully replicated and segregated to daughter cells at every cell division. In the yeast *Saccharomyces cerevisiae*, the spontaneous rate of chromosome loss has been estimated to be approximately 1×10^{-5} per mitotic division (ESPOSITO *et al.* 1982; HARTWELL and SMITH 1985). At least three classes of *cis*-acting elements are thought to function in chromosome replication and segregation: replication origins, centromeres and telomeres (reviewed by NEWLON 1988, 1989; CAMPBELL and NEWLON 1991).

Replication origins are the sites at which DNA replication initiates. In yeast, as in other eukaryotes, there are multiple origins per chromosome. Estimates of the distance between origins, obtained from both electron microscopy and fiber autoradiography, range from 36 to 90 kilobases (kb) (reviewed by NEWLON 1988). The lower estimate is probably most accurate because it was corrected for asynchrony of initiation (NEWLON and BURKE 1980). Recent evidence indicates that *a*utonomously *r*eplicating *s*equence (*ARS*) elements, identified on the basis of their ability to promote the extrachromosomal replication of plasmids in *S. cerevisiae* (HSIAO and CARBON 1979; STINCHCOMB, STRUHL and DAVIS 1979; STRUHL *et al.* 1979), function as replication origins in plasmids (BREWER and FANGMAN 1987; HUBERMAN *et al.* 1987) and in chromosomes (HUBERMAN *et al.* 1988).

Centromeres are required for efficient chromosome segregation. They function as the chromosomal attachment site for the mitotic and meiotic spindles. *S. cerevisiae* centromeres were identified on the basis of their ability to stabilize *ARS* plasmids, and they consist of three conserved DNA elements of approximately 120 bp total length (reviewed by FITZGERALD-HAYES 1987; NEWLON 1988).

Telomeres, the physical ends of chromosomes, have at least two functions. First, they allow the replication of the 5′ ends of linear DNA duplexes, which cannot be accomplished by any known DNA polymerase acting alone. Second, they promote the stable maintenance of chromosome ends, which are distinguished from other free DNA ends by their lack of interaction with sequences internal to the chromosome. As in other eukaryotes, *S. cerevisiae* telomeres consist of an array of a simple G + C-rich repeated sequence (reviewed by ZAKIAN 1989).

ARS elements, centromeres and telomeres have been combined in plasmids with selectable genes to produce artificial minichromosomes. One of the most striking features of these minichromosomes is their instability; small minichromosomes are at least two orders of magnitude less stable than natural chromo-

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somes (reviewed by NEWLON 1988). Two lines of evidence suggest that the instability of minichromosomes may result from their small size: Minichromosomes become increasingly stable as their size increases (HIETER *et al.* 1985; MURRAY and SZOSTAK 1983), and deletion derivatives of chromosome *III* have reduced stability (SUROSKY, NEWLON and TYE 1986). There may also be other elements, as yet unidentified, that account for the high stability of natural chromosomes.

In order to approach questions concerning the functional organization of a eukaryotic chromosome, including whether all *ARS* elements are replication origins and whether there are additional elements required for chromosome stability, it would be useful to have an entire chromosome cloned. A circular derivative of *S. cerevisiae* chromosome *III* that contains all sequences between the *HML* locus on the left arm and the *MAT* locus on the right arm (STRATHERN *et al.* 1979) is advantageous for this purpose because its circularity imparts physical properties that allow it to be purified substantially free from other chromosomal DNAs. In this paper, we report the cloning and restriction mapping of this circular derivative of chromosome *III*, as well as the identification and localization of *ARS* elements, Ty elements and delta sequences. The 210 kb of DNA that extends from approximately 3 kb proximal to the left telomere through the *MAT* locus contains 13 *ARS* elements and two regions that exhibit extensive strain to strain variation.

These results complement and extend the recent report of an ordered clone bank of chromosome *III* by YOSHIKAWA and ISONO (1990). Our collection of chromosome *III* clones includes the region of the left arm that YOSHIKAWA and ISONO were unable to clone. Our use of a shuttle vector for the primary cloning allowed a direct functional analysis in yeast of *cis*-acting sequences that complements the transcript map prepared by YOSHIKAWA and ISONO. A preliminary account of this work has been published (NEWLON *et al.* 1986).

MATERIALS AND METHODS

Strains and culture conditions: The yeast strains used in this study are listed in Table 1. *Escherichia coli* strain JA226 (DEVENISH and NEWLON 1982) was used for the propagation of plasmids, and *E. coli* strain B127 (YEN and GUSSIN 1980) was used for the propagation of bacteriophage λ. The yeast strains were grown in either Y-minimal medium (NEWLON *et al.* 1974) supplemented with glucose (20 g/liter) and required amino acids (50 mg/liter), adenine and uracil (20 mg/liter) or in YEPD (*cf.* SHERMAN, FINK and HICKS 1981) supplemented with uracil and adenine (20 mg/liter). Bacterial strains were grown in LB (*cf.* MANIATIS, FRITSCH and SAMBROOK 1982). For the selection and propagation of plasmid-containing cells, LB was supplemented with 50 mg/liter ampicillin. For the propagation of bacteriophage λ, LB was supplemented with maltose (2 g/liter).

Enzymes: Restriction enzymes were obtained from New England Biolabs, Boehringer Mannheim Biochemicals, or Bethesda Research Laboratories, and used under the conditions recommended by the supplier. T4 DNA ligase was from New England Biolabs, and calf intestinal alkaline phosphatase was from Boehringer Mannheim Biochemicals. Zymolyase was obtained from Kirin Brewery, Japan.

Plasmids: The *URA3* vectors YIp5 (STRUHL *et al.* 1979), YRp12 (STINCHCOMB *et al.* 1980) and YCp50 (ROSE *et al.* 1987) have been described. Plasmids carrying DNA fragments used as probes were obtained from other sources as listed in Table 2.

"Gap-filling" plasmids constructed in YCp50 (Figures 1 and 2) were used to rescue from yeast two segments of the ring chromosome that were not found bacteriophage λ libraries. For each of the three plasmids, fragments flanking the gap were cloned in their chromosomal orientations in such a way that the plasmid could be linearized by cutting at a unique restriction site at the junction of the gap termini. J10A-B9G GF (*cf.* Figure 1) was constructed by inserting the 1.5-kb *Bgl*II-*Bam*HI fragment from the right end of J10A into the *Bam*HI site of YCp50, and then inserting the 1.2-kb *Eco*RI-*Bam*HI fragment from the left end of B9G between the *Eco*RI and *Bam*HI sites of the resulting plasmid. B52D-H9G GF (*cf.* Figure 2) was constructed by cloning the 1.1-kb *Bam*HI-*Xho*I fragment from the left end of H9G between the *Bam*HI and *Sal*I sites of YCp50, and then inserting the 1.4-kb *Eco*RI-*Bam*HI fragment from the right end of 62B5-2D in the resulting plasmid. J11D-K3B GF (*cf.* Figure 2) was constructed by inserting the 0.8-kb *Hin*dIII-*Eco*RI fragment from the right end of J11D between the corresponding sites of YCp50, and then inserting the 0.9-kb *Hin*dIII-*Bam*HI fragment from the right end of K3B in the resulting plasmid.

Preparation of DNA: Plasmid DNAs were prepared from *E. coli* strains by the alkaline lysis procedure as described by MANIATIS, FRITSCH and SAMBROOK (1982). Large scale preparations were purified by centrifugation in CsCl gradients.

Covalently closed circular DNA was prepared from yeast by the alkaline lysis procedure described by DEVENISH and NEWLON (1982).

Yeast genomic DNA was prepared from spheroplasts by a modification of the method of BLOOM and CARBON (1982). Approximately 5×10^9 spheroplasts in 0.5 ml 1 M sorbitol were mixed with 5 ml lysis buffer (0.1 M Tris-Cl, 0.05 M NaEDTA, pH 8.0) and lysed by the addition of 10% (w/v) sodium dodecyl sulfate (SDS) to a final concentration of 0.5%. The lysate was made 0.5 M in NaCl by the addition of a 5 M solution, and then extracted with 0.75 volume of phenol:chloroform:isoamyl alcohol (50:24:1). Following a second extraction with 0.5 volume chloroform:isoamyl alcohol (24:1), DNA was precipitated with 2 volumes of ethanol and recovered by centrifugation. The DNA pellet was dissolved in 0.5 ml STE (10 mM NaCl, 10 mM Tris-Cl, 1 mM EDTA, pH 8.0) and treated with 5 μg/ml pancreatic RNase at 37° for 1 hr. SDS and NaCl were added as above, and the organic extractions were repeated before DNA was precipitated with ethanol.

Construction of chromosome *III* libraries: DNA preparations enriched for circular chromosome *III* were prepared by the alkaline lysis method from approximately 2×10^{10} cells of strains XG1#24 and DC208 as described above and centrifuged to equilibrium in neutral CsCl gradients as described previously (NEWLON *et al.* 1974). Fractions were collected through a 15 gauge needle, and DNA-containing fractions were combined and precipitated with ethanol. DNA from each preparation was digested with *Bam*HI,

TABLE 1

Strains

Strain	Genotype or description	Source or reference
XJ24-24a	*MAT***a** *arg4-17 trp1-1 aro7-1 ade6 lys2 MAL2*	STRATHERN *et al.* (1979)
LS1	*ura3* derivative of XJ24-24a, isolated by growth on 5-FOA plates	SYMINGTON and PETES (1988)
248	*MAT***a** *his4 leu2-1 cry1-3 ade6 lys2 MAL2*	STRATHERN *et al.* (1979)
YNN27	*MATα trp1-289 ura3-52*	STINCHCOMB *et al.* (1980)
CN31C	*MAT***a** *his4 leu2-1 cry1-3 ade6 ura3-52*, segregant of cross between 248 and YNN27	WARMINGTON *et al.* (1987)
XG1#24	*MAT***a**/*MATα-lethal his4/HIS4 leu2-1/LEU2 cry1-3/CRY1 aro7-1/ARO7 lys2/LYS2 ade6/ade6 MAL2/mal2*; carries ring chromosome derived from XJ24-24a chromosome *III*	STRATHERN *et al.* (1979)
A364A	*MAT***a** *ade1 ade2 ura1 tyr1 his7 lys2 gal1*	HARTWELL (1967)
DC208	*MAT***a**/*MATα-lethal* [2 μm^0]; derived from strain CB11, carries ring chromosome *III*	STRATHERN *et al.* (1980)
AB972	*MATα gal2 tra1-0* [rho^0]; derived from S288C without outcrossing	LINK and OLSON (1991)
AH22	*MAT***a** *his4-519 leu2-3 leu2-112 can1*, derived from S288C	HINNEN, HICKS and FINK (1978)

TABLE 2

Sources of chromosome *III* probes

Plasmid	Insert	Source
YIp300	22.8-kb *HIS4 Bam*HI fragment (HINNEN *et al.* 1979)	G. FINK
295	3.6-kb *Eco*RI-*Hin*dIII fragment containing *MAT***a**	J. STRATHERN
366	0.5-kb *Eco*RI-*Hin*dIII fragment centromere-proximal to *MAT*	J. STRATHERN
373	0.8-kb *Hin*dIII-*Bam*HI fragment distal to *HML*	J. STRATHERN
pJL37	1.4-kb *Eco*RI fragment carrying part of *CRY1* (LARKIN and WOOLFORD 1983)	J. WOOLFORD
pTy1-17	*Xho*I fragment of Ty1-17, subcloned from λgtKG17 (KINGSMAN *et al.* 1981)	This study
pCH4	6.5-kb *CEN3* fragment isolated from partial *Sau*3A digest (HSIAO and CARBON 1981)	J. CARBON
pB1	3.1-kb *Hin*dIII fragment carrying *PGK1* (HITZEMAN, CLARKE and CARBON 1980)	J. CARBON

mixed with *Bam*HI-digested, dephosphorylated YIp5, and treated overnight with T4 DNA ligase using standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). *E. coli* strain JA226 was transformed with the ligation mixtures and plated on LB-amp plates. To select transformants carrying plasmids with inserts, transformation plates were replicated to tetracycline-containing plates. Individual ampicillin-resistant colonies that were tetracycline-sensitive were picked and stored in a gridded array in microtiter dishes. The final library from strain XG1#24 contained 624 transformants, and that from DC208 contained 192 transformants. Calculations based on an expected *Bam*HI fragment size of 9.3 kb and assuming that ring chromosome *III* DNA was the only DNA in the preparations cut by *Bam*HI and that all chromosome *III* fragments were cloned with equal efficiency predicted that a library of 97 plasmids has a 99% probability of containing the entire ring chromosome (CLARKE and CARBON 1976).

Screening of λ libraries for chromosome *III* sequences: A λ-Charon 4A library constructed with partially *Eco*RI-digested DNA from *S. cerevisiae* strain A364A (WOOLFORD and ROSBASH 1981) was screened with a probe prepared by nick-translating ring chromosome *III* DNA purified from an agarose gel by the perchlorate method of YANG, LIS and WU (1979). Because the chromosome *III* DNA was contaminated with ribosomal DNA (see DEVENISH and NEWLON 1982), positive plaques were counterscreened using a rDNA probe. A total of 142 chromosome *III*-specific phages with an average yeast DNA insert of about 13 kb were identified.

Gels, transfers, nick translations and hybridizations: Electrophoresis of DNA was in 0.4% or 0.7% agarose gels using a Tris-borate-EDTA buffer (MANIATIS, FRITSCH and SAMBROOK 1982). Transfer of DNA to nitrocellulose filters, nick-translation of DNA probes, and hybridizations were by standard procedures as described previously (DEVENISH and NEWLON, 1982).

***ARS* assays:** *ARS*-containing plasmids were identified on the basis of their ability to transform *S. cerevisiae* strain YNN27 with efficiencies 10^2- to 10^3-fold higher than the YIp5 vector control. DNA was prepared from plasmid-bearing YNN27 transformants that gave a positive result in this assay and was analyzed for the presence of covalently closed circular plasmid DNA by probing blots of uncut DNA that had been subjected to electrophoresis in 0.7% agarose gels with ^{32}P-labeled probe specific for the insert. In all cases, plasmids exhibiting high transformation efficiencies were found to be maintained extrachromosomally.

RESULTS

Construction of a restriction map of ring chromosome *III*: Our strategy for constructing a restriction map of the ring chromosome was to construct a library of fragments from a DNA preparation enriched for ring chromosome *III* in the integrating *URA3* vector YIp5 (STRUHL *et al.* 1979). This vector was chosen to facilitate the later functional analysis of chromosome *III* fragments in yeast, including the presence of *CEN* and *ARS* activity. For the construction of the YIp5 library, covalently closed circular DNA was prepared from *S. cerevisiae* strains carrying the ring chromosome by a gentle alkaline lysis method and digested to completion with *Bam*HI. *Bam*HI was used because it was expected to cut yeast DNA into relatively large fragments and, more importantly, be-

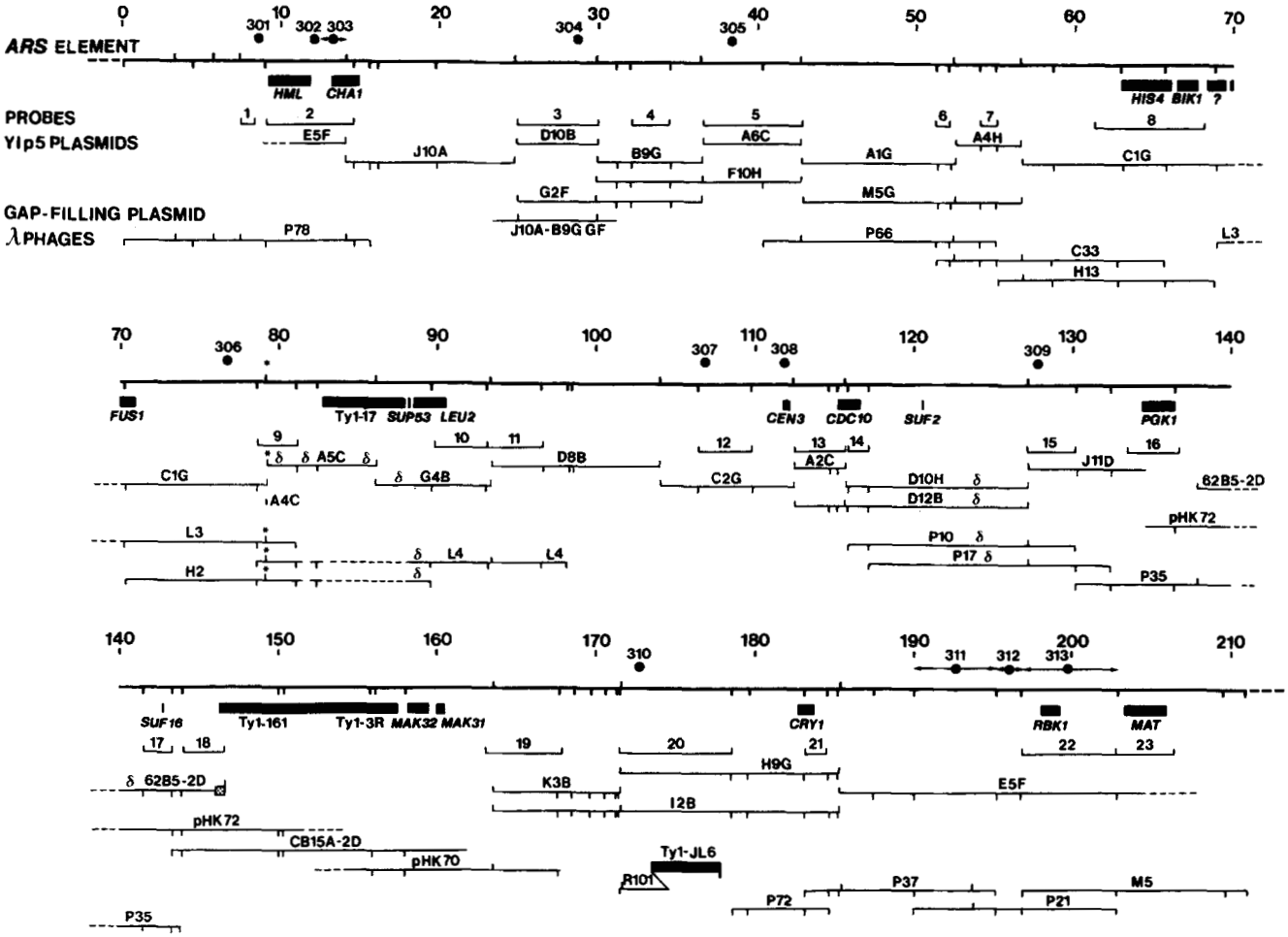

FIGURE 1.—Restriction map of the *HML* to *MAT* region of chromosome *III*. The heavy line represents the deduced restriction map; it is broken into three segments for convenient presentation. The numbers above the restriction map are distances in kilobases. Fragments used as probes for library screening are shown below the restriction map and are numbered 1–23 for cross-referencing with Tables 3 and 4. The *Bam*HI fragments recovered in the YIp5 library prepared from strain XG1#24 are drawn below the probes and are designated according to the position of the plasmid in the gridded library. The stippled segment at the end of the 62B5-2D fragment is not present in the ring chromosome (see Figure 2). The asterisk (*) indicates the position of the 150 bp A4C fragment whose location was confirmed in λ L4. As noted in Table 3, this fragment was initially identified as a second *Bam*HI fragment in a plasmid carrying A6C. This is the only fragment that was found together with nonadjacent chromosome *III* fragments in plasmids in the YIp5 library. The plasmids carrying adjacent genomic *Bam*HI fragments (G2F, F10H, M5G, D12B and I2B) were present as single isolates in the library, and are not listed in Table 3. Except as noted in Table 3 and the text, all plasmids are from strain XG1#24. The "gap-filling" plasmids are described in the text and in Figure 2. The inserts identified in a subset of the λ Charon 4A phages prepared from strain A364A are shown below the plasmids. The dashed lines in λ phages H2 and L4 represent positions occupied by Ty and delta elements in the YIp5 clones prepared from strain XG1#24 that are not present in the λ clones from strain A364A DNA. Similarly, phage R101 carries part of a Ty element (Ty1-JL6) that is not present in the XG1#24 ring chromosome (see text). The filled circles above the restriction map, numbered 301–313, represent *ARS* elements (see Figure 3 and text). The horizontal arrows associated with 303, 311, 312 and 313 represent the extents of the smallest *ARS*-containing subclones defined. The placements of genes, indicated by filled bars below the restriction map, were on the basis of published reports: *HML* and *MAT* (STRATHERN *et al.* 1980; ASTELL *et al.* 1981), *CHA1* (BORNÆS 1988), *HIS4* (DONAHUE, FARABAUGH and FINK 1982), *BIK1*, *?*, *FUS1* (TRUEHART, BOEKE and FINK 1987), Ty1–17 (KINGSMAN *et al.* 1981; WARMINGTON *et al.* 1985), *SUP53* (FISCHOFF, WATERSTON and OLSON 1984; WARMINGTON *et al.* 1986), *LEU2* (RATZKIN and CARBON 1977; ANDREADIS *et al.* 1984), *CEN3* (FITZGERALD-HAYES, CLARKE and CARBON 1982), *CDC10* (CLARKE and CARBON 1980), *SUF2* (CUMMINS and CULBERTSON 1981), *PGK1* (HITZEMAN, CLARKE and CARBON 1980), *SUF16* (GABER and CULBERTSON 1982), Ty1-161 (KINGSMAN *et al.* 1981; WARMINGTON *et al.* 1987), Ty1-3R (WARMINGTON *et al.* 1987), *MAK31*, *MAK32* (TOH-E and SAHASHI 1985), Ty1-JL6, *CRY1* (LARKIN and WOOLFORD 1983), *RBK1* (THIERRY, FAIRHEAD and DUJON 1990). ⊥, *Bam*HI; T, *Eco*RI.

cause it does not cleave the major known contaminants in the circular DNA preparations, 2 μm DNA and ribosomal DNA (see DEVENISH and NEWLON, 1982). To facilitate chromosome "walking," a λ Charon 4A library prepared from a partial *Eco*RI digest of total genomic DNA was screened with gel-purified ring chromosome *III* DNA as a probe. The *Eco*RI fragments in these phages overlapped the *Bam*HI ends of the fragments in the YIp5 library, and allowed identification of YIp5 clones carrying adjacent chromosome *III* fragments.

Using cloned fragments containing the *HIS4*, *CEN3*, *PGK1*, *CRY1* and *MAT***a** loci (see Table 2) to screen the chromosome *III*-specific YIp5 and λ libraries, and

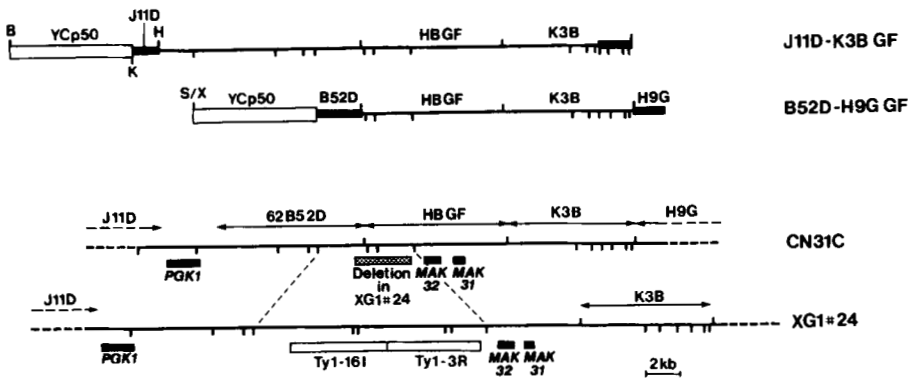

FIGURE 2.—Right arm gap sequences recovered from strain CN31C and their relationship to the XG1#24 ring chromosome. The top part of the figure shows restriction maps of plasmids produced by gap repair in strain CN31C using plasmids J11D-K3B GF and H9G-B52D GF. YCp50 vector sequences are indicated by open boxes and the chromosome *III* fragments cloned to provide homology for gap repair are indicated by filled boxes (see MATERIALS AND METHODS). The lower part of the figure shows the relationship of the deduced structure of this region in strains CN31C and the ring chromosome from strain XG1#24, showing names of plasmids from Figure 1. See WARMINGTON *et al.* (1987) for details of analysis of region around Ty1-161 and Ty1-3R. *Bam*HI and *Eco*RI sites are as in Figure 1; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

walking from those regions, we were able to construct a restriction map of approximately 160 kb of chromosome *III* that contained two gaps. It became apparent in the early screening experiments that the the library prepared from strain XG1#24 was of much higher quality than the DC208 library, and the screening of the DC208 library was not completed. Figure 1 summarizes the final restriction map of the ring chromosome from strain XG1#24 as well as the *Bam*HI fragments carried by individual plasmids and λ Charon 4A phages. The probes used for screening the libraries are also indicated. Table 3 details the plasmids identified, listing the probes used, the sizes of the inserts, and the number of independent plasmids recovered. Table 4 details the λ phages with overlapping inserts and the probes used for their identification. Only the minimal subset of the λ phages recovered are listed in Table 3 and shown in Figure 1. Cloning of sequences from the gaps that remained, one from approximately position 25 to 30 kb and the second from approximately position 145 to 172 kb on the map in Figure 1, is described below.

In addition to overlapping λ clones, two lines of evidence were used to demonstrate that the YIp5 clones represented chromosomal DNA fragments that had not been rearranged in the process of constructing or propagating the clones, and that the order deduced is correct. First, in most cases, multiple plasmids carrying the same insert were identified in the YIp5 library (see Table 3). Analysis of minipreps from all of the isolates of a given fragment with at least three restriction enzymes revealed that the independent clones were identical. It is unlikely that the same rearrangement would occur in all representatives of a given clone. Second, hybridization of segments of the cloned DNA fragments to blots of genomic DNA that had been cut with *Bam*HI, *Eco*RI or *Hin*dIII revealed that genomic *Bam*HI fragments were of the same size as the cloned fragments. In addition, pairs of small fragments flanking a *Bam*HI site that separated adjacent clones were shown to hybridize to the genomic *Eco*RI or *Hin*dIII fragment that contained the genomic *Bam*HI site. These predictions were confirmed for all fragments mapped, using genomic DNA prepared from strains XG1#24, XJ24-24a, A364A and AB972 (data not shown). The only exceptions noted were for restriction fragment polymorphisms among the strains (see below), and in these cases genomic DNA prepared from the strain from which the clones were obtained conformed to expectations.

Two gaps remained after the initial stages of the mapping were complete. We were unable to obtain fragments that overlapped the *Bam*HI sites at each end of the gaps by carefully screening two additional libraries in λ vectors and one additional plasmid library. On the assumption that the sequences in the gaps might be deleterious to *E. coli*, we attempted to clone the sequences directly in yeast by exploiting the ability of yeast to repair "gapped" plasmids by gene conversion from chromosomal sequences (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983).

Because strain XG1#24 is a Ura^+ diploid, it could not be used as the recipient for a gap repair plasmid based on YCp50. Therefore, gapped plasmids were used to transform *ura3* derivatives of the haploid parents of strain XG1#24. LS1 (SYMINGTON and PETES 1984) was derived from strain XJ24-24a, the haploid parent whose chromosome *III* circularized to yield the ring chromosome. Strain CN31C carries the linear chromosome *III* present in strain XG1#24 that originated in its other haploid parent, strain 248 (WARMINGTON *et al.* 1987). No differences in the restriction map of the region around the gap on left arm of these chromosomes have been detected.

For the gap in the left arm flanked by J10A and B9G (position 25 to 30 kb in Figure 1), the plasmid

TABLE 3

Strain XG1#24 ring chromosome *III* plasmids

Plasmid designation	Probe used[a]	Insert size (kb)	Number recovered	Loci present[b]
J10A	2: 6.6-kb *Eco*RI fragment from λP78	10.8	18	Part of *CHA1*
D10B	3: 5.1-kb *Bam*HI fragment from J10A-B9G GF	5.1	15	
B9G	4: 2.5-kb *Eco*RI fragment from F10H	6.6	18	
A6C	5: isolated in screen for *ARS* function; insert used to screen plasmid bank and λ phages	6.2	40	
A1G	6: 0.9-kb *Eco*RI fragment from λC33	9.7	10	
A4H	7: 4.2-kb *Eco*RI fragment from λC33	4.2	11	
C1G	8: 9.3-kb *Pst*I fragment from YIp300	22.8	7	*HIS4*, *BIK1*, *FUS1*, and unidentified ORF
A4C	0.15-kb *Bam*HI fragment found in plasmid with A6C insert	0.15	149	
A5C	9: 2.4-kb *Eco*RI fragment from λL4	6.8	8	Part of Ty1-17
G4B	10: 3.4-kb *Eco*RI fragment from λL4	7.2	29	Part of Ty1-17, *LEU2*
D8B	11: 3.6-kb *Eco*RI fragment from λL4	10.7	12	
C2G	12: 3.4-kb *Eco*RI fragment from pCH4	8.4	5	*CEN3*
A2C	13: 3.2-kb *Eco*RI fragment from D12B	3.2	14	Part of *CDC10*
D10H	14: 1.3-kb *Eco*RI fragment from λP10	11.6	55	Part of *CDC10*, *SUF2*
J11D[c]	16: 3.1-kb *Hin*dIII fragment from pB1 (*PGK1*)	~7.2	1	Part of *PGK1*
62B5-2D[d]	17: 1.6-kb *Eco*RI fragment from λP35	8.6	1	*SUF16*
CB15A-2D[e]	18: 2.8-kb *Bam*HI fragment from 62B5-2D	18	1	
pHK70	See text	22	1	
K3B	19: 4.6-kb *Sac*I-*Xho*I fragment from pHK70	8.0	10	
H9G	21: 1.4-kb *Eco*RI fragment from pJL37 (Table 2)	14.0	8	*CRY1*
E5F	22: 6.1-kb *Eco*RI fragment from λM5	22.5	5	*MAT/HML* fusion, part of *CHA1*

[a] Probes used are indicated and numbered in Figure 1.
[b] References are indicated in the legend to Figure 1.
[c] The single plasmid identified carries only the *CEN3*-proximal 7.2 kb of a 36.5-kb *Bam*HI fragment.
[d] This plasmid was isolated from the library constructed from strain DC208.
[e] Plasmid isolated from YCp50 library constructed by Rose *et al.* (1987).

J10A-B9G GF was used. It was linearized by digestion with *Bam*HI, and the presence of *CEN4* on the plasmid prevented the recovery of transformants carrying integrated plasmid. Uncut DNA from transformants was screened using a pBR322 probe for plasmids that were larger than the starting plasmid. In the case of LS1, screening more than fifty transformants revealed only plasmids of the same size as the starting plasmid. Two of these were recovered in *E. coli*, and they were shown to have a restriction map identical to the starting plasmid. In contrast, more than half of the CN31C transformants carried a larger plasmid. Plasmids were recovered from four independent transformants, and all carried the yeast insert shown in Figure 1 (J10A-B9G GF).

The recovery of these sequences by gap repair confirms that the 5.1-kb fragment carried with the adjacent B9G fragment in plasmid G2F is the only *Bam*HI fragment in the gap. Screening the YIp5 library with this 5.1-kb fragment revealed fifteen plasmids carrying this fragment in the library. The *E. coli* tranformants carrying these plasmids grew slowly, forming colonies less than half the diameter of those carrying other chromosome *III* fragments after overnight growth. This suggests that plasmids and phages carrying this fragment in the other libraries screened may have been lost as a result of their poor growth when the libraries were amplified.

The gap in the right arm represented a more serious challenge because hybridization of genomic DNA from several strains with probes flanking the region revealed the region to be extremely polymorphic when DNA was analyzed from several strains, including the ring strain XG1#24 and its haploid parents as well as A364A and AB972, a strain derived from S288C. The polymorphisms could not be explained by the simple presence or absence of restriction sites. Thus, probe 15 from the J11D fragment hybridized to a *Bam*HI fragment too large to be resolved in XJ24-24a and AB972, to a smaller 10.5-kb fragment in A364A, and to a fragment of intermediate size in strain 248. Probe 17 from λ P35 hybridized to a 9-kb *Bam*HI fragment in A364A and to the same large fragment as probe 15 in the other strains.

Screening of the ring chromosome libraries with probe 17 identified only one positive clone in the

TABLE 4

λ phages carrying chromosome *III* fragments

Phage designation	Probe used[a]	Insert size (kb)
P78	1: 0.8-kb *Hin*dIII-*Bam*HI *HML*-distal fragment from plasmid 373 23: 3.6-kb *Eco*RI-*Hin*dIII *MAT***a** fragment from plasmid 295 (Table 2)	15.8
P66	5: 6.2-kb A6C fragment (Table 3) 6: 0.9-kb *Eco*RI from λC33 7: 1.9-kb *Eco*RI from λC33	14.6
C33	8: 9.3-kb *Pst*I fragment from YIp300	13.8
H13	8: See entry above	13.2
H2	22.8-kb C1G fragment (Table 3)	13.4
L3	9: 2.5-kb *Eco*RI fragment from λH2	12.1
L4	9: See above 10: 3.4-kb *Eco*RI from G4B	3.4
P10	15: 3.4-kb *Bam*HI-*Eco*RI fragment from J11D	13.6
P17	15: 3.4-kb *Bam*HI-*Eco*RI fragment from J11D	14.4
P35	16: 3.1-kb *Hin*dIII fragment from pB1 (Table 2)	13.5
R101	20: 7.0-kb *Eco*RI fragment from H9G	7.4
P72	21: 1.4-kb *Eco*RI fragment from pJL37 (Table 2)	6.2
P37	21: See above	12.2
P21	22: 6.1-kb *Eco*RI fragment from λM5	13.0
M5	23: 3.6-kb *Eco*RI-*Hin*dIII *MAT***a** fragment from plasmid 295 (Table 2); confirmed to contain *MAT* locus by hybridization with *MAT*-proximal fragment in plasmid 366 (Table 2)	13.9

[a] Probes used are indicated and numbered in Figure 1.

library from strain DC208 and none in the XG1#24 library. Hybridization of sequences from the distal end of this clone (62B5-2D, probe 18) to *Bam*HI-digested genomic DNA revealed that the particular *Bam*HI sites that flank the ends of this clone are polymorphic, and are not present in the XG1#24 ring chromosome but are present in strain A364A. This probe also failed to reveal positive clones in the chromosome *III*-specific plasmid and λ libraries as well as two other λ libraries. Similarly, use of probe 20 from the proximal end of the H9G fragment failed to identify phages that overlapped the other end of the gap.

KLEIN and PETES (1984) had used the integration of a plasmid carrying the *LEU2* gene and part of the adjacent Ty element, Ty1-17, to mark and genetically map several Ty elements in the genome of strain AH22, which was derived from the commonly used laboratory strain S288C (HINNEN, HICKS and FINK 1978). One of the mapped integrants, AH22(CV9)-32, identified a Ty element between *CEN3* and *MAT*. By cutting genomic DNA from this transformant with *Sac*I, an enzyme that cuts once within plasmid sequences, we were able to recover a plasmid, pHK70, carrying genomic sequences flanking the integration site. The use of *Pvu*I allowed recovery of genomic sequences flanking the other side of the integration site, in plasmid pHK72 (see Figure 1). pHK72 was shown to overlap plasmids J11D and 62B5-2D, and a unique fragment from pHK70 (probe 19) was used to identify fragment K3B and nine other plasmids with the same insert in the YIp5 ring chromosome library. The use of probe 18 to screen a YCp50 library constructed from another S288C-derived strain by ROSE *et al.* (1987) identified plasmid CB15A-2D, which spans the tandem Ty elements.

Since the K3B fragment was found together with the H9G fragment in plasmid I2B, it seemed likely that the gap had been bridged. This was confirmed by using two gap-filling plasmids to recover gap sequences from strain CN31C. We also tried to recover gap sequences from strain LS1 but, as was the case with attempts to rescue sequences from the left gap in this strain, we failed to recover anything other than recircularized vector after screening more than 100 Ura^+ transformants obtained with the J11D-K3B GF plasmid. It is not clear why we failed to recover gap-repaired plasmids from strain LS1. Perhaps the strain is deficient in gap repair; alternatively, recircularization of linear plasmids may be more efficient in strain LS1.

Figure 2 summarizes the deduced structure of the sequences recovered from the gap region of chromosome *III* from strain CN31C compared with the structure of the region in the ring chromosome of strain XG1#24. Analyses of the sequences recovered in the J11D-K3B GF plasmid by hybridization with digests of the J11D, 62B5-2D and CB15A-2D plasmids and comparisons of their restriction maps were consistent with the map shown in Figure 1 for the region between positions 130 and 165 kb. Analysis of the sequences recovered in the B52D-H9G GF plasmid confirmed that fragment K3B is adjacent to fragment H9G as shown in Figures 1 and 2. The details of the hybridization experiments and DNA sequence analysis demonstrating that the ring chromosome in strain XG1#24 differs from chromosome *III* of strain CN31C by the deletion of approximately 3.5 kb of DNA and the presence of a tandem pair of Ty1 elements have been published previously (WARMINGTON *et al.* 1987).

In summary, we have shown that the ring chromosome *III* derived from strain XJ24-24a and isolated from strain XG1#24 (STRATHERN *et al.* 1979) is 194 kb in length and contains 17 *Bam*HI fragments that vary in length from 0.15 to 36.5 kb. An additional 10 kb of DNA distal to *HML* and 5 kb of DNA distal to *MAT* were recovered in λ clones. The library prepared from strain XG1#24 was complete except that only part of the 36.5-kb *Bam*HI fragment was recovered.

The smaller library prepared from the 2 μm° strain, DC208 was incomplete, but allowed recovery of a portion of the large *Bam*HI fragment.

Restriction fragment length polymorphisms: The YIp5 ring chromosome library and the λ Charon 4A library used for chromosome walking were prepared from different strains, and it was not surprising to find some differences in the restriction maps of the two sets of clones. Some of the differences could be explained by single base pair changes that created or abolished a restriction site without changing the overall length of the region. Three such restriction site polymorphisms were identified: the *Bam*HI site present at position 138 in A364A (λ phage P35) but not in the XG1#24 ring chromosome, the *Bam*HI site present at position 194 kb in A364A (λ phages P37 and P21) but not in the XG1#24 ring chromosome (YIp5 plasmid E5F), and the *Eco*RI site present at position 188 kb in the XG1#24 ring chromosome (YIp5 plasmid E5F) but not in A364A (λ phage P37.

Other length polymorphisms could not be explained by single base pair changes, but instead changed the physical lengths of the region in question; *e.g.*, compare the λ clones and the YIp5 clones in the region containing *LEU2* between positions 80 and 90 kb (Figure 1). The presence or absence of transposable elements in a region could explain these polymorphisms, and the observation that fragments from the polymorphic regions often hybridized to many DNA bands on genomic blots further suggested that transposons were involved. We therefore analyzed the hybridization of a Ty1-17 probe and a solo δ probe from plasmid pPM5 (Olson *et al.* 1981) to blots containing *Bam*HI-*Eco*RI double digests of the chromosome *III* plasmids and phages. These results are summarized in Figure 1. The Ty elements found in these two strains had all been previously identified (see Figure 1). Chromosome *III* positions occupied by Ty elements differ in A364A and the XG1#24 ring chromosome. The ring chromosome has a Ty2 element distal to *LEU2* on the left arm (Ty1-17[5]) and a pair of Ty1 elements distal to *PGK1* on the right arm. A364A lacks the Ty element distal to *LEU2*, but has a Ty1 element proximal to *CRY1* on the right arm, and may also carry the Ty1-3R element. Hybridization of genomic blots with the 8.5-kb HBGF fragment shown in Figure 2 showed that the homologous fragment in A364A is approximately 14 kb in length, consistent with the presence of a Ty1 element in the fragment. The only solo δ elements identified fall within the previously characterized right arm and left arm hot spots for Ty transposition (Warmington *et al.* 1986, 1987).

Identification and localization of *ARS* elements: Addition of an *ARS* element to an integrating plasmid results in a 100–1000-fold increase in its efficiency of transformation in *S. cerevisiae*. Since the ring chromosome libraries were constructed in an integrating vector, YIp5, the efficiency of transformation of the plasmids should allow identification of the chromosome *III* *Bam*HI fragments that contain *ARS* elements. The results of one such experiment are shown in Table 5, where it can be seen that the control plasmids gave the expected result: YIp5 showed a very low transformation efficiency (<1 transformant/5 μg DNA), while YRp12, a derivative of YIp5 that carries *ARS1* (Stinchcomb *et al.* 1980), transformed 1000-fold more efficiently. Plasmids carrying six of the chromosome *III* fragments, E5F, D10B, A6C, C2G, J11D and H9G, transformed with high efficiency, while the remainder behaved more like the YIp5 vector. Analysis of DNA isolated from these transformants showed that the plasmids exhibiting high frequency transformation were maintained as extrachromosomal circular DNA (data not shown).

In a separate study that examined the time of replication of chromosome *III* sequences (Reynolds *et al.* 1989) a region of the C1G clone was found to replicate early, suggesting that it might contain a replication origin. Therefore, despite the failure to

TABLE 5

Transformation efficiencies of chromosome *III* fragments

Plasmid[a]	Transformants per μg DNA
YIp5	1
YRp12	8,026
J10A	2
D10B	3,820
B9G	7
A6C	15,000
A1G	4
A4H	10
C1G	4
G4B	1
D8B	2
C2G	1,268
A2C	1
D10H	2
J11D	2,980
62B5-2D	2
HBGF[b]	3
K3B	5
H9G	3,376
E5F	579
M5G	3
D12B	7
λC33-4.4	5
λL4-2.4	6

Strain YNN27 was transformed using the lithium acetate procedure of Ito *et al.* (1983).

[a] Plasmids carry one or more chromosome *III* *Bam*HI fragments except λC33-4.4 and λL4-2.4 which carry *Eco*RI fragments subcloned from the λ phages indicated. See Figure 1 for locations of fragments.

[b] HBGF fragment subcloned from J11D-K3B GF (see Figure 2).

[5] Ty1-17 is a class II (Ty2) element (see Warmington *et al.* (1985).

observe high efficiency transformation by the YIp5 plasmid carrying the 22-kb C1G fragment, we examined subclones of the fragment, and discovered that the 8.1-kb *Eco*RI fragment from C1G, in fact, contains an *ARS* element.

To exclude the possibility that the ring chromosome contains additional *ARS* elements whose activity was disrupted by cloning *Bam*HI fragments, we tested plasmids carrying fragments that overlapped the *Bam*HI junctions. In some cases we had recovered YIp5 clones containing two adjacent *Bam*HI fragments derived from partial digestion products (*e.g.*, M5G that contains A1G and A4H, and D12B that contains A2C and D10H, see Figure 1). In other cases, we subcloned *Eco*RI fragments from λ phages (*e.g.*, the 4.4-kb *Eco*RI fragment from λ C33, and the 2.4-kb *Eco*RI fragment from λ L4). In no case did we find evidence of an *ARS* element disrupted by a *Bam*HI site.

Since the chromosome *III Bam*HI fragments that exhibit *ARS* activity are large, varying from 5.1 to 22.5 kb, it seemed possible that some fragments might contain more than one *ARS* element. We therefore subcloned the fragments using the YIp5 vector as shown in Figure 3. Subclones were tested for *ARS* activity as described above. We also subcloned the yeast insert in λ P78 to map *ARS* elements distal to the *HML* locus. For five of the *Bam*HI fragments (D10B, A6C, C1G, J11D and H9G), subcloning revealed a single *ARS* element that could be localized within a fragment 0.1 to 1 kb in length. The C2G fragment contains two *ARS* elements, one of which is tightly associated with *CEN3*. Limited subcloning of the E5F fragment, which contains the *MAT/HML* fusion that created the ring chromosome, allowed identification of at least five *ARS*-containing subclones. Two of these *ARS* elements are centromere proximal to the *HML* locus and three are centromere proximal to the *MAT* locus in the wild-type, linear chromosome. Subcloning the λ P78 clone revealed one *ARS* element distal to *HML* as well as the two found proximal to *HML* in the E5F plasmid. The two *ARS* elements flanking *HML* are likely to correspond to the previously identified *ARS* elements associated with the *HML* transcriptional silencers, E and I (Broach *et al.* 1984).

The positions of the thirteen *ARS* elements identified by this analysis are indicated above the chromosome *III* restriction map in Figure 1. An additional *ARS* element associated with the left telomere, and located distal to the sequences contained in λ P78 has been identified by Button and Astell (1988). We have used a convention to name these *ARS* elements that should be generally applicable (Campbell and Newlon 1991). The first number in the designation indicates that these *ARS* elements are from chromosome *III*. The second two numbers uniquely identify each *ARS*. Since we believe that we have identified all the *ARS* elements in this 210-kb region of chromosome *III*, we have numbered them from left to right according to their chromosomal position. The *ARS* element associated with the left telomere would become *ARS300*. For other chromosomes we propose that the first one or two digits in an *ARS* element refer to the chromosome on which it resides, and the last two digits reflect the order of its identification.

DISCUSSION

This project was undertaken in 1980 before the development of pulsed-field gel electrophoresis methods for the separation of intact chromosomal DNAs (Schwartz and Cantor 1984; Carle and Olson 1984, 1985; Chu, Vollrath and Davis 1986), and we took advantage of the physical properties of a circular derivative of chromosome *III* to purify a single chromosome. Seventy percent of the clones in the library constructed from the purified ring chromosome were ultimately shown to be derived from chromosome *III*, as were 97% of the 143 λ clones identified by screening with gel-purified ring chromosome.

Our map covers approximately 60% of the chromosome (210 kb of the estimated 350-kb total length–Link and Olson 1991). The λ clones extend to within approximately 3 kb of the left telomere (Button and Astell 1986) and extend approximately 5 kb beyond the *MAT* locus on the right arm. Analysis of these clones has allowed us to identify and map *ARS* elements, compare physical and genetic maps, and examine the nature of chromosome polymorphisms in related laboratory strains.

***ARS* elements on chromosome *III*:** The data presented here provide the first long-range map of potential replication origins on a eukaryotic chromosome. *ARS* elements are identified by their ability to promote high frequency transformation and to allow the extrachromosomal maintenance of plasmids. The density of *ARS* elements in the 210-kb region of chromosome *III* analyzed (13) is twice that previously reported (Beach, Piper and Shall 1980; Chan and Tye 1980), perhaps because of the extensive subcloning done in the present study. Two of the seven *ARS*-containing *Bam*HI fragments from chromosome *III* were shown to have more than one *ARS* element, with one of them having at least five.

A long-standing question is whether *ARS* elements, identified on the basis of a plasmid transformation assay, function as chromosomal replication origins. The 13 *ARS* elements identified in the region of chromosome *III* analyzed is about twice the number of replication origins expected on the basis of previous estimates of the average spacing between active rep-

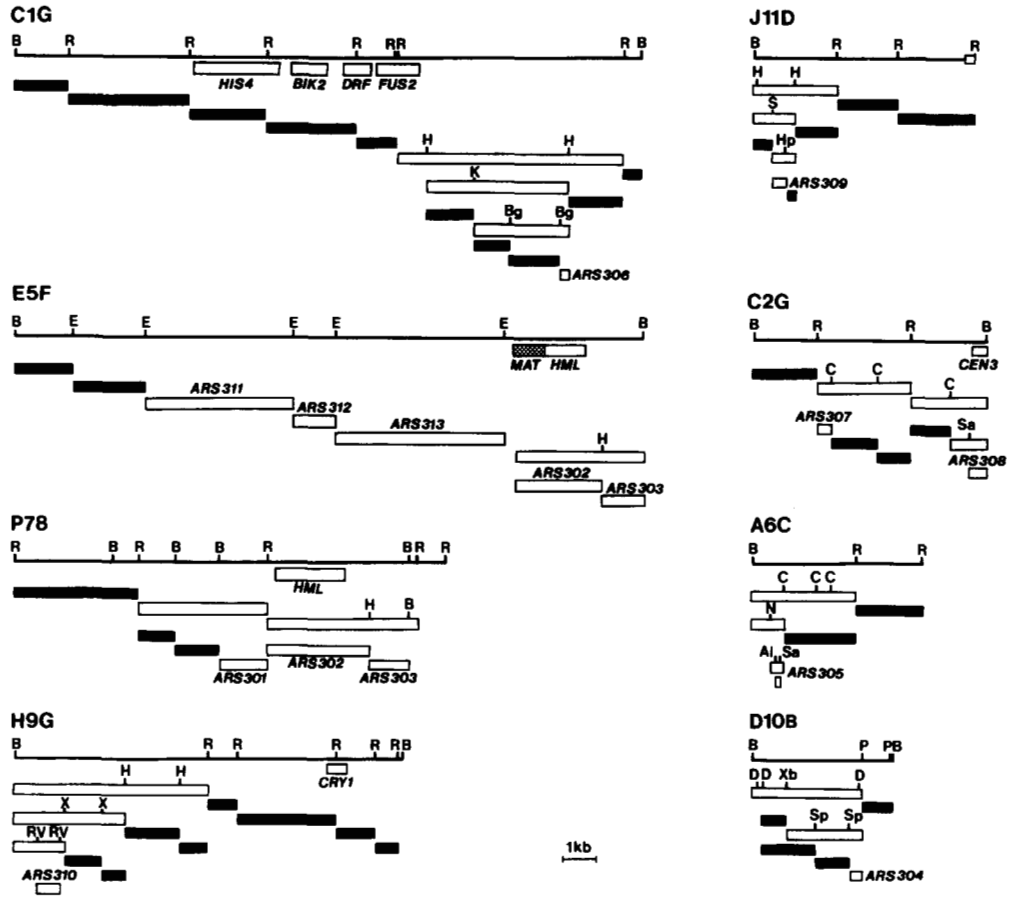

FIGURE 3.—Subcloning analysis of *ARS* localization in chromosome *III* clones. *Bam*HI fragments found to promote high frequency transformation were subcloned in YIp5, using corresponding restriction sites. *Bgl*II fragments were cloned using the vector *Bam*HI site, and *Xho*I fragments were inserted in the vector *Sal*I site. Open bars represent fragments exhibiting *ARS* function (high frequency transformation), and filled bars show subclones lacking *ARS* function. *ARS* elements are numbered as described in the text. B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; R, *Eco*RI; RV, *Eco*RV; H, *Hin*dIII; Hp, *Hpa*I; N, *Nru*I; S, *Sal*I; Sp, *Ssp*I; Xb, *Xba*I; X, *Xho*I.

lication origins obtained by electron microscopic and autoradiographic analysis of replicating DNA (1 per 36 kb; reviewed by NEWLON 1988, 1989). A question of obvious importance is which, if any, of the *ARS* elements we have identified function as chromosomal replication origins. Using two-dimensional gel electrophoresis methods that allow a direct assessment of replication patterns of chromosomal DNA molecules (BREWER and FANGMAN 1987; HUBERMAN *et al.* 1987), we have found that of ten *ARS* elements examined (*ARS301-ARS310*), only five function as chromosomal origins at a detectable level (HUBERMAN *et al.* 1988; DUBEY *et al.* 1991 ; S. A. GREENFEDER, A. DESHPANDE, I. COLLINS and A. DERSHOWITZ, unpublished data; reviewed by CAMPBELL and NEWLON 1991). This finding, that about half of the *ARS* elements identified do not function as chromosomal replication origins, suggests that the density of active replication origins on chromosome *III* is similar to that of the entire genome. It also raises the interesting question of what distinguishes *ARS* elements that function as chromosomal replication origins from those that do not.

Genetic *vs.* physical map: The region of chromosome *III* between *HML* and *MAT* comprises approximately 57% of its physical length and approximately 56% of its genetic length, with an average of 0.43 cM/kb (genetic data from MORTIMER and SCHILD 1985; MORTIMER *et al.* 1989). However, the relationship between physical and genetic length is not constant. In agreement with the recent study of chromosome *I* (KABACK, STEENSMA and DE JONGE 1989), the intervals flanking the centromere show a lower than average rate of recombination (*CEN3* to *PGK1*: 2 cM and 24 kb, 0.08 cM/kb; *CEN3* to *LEU2*: 5 cM and 22 kb, 0.23 cM/kb). These results are consistent with the previous report of LAMBIE and ROEDER (1988) who showed that moving *CEN3* to near the *HIS4* locus represses recombination around *HIS4*. However, lower than average recombination rates are not confined to the centromeric region. The region between *CRY1* and *MAT* is also relatively cold (0.25 cM/kb). In contrast, the *HIS4-LEU2* interval shows a high rate of recombination (0.73 cM/kb), with the *SUP53-LEU2* interval very high (2 cM/kb from *SUP53* to the middle

of the *LEU2* coding region). Thus there is at least a tenfold variation in the rate of recombination along chromosome *III*, and it seems likely that even greater variation may be found as more detailed studies are made. In a study of the relatively cold *LEU2-CEN3* region, SYMINGTON and PETES (1988) found evidence of a recombination hot spot.

Chromosome polymorphisms: An issue that is largely unresolved is the question of how much strain variation exists within a species at the level of chromosome organization. This question can be approached at several levels, including gene order, the number and location of transposable elements, the conservation of *cis*-acting elements like centromeres and replication origins, and DNA sequence conservation within homologous regions. It is clear from pulsed-field gel analysis that length polymorphisms are common for at least some *S. cerevisiae* chromosomes (SCHWARTZ and CANTOR 1984; CARLE and OLSON 1985; DE JONGE *et al.* 1986), but the reason(s) for the polymorphisms have not been examined. The observation that crosses between strains exhibiting length polymorphisms are completely fertile suggests that the polymorphisms do not result from gross genetic rearrangements but rather from duplications of DNA and the presence of varying numbers of repetitive elements (Ty and Y′ sequences). For example, there are two reports of duplications of sequences mapping near the left end of chromosome *III*, presumably as a result of translocations. In one case, the *HIS4* locus and sequences distal to it were found duplicated on chromosome *I* both genetically (MIKUS and PETES 1982) and physically (SCHWARTZ and CANTOR 1984). In the second case, a fragment distal to *HML* has been reported to be duplicated elsewhere in the genome of at least two strains (STRATHERN *et al.* 1980; HOLMBERG 1982). Results of the analysis of a brewers' yeast chromosome that can substitute for *S. cerevisiae* chromosome *III* emphasize the long-term stability of gene order and function in Saccharomyces (NILLSON-TILLGREN *et al.* 1981; HOLMBERG 1982; RESNICK, SKAANILD and NILLSON-TILLGREN 1989). The left half of the brewers' yeast chromosome has diverged extensively at the nucleotide level as revealed by weak hybridization of *S. cerevisiae* chromosome *III* probes and differences in the restriction maps of regions tested. Despite the divergence, all chromosome *III* genes essential for *S. cerevisiae* are present on the brewers' yeast chromosome, and no differences in the genetic map have been identified.

In conjunction with the recent report of a chromosome *III* physical map by YOSHIKAWA and ISONO (1990), the data reported here extend to three the number of *S. cerevisiae* strains for which the physical map of the region of chromosome *III* between the *HML* and *MAT* loci is known. They allow an assessment of the strain variation in a region covering approximately two-thirds of a eukaryotic chromosome. While these three strains were not specifically chosen to represent the extremes of the species *S. cerevisiae*, they derive from strain backgrounds in common use in basic research. Strain DC5 used by YOSHIKAWA and ISONO was constructed by J. HICKS using strains derived from S288C. The genealogy of S288C, from which most of the auxotrophic mutations used in yeast genetic analysis were derived, has been traced (MORTIMER and JOHNSTON 1986). A364A is also thought to be closely related to S288C (MORTIMER and JOHNSTON 1986). The lineage of strain XJ24-24a, which gave rise to the ring chromosome cloned in this study, can be traced back through five generations, and its progenitor strains include several strains used in the Cold Spring Harbor Yeast Course and strains from I. HERSKOWITZ, T. MANNEY and C. MCLAUGHLIN (J. STRATHERN, personal communication). The auxotrophic markers carried by the progenitor strains are largely from the S288C background.

The three physical maps of chromosome *III* are remarkably similar. Differences found in the physical lengths of segments of chromosome *III* all relate directly or indirectly to differences in the distribution of transposable elements (Ty) or the delta (LTR) sequences of the Ty elements. These length polymorphisms fall in or near previously characterized "hotspots" for transposable elements (WARMINGTON *et al.* 1986, 1987). Thus in the "left-arm hot-spot" strain A364A has no Ty elements and only one solo delta detected by our probe, and the XG1#24 ring chromosome has one Ty2 element and several solo deltas (WARMINGTON *et al.* 1986). The precise structure of DC5 in this region is unclear because clones spanning the region were not recovered by YOSHIKAWA and ISONO (1990). However, their data reveal the presence of at least one Ty1 and one Ty2 element in the region, and comparison with our complete map of the region shows that their ordered clones are not missing more than a few kilobases of unique sequence. Similarly, on the right arm, strains DC5 and A364A have a Ty1 element near *CRY1* that is not present in the XG1#24 ring chromosome, and A364A and the XG1#24 ring differ in the region occupied by tandem Ty1 elements.

It seems clear that even within strains that have been directly derived from S288C there are differences in the number of Ty elements on the right arm of chromosome *III*. We have reported on a strain from this background with a single, hybrid Ty in place of the tandem array of Ty1-161 and Ty1-3R present in strains GRF88 and DC5 (WARMINGTON *et al.* 1987). From the structure of the pHK70 and pHK72 plasmids (Figure 1) and the size of the *Bam*HI fragment homologous to probe 17 (data not shown), it appears

that strain AH22 carries three tandem Ty elements at this position.

Thus, differences in the number of Ty elements on chromosome *III* contribute to the length polymorphisms observed on pulsed-field gels. However, length polymorphisms of at least 100 kb have been observed among meiotic segregants of diploids constructed from strains derived by SIKORSKI and HIETER (1989) from S288C (P. HIETER, personal communication; A. DERSHOWITZ and C. S. NEWLON, unpublished results). It is likely that these large differences in length result from unequal crossing over between tandem iterations of the *ca.* 25-kb region between Ty1-161 and Ty1-JL6 (see Figure 1). These strains carry two or more copies of *ARS310* on chromosome *III* (A. DERSHOWITZ and C. S. NEWLON, unpublished results), and chromosome fragmentations (VOLLRATH *et al.* 1988) map the length polymorphisms proximal to a fragment at position 176 kb (P. HIETER, personal communication). The initial duplication of approximately 25 kb of chromosome *III* sequences could have been generated by a unequal sister chromatid exchange resulting from the pairing of Ty1-JL6 and either Ty1-161 or Ty1-3R. Alternatively, the same duplication could have been generated by a similar unequal mitotic crossover between homologous, non-sister chromatids. In this case the polymorphism results not from differences in the number of Ty elements, but from unequal recombination between Ty elements in direct orientation causing the duplication of intervening DNA sequences.

If polymorphisms resulting from Ty elements are excluded, then differences in restriction enzyme recognition sites can provide one assessment of the degree of DNA sequence divergence. A comparison of our maps for the XG1#24 ring chromosome and A364A with the YOSHIKAWA and ISONO (1990) map of strain DC5 revealed that of the 57 *Eco*RI sites present in at least one strain, four differences (7%) were found. In contrast, of 26 positions where a *Bam*HI site was found in at least one of the three strains, seven positions (27%) showed variation. It is not clear whether the difference in the fraction of polymorphic sites for the two enzymes is significant. In the case of the *Bam*HI polymorphisms, 5 of the 7 found result from a site in strain DC5 that is not present in A364A or the XG1#24 ring chromosome. We did not find these polymorphisms in our analysis of other S288C-related strains, raising the possibility that they are peculiar to strain DC5 or the result of *Bam*HI* activity in the DC5 mapping studies. A further assessment of this issue will be possible when sites for the other six enzymes mapped by YOSHIKAWA and ISONO (1990) can be identified in the DNA sequence of these YIp5 clones as it becomes available from the European Economic Community (EEC) Consortium later this year.

The extent to which the positions of *ARS* elements vary from strain to strain is an important question. It has been difficult to define precisely the DNA sequences required for *ARS* activity. An 11-bp consensus sequence and sequences 3′ to the T-rich strand of the consensus are essential (reviewed by CAMPBELL and NEWLON 1991). The finding that *ARS* elements can be created in prokaryotic vector sequences by mutation (KIPLING and KEARSEY 1990) and the occurrence of *ARS* consensus sequences in yeast DNA without *ARS* function raise the possibility that substantial variation might be found in the location of *ARS* elements. Several of the *ARS* elements we have identified in the ring chromosome from strain XG1#24 clearly have counterparts in other strains. For example, *ARS301*, *ARS302* and *ARS303*, which flank *HML*, have been identified in four strains (BROACH *et al.* 1984; DUBEY *et al.* 1991; this study). Similarly, *ARS306* has also been identified in clones from A364A (A. REYNOLDS, personal communication). In addition, although *ARS306*, *ARS307* and *ARS309* have been identified by the high frequency transformation assay only in the XG1#24 ring chromosome clones, the origins associated with them have been visualized by two-dimensional gels in at least three strains (S. Greenfeder, A. Deshpande, I. Collins and C. S. NEWLON, unpublished results). We are aware of only one possible strain variation in *ARS* location. TOH-E and SAHASHI (1985) reported an *ARS* element in a plasmid that complements a deletion that removes *MAK31* and *MAK32* (see Figure 1). We have not been able to find an *ARS* element in the HBGF fragment that contains this region (see Figure 2). TOH-E and SAHASHI (1985) were unable to localize this putative *ARS* element, raising the possibility that the activity they measured resulted from some conjunction of vector sequences with the yeast DNA in the plasmid. An alternative that cannot presently be excluded is that subcloning of the HBGF fragment would reveal an *ARS* element, as was the case with the C1G fragment. In summary, the data presently available support the view that the locations of *ARS* elements show very little, if any, strain to strain variation.

For the first time we now have an almost complete picture of the structure of a eukaryotic chromosome: a physical map, a transcription map, a map of the *ARS* elements, and a partial map of the replication origins (YOSHIKAWA and ISONO 1990; this study). The forthcoming DNA sequence, determined by the EEC Consortium, of the clones reported here and the sequence of clones covering the rest of the right arm provided by M. V. OLSON will allow a further refinement of the positions of open reading frames and an assessment of the positions of *cis*-acting elements with respect to

coding regions. The ordered clones and the wealth of information accumulating about chromosome *III* provide powerful tools for probing issues relating to chromosome structure and dynamics. The differences found in the number and position of Ty elements in laboratory strains will allow an assessment of the effects of these elements on recombination frequencies. With respect to DNA replication, it should now be possible to probe the determinants of origin function and to systematically alter and delete origins to understand their role in chromosome stability and segregation.

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