Physical Map of the *Saccharomyces cerevisiae* Genome at 110-Kilobase Resolution

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ABSTRACT

A physical map of the *Saccharomyces cerevisiae* genome is presented. It was derived by mapping the sites for two restriction endonucleases, *Sfi*I and *Not*I, each of which recognizes an 8-bp sequence. DNA-DNA hybridization probes for genetically mapped genes and probes that span particular *Sfi*I and *Not*I sites were used to construct a map that contains 131 physical landmarks—32 chromosome ends, 61 *Sfi*I sites and 38 *Not*I sites. These landmarks are distributed throughout the non-rDNA component of the yeast genome, which comprises 12.5 Mbp of DNA. The physical map suggests that those genes that can be detected and mapped by standard genetic methods are distributed rather uniformly over the full physical extent of the yeast genome. The map has immediate applications to the mapping of genes for which single-copy DNA-DNA hybridization probes are available.

UNTIL recently, knowledge of the structure of the yeast genome has come primarily from linkage mapping. Since the pioneering studies of LINDEGREN *et al.* (1962) and HAWTHORNE and MORTIMER (1960), the map has grown to include data on 769 markers with an average spacing of 6 cM (MORTIMER *et al.* 1989). Because the average ratio of physical to genetic distance in yeast is only 2.5–3.0 kb/cM (CARLE and OLSON 1985; MORTIMER and SCHILD 1985), this resolution corresponds to an average spacing between markers of 15–20 kb. As measured in base pairs, the resolution of the yeast linkage map exceeds by several hundredfold that of current genetic maps of higher organisms (see, for example, DONIS-KELLER *et al.* 1987; YOUNG, MILLER and TANKSLEY 1987).

Genetic, cytogenetic and electrophoretic data support the view that yeast has 16 metacentric chromosomes. The only discordant data concern a single centromere-linked dominant mutation (WICKNER, BOUTELET and HILGER 1983) which appears either to involve an aberration in the wild-type karyotype (MORTIMER and SCHILD 1985) or to define a small chromosome that has escaped physical detection. A number of light- and electron-microscopic studies provide good visualization of the yeast chromosomes (BYERS and GOETSCH 1975; KUROIWA *et al.* 1984; DRESSER and GIROUX 1988). Particularly at pachytene, when the synaptonemal complexes are readily traced in electron micrographs, 16 bivalents are clearly visible. Only chromosome *XII*, which encodes the rDNA and is associated with the nucleolus, can be individually recognized in cytogenetic preparations.

Pulsed-field gel electrophoresis has allowed the development of a more detailed karyotype (SCHWARTZ *et al.* 1983; SCHWARTZ and CANTOR 1984; CARLE and OLSON 1984). In typical strains, the 16 chromosomal DNA molecules can be separated into 13 bands (10 singlets and 3 doublets). Because naturally occurring chromosome-length polymorphisms allow each of the three doublets to be resolved in particular laboratory strains, all 16 chromosomes have been identified as individual entities (CARLE and OLSON 1985, 1987).

Further progress on a unified physical and genetic map for yeast requires more refined physical mapping. The separation and identification of the chromosomal DNA molecules provide a physical map with an average resolution of approximately 900 kb; in this map, the physical ends of the chromosomes are the cartographic landmarks. In the present paper, we describe higher resolution mapping using the restriction endonucleases *Sfi*I and *Not*I, both of which have 8-bp recognition sites composed entirely of G/C base pairs (QIANG and SCHILDKRAUT 1987). In combination, the sites for these two enzymes define a map with an average resolution of 110 kb. Most of the mapping was done using hybridization probes to identify particular *Sfi*I or *Not*I fragments with corresponding segments of the linkage map. Similar methodology has been used to determine the *Not*I maps of the *Escherichia coli* and *Schizosaccharomyces pombe* chromosomes (SMITH *et al.* 1987; FAN *et al.* 1988).

MATERIALS AND METHODS

Strains: Mapping was carried out on *S. cerevisiae* strain AB972, derived by treating X2180-1B-*trp*1-o (E. JONES,

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TABLE 1

DNA-DNA hybridization probes that detect genetically mapped genes

Locus	Chr	Original name	Local name	Reference
ACT1	*VI*	pYact1	pPM820	NG and ABELSON (1980)
ADE1	*I*	YEp13:(*ADE1*)	pPM844	CROWLEY and KABACK (1984)
ADH1	*XV*	pJD14	pPM995	BENNETZEN and HALL (1982)
ADR1	*IV*	YRp-7-*ADR1*-411	pPM940[a]	DENIS and YOUNG (1983)
ARG4	*VIII*	pGT30	pPM450	TSCHUMPER and CARBON (1982)
CAN1	*VIII*	TLC1	pPM924	BROACH, STRATHERN and HICKS (1979)
CDC3	*XII*	pBR3-SR1(*CDC3*)	pPM460	J. PRINGLE (personal communication)
CDC11	*X*	YIp5(*CDC11*)-6	pPM932	J. PRINGLE (personal communication)
CDC12	*VIII*	YIp5(*CDC12*)-1	pPM934	J. PRINGLE (personal communication)
CDC19	*I*		λPM4237	CARLE and OLSON (1984)
CDC40	*IV*	pYK7-R2	pPM963[b]	KASSIR *et al.* (1985)
CDC42	*XII*	pBR4-10	pPM462	J. PRINGLE (personal communication)
CEN5	*V*		pPM957	OLSON *et al.* (1986)
CEN10	*X*	p180-*CEN10*	pPM852	HIETER *et al.* (1985)
CEN11	*XI*	pYe(*CEN11*)10	pPM862[c]	FITZGERALD-HAYES *et al.* (1982)
CEN14	*XIV*		pPM408	CARLE and OLSON (1985)
CHO1	*V*	pAB306	pPM972	BAILIS *et al.* (1987)
CPA1	*XV*	JL113/ST15	pPM908[d]	NYUNOYA and LUSTY (1984)
CUP1	VIII	plw6	pPM876	*FOGEL and WELCH (1982); S. FOGEL and J. W. WELCH (personal communication)
CYC7	*V*	pYe*CYC7*(0.66)	pPM836	MONTGOMERY *et al.* (1982)
CYH2	*VII*	pCYH(2.2)	pPM902[e]	KAUFER *et al.* (1983); FRIED and WARNER (1982)
EDR1	*XII*			R. ROTHSTEIN (personal communication)
GAL1-10	*II*	pBM126	pPM615[f]	*CITRON and DONELSON (1984); M. JOHNSTON (personal communication)
GAL2	*XII*	pTLG2	pPM404[g]	TSCHOPP *et al.* (1986)
GAL4	*XVI*	pG525	pPM440	LAUGHON and GESTELAND (1984)
GAL80	*XIII*	pBM320	pPM406	*YOCUM and JOHNSTON (1984); M. JOHNSTON (personal communication)
HIS1	*V*	pAH7	pPM900	*HINNEBUSCH and FINK (1983); G. R. FINK (personal communication)
HIS3	*XV*	pBM394	pPM815[h]	*STRUHL and DAVIS (1980); M. JOHNSTON (personal communication)
HIS4	*III*	pBM127	pPM825	*DONAHUE, FARABAUGH and FINK (1982); M. JOHNSTON (personal communication)
HO	*IV*	YCp50-HO6	pPM805[i]	*JENSEN, SPRAGUE and HERSKOWITZ (1983); M. JOHNSTON (personal communication)
HXK1	*VI*	mp19-hxk19.4	fPM281	*WALSH, KAWASAKI and FRAENKEL (1983); R. B. WALSH and D. FRAENKEL (personal communication)
HXK2	*VII*	18-1j	fPM282	*WALSH, KAWASAKI and FRAENKEL (1983); A. VOJTEK and D. FRAENKEL (personal communication)
ILS1	*II*	pFM20	pPM892	MEUSSDOERFFER and FINK (1983)
KEX2	*XIV*	YEp24-pJ2B	pPM854	JULIUS *et al.* (1984)
LEU1	*VII*	λ2-14-4	λPM4248	J. MARGOLSKEE and I. HERSKOWITZ (personal communication)
LYS2	*II*	YIp333	pPM430	EIBEL and PHILIPPSEN (1984)
MES1	*VII*	pFM5	pPM904[j]	MEUSSDOERFFER and FINK (1983)
PEP3	*XII*	A1	pPM456	E. JONES (personal communication)
PHO3	*II*	PAP20	pPM944	*ROGERS, LEMIRE and BOSTIAN (1982); K. BOSTIAN (personal communication)
PHO4	*VI*	pBR4-HS	pPM946	KOREN, LEVITRE and BOSTIAN (1986)
PHO5	*II*	PAP20	pPM944	*ROGERS, LEMIRE and BOSTIAN (1982); K. BOSTIAN (personal communication)
PHR1	*XV*	YEp13-*PHR1*	pPM978[k]	SCHILD *et al.* (1984)
PPR1	*XII*	puc18-*PPR1*	pPM454	*LOSSON and LACROUTE (1981); F. LACROUTE (personal communication)
PUT2	*VIII*	pKB11	pPM874	BRANDRISS (1983)
RAD1	*XVI*	pNF1000	pPM916	YANG and FRIEDBERG (1984)
RAD3	*V*	pNF3005	pPM921	NAUMOVSKI *et al.* (1985)
RAD6	*VII*	YH6	pPM964	KUPIEC and SIMCHEN (1984a)

Locus	Chr	Original name	Local name	Reference
RAD50	*XIV*	pMK50-3	pPM965	*KUPIEC and SIMCHEN (1984b); G. SIMCHEN (personal communication)
RAD54	*VII*		pPM975	*CALDERON, CONTOPOULOU and MORTIMER (1983); R. MORTIMER (personal communication)
RAD55	*IV*	YEp13-*RAD55*-13c	pPM976	CALDERON, CONTOPOULOU and MORTIMER (1983)
RAS1	*XV*	p*RAS1*	pPM928	KATAOKA *et al.* (1984)
RAS2	*XIV*	p*RAS2*	pPM930	KATAOKA *et al.* (1984)
RDN1	*XII*		λPM5617	M. OLSON (unpublished)
SIR1	*XI*	pJR63	pPM997[l]	J. RINE (personal communication)
SIR3	*XII*		pPM458	J. RINE (personal communication)
SIR4	*IV*	pRS26	pPM999[m]	J. RINE (personal communication)
SNF1	*IV*	pCE9	pPM880[n]	CELENZA and CARLSON (1984)
SPT2	*V*	pTB107	pPM878	*ROEDER *et al.* (1985); G. ROEDER (personal communication)
SST2	*XII*			F. CROSS (personal communication)
SUC2	*IX*	pSEY125	pPM810	EMR *et al.* (1984)
SUP2	*IV*		λPM1405	M. OLSON (unpublished)
SUP3	*XV*		λPM1420	M. OLSON (unpublished)
SUP8	*XIII*		λPM3290	M. OLSON (unpublished)
SUP16	*XVI*		λPM975	M. OLSON (unpublished)
SUP17	*IX*	14g	pPM410	BECKMAN, JOHNSON and ABELSON (1977); BROACH, STRATHERN and HICKS (1979)
SUP19	*V*		λPM4741	M. OLSON (unpublished)
SUP45	*II*		pPM890[o]	*HIMMELFARB, MAICAS and FRIESEN (1985); J. FRIESEN (personal communication)
SUP61	*III*		λPM680	M. OLSON (unpublished)
TOP2	*XIV*	pY-1	pPM990	*GOTO and WANG (1984); T. GOTO and J. WANG (personal communication)
TRP1	*IV*	YRp7	pPM260[p]	STRUHL *et al.* (1979)
TRP2	*V*	pAS4	pPM872	ZALKIN *et al.* (1984)
TRP3	*XI*	pJP11	pPM866	ZALKIN *et al.* (1984)
TRP5	*VII*	p*TRP5*	pPM868	*ZALKIN and YANOFSKY (1982); H. ZALKIN (personal communication)
URA1	*XI*	pRG4	pPM401	LOISON *et al.* (1981)
URA2	*X*	pJLS1	pPM402	SOUCIET, HUBERT and LACROUTE (1982)
URA3	*V*		λPM910	CARLE and OLSON (1984)
X seq.			pPM958[q]	
Y seq.			pPM959[r]	
$C_{1-3}A$			fPM280[s]	
pZ1[t]	*XII*	pZ1	pPM1035	ZAMB and PETES (1982)

[a] A 5-kb *Bam*HI fragment was gel-purified.
[b] A *Bam*HI-*Cla*I fragment was gel-purified.
[c] A *Hin*dIII-*Sal*I fragment was gel-purified.
[d] A 3.6-kb *Sph*I-*Hin*dIII fragment was gel-purified.
[e] A 2.2-kb *Eco*RI fragment was gel-purified.
[f] An *Eco*RI-*Bam*HI fragment was gel-purified.
[g] A 3.9-kb *Bam*HI fragment was gel-purified.
[h] A 1.35-kb *Bam*HI fragment was gel-purified.
[i] A 2.5-kb *Hin*dIII fragment was gel-purified.
[j] A 3-kb *Bam*HI-*Sal*I fragment was gel-purified.
[k] A *Pvu*II fragment was gel-purified.
[l] A 1.9-kb *Eco*RI-*Hin*dIII fragment was gel-purified.
[m] A 4.3-kb *Hin*dIII fragment was gel-purified.
[n] A 4.5-kb *Hin*dIII-*Bam*HI fragment was gel-purified.
[o] A *Bgl*II fragment was gel-purified.
[p] An *Eco*RI fragment was gel-purified.
[q] A 600-bp *Bam*HI-*Hpa*I fragment of plasmid L5-8-CN5 (B. TYE, personal communication) was subcloned into pBR322.
[r] A 1-kb *Sau*3A fragment of plasmid pSZ220 (SZOSTAK and BLACKBURN, 1982) was subcloned into pBR322.
[s] A 400-bp *Hpa*I-*Sph*I fragment of plasmid L5-8-CN5 (B. TYE, personal communication) was subcloned into m13.mp18.
[t] rDNA distal junction.
* The reference cites the original cloning of the gene; the notation "personal communication" indicates that the cited reference does not contain a description of the precise clone used as a probe.

personal communication) with ethidium bromide to make it ρ^0. The genotype of AB972 is *MATα* ρ^0 *trp*1-o; its lineage traces directly to strain S288C (MORTIMER and JOHNSTON 1986) with no intervening outcrosses. As described previously (CARLE and OLSON 1985; VOLLRATH *et al.* 1988), the following strains were used to resolve chromosomes that co-

migrate on pulsed-field gels when derived from AB972: *V & VIII*, A364a (HARTWELL 1967); *XIII & XVI*, YNN281 (D. VOLLRATH and R. W. DAVIS, personal communication); *VII & XV*, DC04α (BROACH and HICKS 1980). Strain YP148, which was a gift from P. HIETER, was used to assist in the assignment of linking probes to chromosomes. In YP148, chromosome *VII* has been fragmented into two chromosomes; these aberrant chromosomes and the 15 wild-type chromosomes present all form readily resolvable electrophoretic bands. The electrophoretic karyotype of YP148 is similar or identical to that of YPH149 (VOLLRATH *et al.* 1988).

DNA-DNA hybridization probes: Table 1 lists genetically mapped DNA-DNA hybridization probes that were assembled from the yeast community and used to order *Sfi*I and *Not*I restriction fragments. Table 2 lists *Sfi*I and *Not*I linking probes that were assigned to particular sites on the *Sfi*I/*Not*I map.

DNA preparations: Yeast DNA was prepared either in agarose plugs or microbeads, as described previously (CARLE and OLSON 1987). Double-stranded plasmid DNA was prepared by the alkaline-lysis method (BIRNBOIM and DOLY 1979). Single-stranded DNA from M13-derived vectors was prepared following precipitation of the virions with polyethylene glycol as described by BARNES, BEVAN and SON (1983). Lambda DNA was prepared after purification of the virions on DEAE cellulose columns (HELMS *et al.* 1985).

Size markers: Lambda oligomers were prepared by either the method of VAN OMMEN and VERKERK (1986) or VOLLRATH and DAVIS (1987). With the latter method, we had good success using commercial preparations of λ DNA (Bethesda Research Laboratory, BRL). In addition to the λ oligomers, which provided a ladder with 48.5-kb spacing between the rungs, we employed *Xho*I-cleaved λDNA (33.5 and 15.0 kb), *Bst*EII-cleaved λ DNA (8.5, 7.2, 6.4, 5.7, 4.8, 4.3 and 3.7 kb), and bacteriophages T5 (125 kb) and T4 (170 kb) as size markers.

Experimental samples were run in blocks of up to six lanes flanked on both sides by identical sets of size markers. Size estimations were carried out by a procedure similar to that described in OLSON *et al.* (1986) except that the relationship between size and distance migrated was assumed to be locally linear. In carrying out linear interpolation to estimate the size of an unknown fragment, the only mobilities considered were those of the unknown and of the two size markers that most closely bracketed it.

Electrophoresis: Pulsed-field gels that contained 1.0% agarose were cast in 0.5 × TBE (45 mM Tris, 45 mM boric acid, 1.25 mM EDTA). The gels were loaded and run as described (CARLE and OLSON 1985, 1987). Most separations employed a contour-clamped homogeneous electric field (CHEF) apparatus whose design involved minor modifications of that described by CHU, VOLLRATH and DAVIS (1986). The circuitry is described in OLSON (1989); the perpendicular distance between opposing faces of the hexagonal array of electrodes was 26 cm. The gels were run at an applied voltage of 160 V, which results in a voltage gradient—as measured in the gel (OLSON 1989)—of 5.5 V/cm.

Field-inversion gels (CARLE, FRANK and OLSON 1986) were used to screen lambda clones for the presence of *Sfi*I and *Not*I sites, an application that requires the ability to separate 25-kb from 50-kb molecules in a short gel run. A forward voltage gradient of 14.0 V/cm and a reverse voltage gradient of 9.5 V/cm were employed. The switching interval was constant at 0.3 sec and the running time was 12 hr.

Southern transfer and hybridization: The DNA was transferred to nitrocellulose as described by CARLE and OLSON (1984). The plasmid and lambda hybridization probes were prepared by nick translation (MANIATIS, FRITSCH and SAMBROOK 1982) or hexamer labeling (FEINBERG and VOGELSTEIN 1983) with [^{32}P]dCTP. M13-derived hybridization probes were prepared by primed extension on the single-stranded template using a vector-specific primer.

All DNA-DNA hybridization was carried out in 10 ml of hybridization buffer [1% Sarkosyl (sodium *N*-lauroylsarcosinate), 0.5 M NaCl, 0.1 M NaH_2PO_4, 5 mM EDTA, 0.1 mg/ml sonicated calf thymus DNA, pH 7.0] for 15–18 hr at 64°. The filters were rinsed four times at room temperature in 10 ml of 1% Sarkosyl, 1 mM Tris-HCl (pH 8.0) followed by four rinses of 5 min each in 500 ml of 1 mM Tris-HCl (pH 8.0). Autoradiograms were exposed at −70° using an intensifying screen.

Digestion of individual yeast chromosomes with restriction enzymes: As described in the text, *Sfi*I and *Not*I digests of individual yeast chromosomes were carried out by "band transplantation" (*i.e.*, excision of a band from one pulsed-field gel, digestion with the restriction enzyme, and transfer of the band to the well of a second pulsed-field gel without intervening DNA purification). Fresh (<1 week old) solid-plug preparations of yeast DNA were prepared using a wet weight of at least 0.6–0.8 g of cells per small (6-cm diameter) Petri plate.

After electrophoresis, the gels were stained for 20 min in 0.1 μg/ml ethidium bromide and then destained for 10 min in distilled water. A 302-nm transilluminator was used to visualize the chromosome bands; individual bands were cut out of the gel in a plug measuring 5 mm × 2 mm × 30 mm, using a pair of single-edged razor blades separated by a 1/16-inch spacer. The agarose plug was equilibrated three times for 1 hr each time in 10 ml of TE8 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 4° with gentle agitation. The plug was then equilibrated three times for 1 hr each time in 10 ml of the 1 × enzyme buffer [for *Sfi*I, 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol; for *Not*I, 150 mM NaCl, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$]. The plug was then transferred to a 1.5-ml microcentrifuge tube containing 600 μl of enzyme buffer, 500 μg/ml of bovine serum albumin (BRL), and 100 units/ml of restriction enzyme. The tube was gently agitated for 8–12 hr at the temperature recommended by the manufacturer (New England Biolabs; 50° for *Sfi*I, 37° for *Not*I).

The plug was equilibrated with 10 ml of electrophoresis buffer for 1 hr at 4° and then inserted into a well of a new pulsed-field gel for separation of the digestion products. If this step was not performed immediately following digestion, the plug was equilibrated with 0.5 M EDTA (pH 9.0) at 4° and then stored, also at 4°.

The microbead DNA samples were prepared for digestion by three washings with water, then with TE8, and finally with 1 × enzyme buffer. Each washing step involved equilibration for 5 min at room temperature and then centrifugation for 5 min at 2500 rpm in a tabletop centrifuge. Then the beads were resuspended in an equal volume of 1 × enzyme buffer, and 1 ml of the suspension was transferred to a 1.5-ml microcentrifuge tube. The beads were spun down at 2000 rpm for 5 min, the supernatant was discarded, and then the beads were resuspended in 500 μl of 1 × enzyme buffer containing 500 μg/ml of bovine serum albumin. Finally, the beads were spun down by a very brief spin in a microcentrifuge, the supernatant was poured off, and 60 units of restriction enzyme (in a volume of <10 μl) were added with mixing to form a slurry. After incubation as described for "band transplantation," the beads were stored in 500 μl of 0.5 M EDTA (pH 9.0) at 4°.

RESULTS

Basic mapping strategy: The fragmentation patterns obtained when high molecular weight yeast DNA is digested with either *Sfi*I or *Not*I are too complex to analyze directly on stained gels. Consequently, we subdivided the genome on a chromosome-separation gel before beginning the analysis. As a practical matter, we relied on band transplantation for this step: a band from a chromosome-separation gel was excised, digested with restriction enzyme in the agarose, and then transplanted to the origin of a new gel on which the digestion products were separated. Typical data for *Not*I are presented in Figure 1. In Figure 1A, a pulsed-field gel is shown on which the chromosomes of our standard yeast strain, AB972, were separated; in Figure 1B, a second gel is shown on which 13 different bands from a chromosome-separation gel were analyzed by *Not*I digestion. Chromosomes *V* & *VIII* were pooled, as were *XIII* & *XVI* and *VII* & *XV*. We were unable to separate the *V* & *VIII* or the *VII* & *XV* doublets of AB972. Although we could separate the *XIII* & *XVI* doublet under optimum conditions, these two molecules were also pooled.

The power of band transplantation is evident in Figure 1B. In the larger size range, where discrete bands are obtained when a *Not*I digest of total yeast DNA is fractionated (lane 4), the bands are readily correlated with bands in particular band-transplantation lanes. Below approximately 350 kb, only the band-transplantation samples produce readily interpretable digests. In general, these digests are no more difficult to interpret than are complete digests of small clones that have been separated on conventional agarose gels. However, some caveats are evident from the examples in Figure 1B. Interpretation of band multiplicities is not always straightforward. In principle, the intensities of single bands should increase linearly with size. However, this effect is compromised by the increased vulnerability of large fragments to degradation (*e.g.*, the bands for chromosome *X* at 380, 260, and 90 kb are all singlets, but the 260-kb band is the most intense). Consequently, quantitation of band intensities rarely improves on subjective interpretation. Subjective interpretation is probably adequate when closely spaced comparison bands are available (*e.g.* the 255, 150, 105 and 65-kb series from chromosome *XII* was interpreted as singlet, singlet, doublet, doublet) but becomes problematic when they are not.

The gels in Figure 1 also document the method used to estimate fragment sizes. Ladders of bacteriophage lambda DNA, produced by ligation of lambda monomers, provided the primary size standards (CARLE and OLSON 1984; VOLLRATH and DAVIS 1987). These ladders were useful for estimating the sizes of the intact chromosomal DNA molecules for all chromosomes smaller than *IV* and *XII* and of nearly all the *Sfi*I and *Not*I fragments. For example, in Figure 1A, the chromosome *VII* & *XV* doublet comigrates with rung 23 of the lambda ladder; since the interrung spacing is 48.5 kb (SANGER *et al.* 1982), the sizes of chromosomes *VII* and *XV* are estimated to be 1120 kb. Above this position in the gel, size estimates are impossible both because the lambda ladder is not well resolved and also because the relationship between size and mobility becomes double-valued (OLSON 1989).

The mobility of test molecules relative to the rungs of the lambda ladder appears to be independent of the switching interval, as would be expected if size is the only significant determinant of mobility. The gel in Figure 1B was run at a shorter switching interval than the one in Figure 1A to optimize the separation of molecules in the size range of most of the *Not*I fragments (with two exceptions, <620 kb). Nevertheless, the relative mobilities of molecules that are resolved in Figures 1A and 1B are identical (*e.g.*, the band for the chromosome *V/VIII* doublet migrates slightly behind rung 12 both in lane 2 of Figure 1A and lane 3 of Figure 1B).

Comparable data were collected for *Sfi*I, which generates considerably more fragments than *Not*I. The pattern of bands obtained when total yeast DNA is cleaved with *Sfi*I is shown in Figure 2A, while illustrative band-transplantation data are shown for chromosomes *XI* and *XII* in Figure 2, B and C, respectively. The overall *Sfi*I pattern is quite complex, particularly below the prominent gap from 400 to 500 kb. In some instances, even the patterns for individual chromosomes are too complex to analyze unequivocally. For example, the left panel of Figure 2B shows band-transplantation data from chromosome *XI*, which yields *Sfi*I fragments of 170, 140 and 105 kb, as well as a cluster of bands in the vicinity of 50 kb. Expansion of the region between the first two rungs of the lambda ladder shows that the cluster of *Sfi*I fragments on chromosome *XI* includes at least 5 bands, ranging in size from 45 to 65 kb (right panel, Figure 2B). Because it is easy to undercount bands in clusters of this type, the most common mapping error is likely to involve the omission of relatively small fragments that comigrate in particular band-transplantation samples. Fragments below 20–30 kb are not detectable on stained gels whether they are present in clusters or not. Consequently, two or more sites spaced more closely than 20–30 kb would be mapped as a single site.

A striking feature of the *Sfi*I digest of total yeast DNA is the intense band at the bottom of the gel (Figure 2A). This band, which is approximately 9 kb, arises from the tandem array of rDNA repeats, which contain one *Sfi*I site per repeat unit. In Figure 2C,

TABLE 2

Linking probes

Chromosome	Clone ID[a]	Type[b]	Junction fragments[c] *Sfi*I	Junction fragments[c] *Not*I	Gene[d]
I	λPM4237	s	2 3	1	*CDC19*
I	λPM5084	s	4 5	2	
I	cPM9218	n	NT	1 2	
II	λPM5891	s	1 2	1	
II	λPM4350	s	2 3	1	
II	λPM5530	n	3	1 2	
II	λPM3991	n	3	2 3	
II	λPM6002	s	3 4	3	
II	λPM2976	s	4 5	4	
III	cPM9172	s	1 2	1	
III	λPM3632	s	2 3	1	*SUP61*
IV	λPM5113	n	1	1 2	
IV	λPM5804	s/n	1 2	2 3	
IV	λPM1340	n	2	3 4	
IV	λPM5912	s/n	2 3	4 5	
IV	λPM1405	n	4	6 7	*SUP2*
V	λPM5917	s/n	1 2	1 2	
V	λPM7150	s	2 3	2	
V	λPM4798	n	3	2 3	
V	λPM3115	n	3	3 4	
VI	λPM3655	s	1 2	1	
VII	λPM3141	s	1 2	1	
VII	λPM5512	n	2	1 2	
VII	λPM6081	n	2	3 4	
VII	λPM5315	s	2 3	4	
VIII	λPM3008	s	1 2	1	
VIII	λPM1617	n	2	1 2	
VIII	λPM6446	s	2 3	2	
VIII	λPM5091	n	3	2 3	
IX	λPM3564	s	1 2	1	
IX	λPM6675	s	2 3	1	
X	λPM2958	s	1 2	1	
X	λPM3471	n	2	1 2	
X	λPM2937	n	2	3 4	
XII	λPM3609	s	1 2	2	
XII	λPM6234	s	2 3	2	
XII	λPM5528	n	3	2 3	
XII	λPM4016	s/n	6 7	3 4	
XII	λPM3000	n	7	4 5	
XII	λPM5886	n	7	5 6	
XIII	λPM6202	n	2	1 2	
XIII	λPM5991	s	2	2 3	
XIII	λPM6112	s	3 4	2	
XIV	pPM854	s/n	1 2	1 2	*KEX2*
XIV	λPM6200	n	2	2 3	
XIV	λPM2008	n	2	3 4	
XIV	λPM3886	s	2 3	4	
XIV	pPM990	s	3 4	4	*TOP2*
XIV	λPM3958	s	5 6	4	
XIV	λPM6054	s	4 5	4	
XV	λPM5448	s	1 2	1	
XV	λPM5893	s	2 3	1	
XV	λPM3578	n	3	1 2	
XV	λPM5897	s	3 4	2	
XV	λPM5385	s	5 6	3	
XV	λPM3117	n	8	3 4	
XV	λPM5648	s	8 9	4	
XV	λPM5558	s	9 10	4	
XVI	λPM6168	s/n	1 2	1 2	
XVI	λPM2742	s	3 4	2	
XVI	λPM5149	s	5 6	2	

[a] The λPM clones are lambda clones, cPM clones are cosmids, and pPM clones are plasmids. The λPM and cPM clones are from our laboratory collection. The two pPm clones are described in Table 1.

[b] s = *Sfi*I, n = *Not*I, s/n = *Sfi*I and *Not*I.

[c] The fragment number identifies the restriction fragment to which the linking probe hybridizes; fragment numbers are from left-to-right on the chromosome, as in Figure 7 and Table 3. NT = not tested.

[d] The linking probe contains the indicated genetically mapped gene, thereby providing a precise point of alignment between the physical and genetic maps.

this band is shown by band transplantation to derive, as expected, from chromosome *XII* (PETES 1979). In addition to the multicopy 9-kb fragment, chromosome *XII* gives a relatively simple pattern of six *Sfi*I fragments ranging in size from 25 to 535 kb, all of which appear to be present in equimolar amounts. The 535-kb fragment from chromosome *XII* is also clearly visible as a distinct band in the *Sfi*I digest of total yeast DNA, running just below rung 11 of the lambda ladder in Figure 2A.

Mapping with gene-specific probes: The main approach to ordering the *Sfi*I and *Not*I fragments in individual band-transplantation samples was by using cloned, genetically mapped genes as DNA-DNA hybridization probes. The process is illustrated in Figure 3 for chromosome *X*. A band-transplantation digest of chromosome *X* with *Sfi*I is shown in the left panel of Figure 3A. There are two fragments of 505 and 255 kb. Gel-transfer hybridization data are shown for the telomere-associated repetitive sequence Y (SZOSTAK and BLACKBURN 1982; HOROWITZ and HABER 1984), the *URA2* gene (SOUCIET, HUBERT and LACROUTE 1982), and the CEN10 sequence (HIETER *et al.* 1985). Similar data were acquired using probes for the *CDC11* gene (J. PRINGLE, personal communication), which hybridizes to the 505-kb fragment, and for the telomere-associated repeat X (CHAN and TYE 1983), which hybridizes to both *Sfi*I fragments.

As shown in Figure 3B, the *Not*I digest of chromosome *X* contains four fragments of 380, 260, 90 and 30 kb. The Y and URA2 probes hybridize to the 380-kb fragment, while CEN10 hybridizes to the 260-kb fragment. CDC11 also hybridizes to the 260-kb fragment, while the X sequence hybridizes to the 90-kb fragment. With the exception of the placement of the 30-kb *Not*I fragment, to which none of the probes

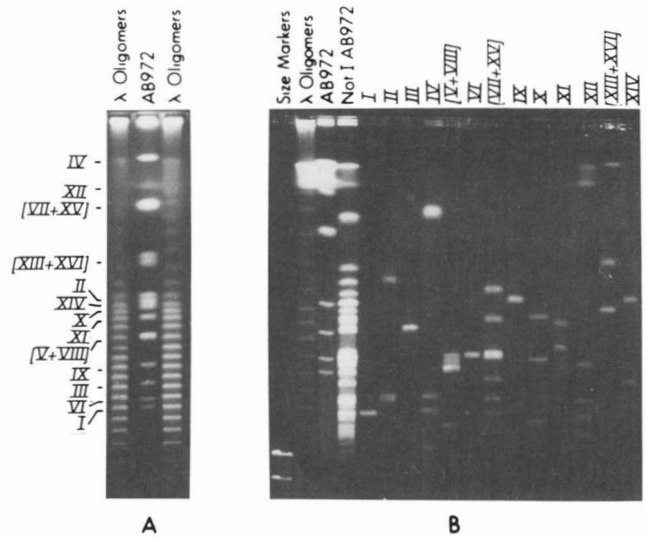

FIGURE 1.—Electrophoretic separations of intact and *Not*I-digested yeast chromosomes. A, Fourteen ethidium bromide (EtBr)-stained bands corresponding to the 16 physically defined yeast chromosomes of AB972. The CHEF gel was run for 24 hr using a switching interval of 60 sec. B, The *Not*I restriction fragments generated by band-transplant digestion of 13 bands from a chromosome-separation gel. In the lane labeled "*Not*I AB972," genomic DNA was prepared by the solid-plug method and digested as described for the band-transplantation protocol. The lane labeled "Size Markers" contains λ DNA cleaved with *Xho*I. The CHEF gel was run for 21.5 hr using a switching interval of 30 sec.

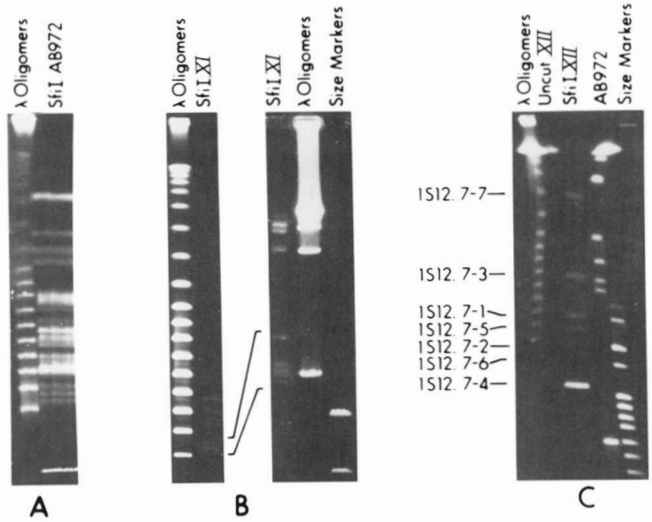

FIGURE 2.—Electrophoretic separations of *Sfi*I digests of total yeast DNA and DNA from chromosomes *XI* and *XII*. A, *Sfi*I digest of genomic DNA from the yeast strain AB972. The DNA was prepared and digested with *Sfi*I using the microbead protocol. The CHEF gel was run for 21 hr using a switching interval of 35 sec. B, *Sfi*I restriction fragments of chromosome *XI* separated under different switching conditions. The left and right CHEF gels were run for 21 hr (switching interval 35 sec) and 22 hr (switching interval 2.5 sec), respectively. The lane labeled "Size Markers" is λ DNA cleaved with *Xho*I. C, *Sfi*I digest of chromosome *XII*. The restriction fragments are numbered from the left arm to the right arm of the chromosome, so that 1S12.7-1 identifies the left-most *Sfi*I fragment. The lane labeled "Size markers" is a mixture of bacteriophages T4 and T5, λ DNA cleaved with *Xho*I, and λ DNA cleaved with *Bst*EII. The gel was run on a pulsed-field gel electrophoresis apparatus that produced less uniform fields than a CHEF apparatus and the angle between the applied fields was 115°. The gel was run for 20 hr at a switching interval of 35 sec.

hybridized, these data define the map shown in Figure 4. The mapping of the 30-kb *Not*I fragment is discussed below.

Mapping with linking probes: A variety of methods were used to map fragments that failed to hybridize to any of the available genetically mapped probes. Primarily, we relied on a large collection of *Sfi*I and *Not*I "linking probes" (*i.e.*, probes that span particular *Sfi*I or *Not*I sites). Such probes are expected to hybridize to fragments on both sides of the site, thereby demonstrating their adjacency (POUSTKA and LEHRACH 1986). The linking probes were obtained by screening a large collection of lambda clones for the presence of *Sfi*I or *Not*I sites. Because the lambda-clone collection covers most of the yeast genome and is organized into sets of overlapping clones (OLSON *et al.* 1986), it was possible to obtain probes spanning most of the *Sfi*I and *Not*I sites by screening only 1000 clones. A list of the clones used as linking probes is presented in Table 2 (See MATERIALS AND METHODS). Linking probes were only included in Table 2 if they hybridized to two bands in Southern experiments carried out on digests of genomic DNA with the appropriate enzyme or enzymes. Some clones that cleave with *Sfi*I or *Not*I were excluded because they hybridized to a single band with both *Sfi*I and *Not*I. This behavior could be due to the proximity of the site to one end of the clone or to the presence of undetected small *Sfi*I or *Not*I fragments in the genome (see DISCUSSION).

Figure 5 illustrates the hybridization of one *Not*I (λPM2937) and one *Sfi*I (λPM2958) linking probe to a standard set of filter strips. These filter strips contained chromosomal DNA molecules, *Not*I fragments, and *Sfi*I fragments that had been separated on pulsed-field gels. Preparative pulsed-field gels were used to fractionate samples containing either intact chromosomal DNA molecules (Figure 5A, top panel), or *Sfi*I or *Not*I digests of total yeast DNA (bottom panel). In either case, the samples were loaded across large blocks of size-calibrated gels. These gels were transferred to single sheets of nitrocellulose and cut into 5-mm strips for use in the hybridization experiments.

For each of the linking probes for which data are shown in Figure 5B, the first four lanes contain size-fractionated chromosomal DNA molecules separated at different switching intervals and derived from different yeast strains. This redundancy ensures that any single-copy linking probe can be unambiguously assigned to a chromosome in one hybridization experiment. The particular pattern of hybridizing chromosomal DNA molecules observed for both λPM2937 and λPM2958 identifies the hybridizing chromosome as *X*.

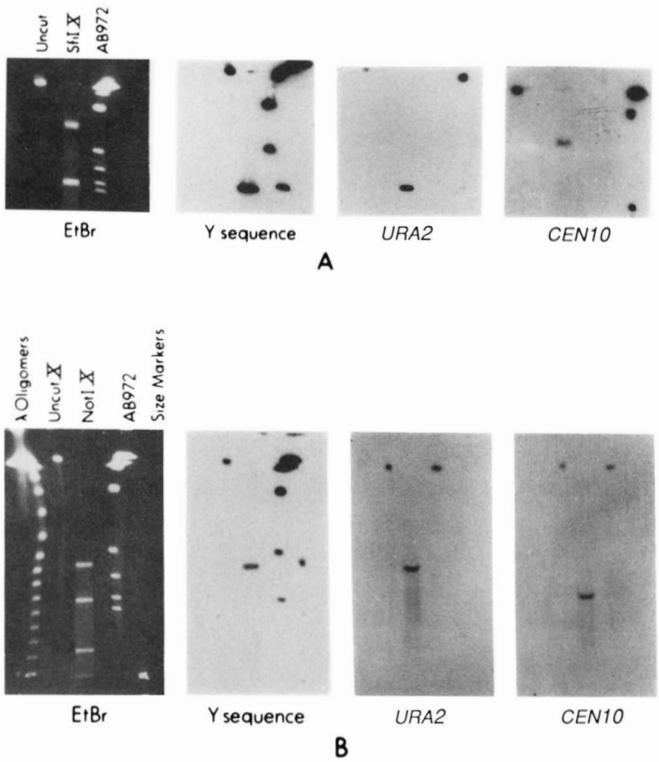

FIGURE 3.—Identification of the *Sfi*I and *Not*I restriction fragments derived from chromosome *X* by gel-transfer hybridization. A, An ethidium-bromide (EtBr)-stained gel. B, Hybridization with the Y and *CEN10* probes using the actual gel shown in A and *URA2* hybridization using a different, nearly identical gel. The Y sequence hybridizes to some of the small AB972 chromosomes as well as to the intact and digested chromosome *X* samples. All gels were run for 20 hr at a 35-sec switching interval on the gel apparatus described in the legend to Figure 2C. The lane labeled "Size Markers" is λ DNA cleaved with *Xho*I.

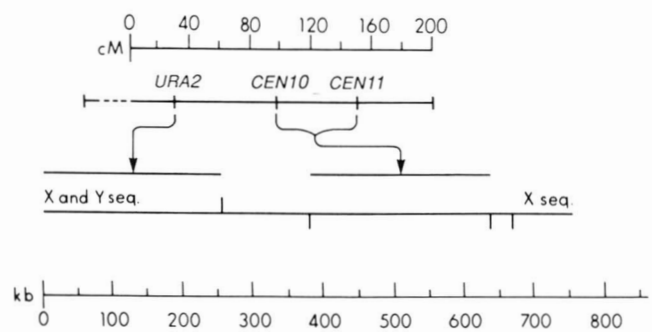

FIGURE 4.—Correlation between the genetic map of chromosome *X* and the *Sfi*I and *Not*I maps. The line drawn from the genetic map to the restriction fragment identifies the fragment to which the indicated probe hybridized. A tick mark above the main line of the physical map indicates a *Sfi*I site, a mark below the line indicates a *Not*I site. The dashed line on the genetic map represents mitotic linkage. The genetic map in this figure was drawn from Edition 9 of the Saccharomyces genetic map (MORTIMER and SCHILD 1985). In Edition 10, a new marker, *dal*5, defines the right end of the map but there is still no information about the physical or genetic distance from *dal*5 to the end of the chromosome.

The results obtained when hybridizing λPM2937 to the *Not*I and *Sfi*I filter strips are those expected for a *Not*I linking probe: the probe recognizes one *Sfi*I and two *Not*I fragments. The *Not*I results demonstrate

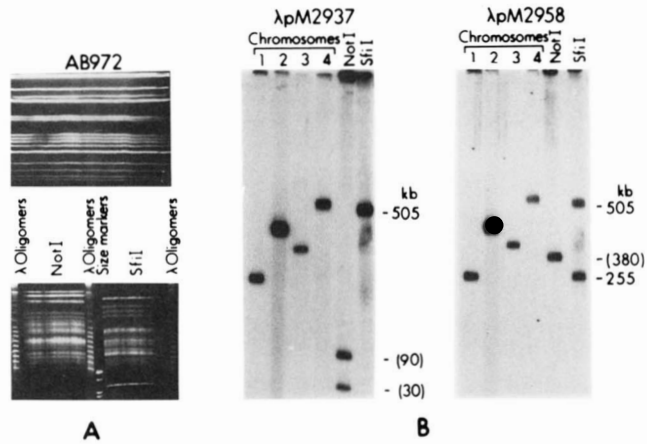

FIGURE 5.—Analysis of two linking probes from chromosome *X* by gel-transfer hybridization. A, Typical gels that were run on the CHEF apparatus and transferred to nitrocellulose to produce the filter strips for the hybridization experiments. The AB972 chromosomes were prepared by the solid-plug protocol, the DNA for the *Not*I and *Sfi*I digests by the microbead method. The lane labeled "Size markers" is a mixture of λ DNA cleaved with *Xho*I and λ DNA cleaved with *Bst*EII. The top gel was run for 28 hr (switching interval 70 sec), the bottom gel for 21 hr (switching interval 35 sec). B, Results from hybridizing a *Not*I linking probe, λpM2937, and a *Sfi*I linking probe, λpM2958, to 5-mm strips derived from gels such as those shown in (A). Lanes 1 and 2, AB972 chromosomes separated using switching intervals of 60 and 50 sec, respectively. Lanes 3 and 4, YP148 chromosomes separated using switching intervals of 60 and 50 sec, respectively. The lanes labeled "*Not*I" and "*Sfi*I" contain AB972 genomic DNA digested with the indicated enzyme.

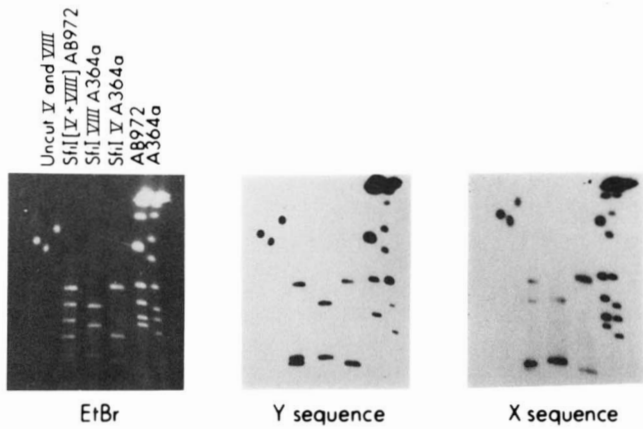

FIGURE 6.—Analysis by gel-transfer hybridization of *Sfi*I fragments generated from chromosomes *V* and *VIII* with probes to the subtelomeric sequences X and Y. The Y-sequence hybridization used the gel shown on the left, the X-sequence hybridization a nearly identical gel. The uncut chromosomes are from the same plug used for *Sfi*I digestions. The DNA was separated using the apparatus described in the legend to Figure 2C. The gel was run for 18 hr at a switching interval of 35 sec.

the adjacency of the 90 and 30-kb *Not*I fragments on chromosome *X*, resolving the last ambiguity in the chromosome *X* map (Figure 4). In contrast, λPM2958 displays the behavior expected for a *Sfi*I linking probe, hybridizing to one *Not*I and two *Sfi*I fragments. The results with λPM2958 confirm, but do not extend, previously inferred features of the map in Figure 4.

Map representation: Taken alone, the *Sfi*I and *Not*I maps of the yeast chromosomes lend themselves to simple graphical representations. Difficulties arise, however, when one attempts the more useful task of representing the relationship between the physical and genetic maps of a chromosome without implying more knowledge than actually exists. This point is illustrated for the case of chromosome *X* in Figure 4. The first problem is that there is no basis for aligning the ends of the physical and genetic maps. The most distal genetic markers on chromosome *X* are *mak*17 on the left arm and the (*met*5, *hom*6) cluster on the right arm. In neither case is anything known about the distance from these markers to the physical ends of the chromosome. Furthermore, *mak*17 is meiotically unlinked to the more proximal markers on the left arm, having been assigned to its distal position by the analysis of aneuploids and mitotic recombinants (WICKNER 1979). In deference to these uncertainties, both the alignment and the relative scaling of the genetic and physical maps in Figure 4 are arbitrary. A final caveat about map representation is that the ordering of closely spaced *Sfi*I and *Not*I sites is often unknown, since the sites for each enzyme were mapped independently. This problem does not arise on chromosome *X*, where the spacing between sites always exceeds the uncertainty in their positions, but it is common elsewhere in the genome. We found the double digests that are required to determine the ordering and spacing of closely adjacent sites too complex to analyze reliably.

Analysis of chromosome-length polymorphisms: Because some chromosomal DNA molecules are not electrophoretically separable in AB972, chromosomes *V* & *VIII*, *XIII* & *XVI*, and *VII* & *XV* had to be analyzed in pairs. It proved useful to carry out this analysis side-by-side with analysis of the individual chromosomes obtained from strains in which the two chromosomes could be separated. The strains that provided the needed chromosome-length polymorphisms have been described previously (CARLE and OLSON 1985): A364a for *V* & *VIII*, YNN281 for *XIII* & *XVI*, and DC04α for *VII* & *XV*. Only the single pair of chromosomes indicated was analyzed in each of the three alternative strains. For the six chromosomes analyzed in this way, the *Sfi*I/*Not*I maps were remarkably well conserved between AB972 and the alternative strains: there were, of course, minor differences between the intersite spacings, but the numbers of sites and the qualitative appearances of the maps were identical.

A typical case is illustrated in Figure 6, which displays *Sfi*I digests of chromosomes *V* & *VIII*, as derived from AB972 and A364a. While all the gene-specific probes recognized corresponding fragments in the two strains, the subtelomeric Y probe detected a qualitative difference between them: while this probe recognized all four of the terminal fragments derived from chromosomes *V* & *VIII* of A364a, it only hybridized to three of the AB972-derived fragments. This variation, which is associated with the right end of chromosome *VIII*, does not contribute significantly to the length polymorphism for this chromosome. The size of chromosome *VIII* is approximately 30 kb smaller in A364a than in AB972; most of this difference is associated with the middle-sized *Sfi*I fragment, which maps to the central region of the chromosome. The size of chromosome *V* is approximately 20 kb larger in A364a than in AB972; this difference appears to be distributed between the two larger *Sfi*I fragments, which map to the middle and right end of the chromosome.

It is apparent from the two left-hand panels of Figure 6 that the strain-specific distribution of Y sequences is a general phenomenon. In the rightmost lanes, in which the smaller of the intact chromosomal DNA molecules from AB972 and A364a are run side-by-side, there are obvious differences in the hybridization of particular chromosomes to the Y probe. Similar data have been published by ZAKIAN and BLANTON (1988), JAGER and PHILIPPSEN (1989), and LOUIS and HABER (1990).

Genomic map: The mapping data for all the chromosomes with both *Sfi*I and *Not*I are summarized in Figure 7. Sites for which linking probes were unequivocally identified are annotated with a horizontal bar. In two cases—on chromosomes *XI* and *XV*—we could not unambiguously determine the order of local clusters of *Sfi*I fragments, which are enclosed in brackets. The length of the rDNA cluster, which is represented by a dashed line, is arbitrary. The left-right orientation of the chromosomes follows conventions established for the linkage map (MORTIMER *et al.* 1989). Tables 3 and 4 summarize the sizes of the 77 *Sfi*I and 54 *Not*I fragments that appear on the map, as well as the identity of all probes that were shown to hybridize to particular fragments in band-transplantation experiments. Further information about the probes is presented in Table 1 (see MATERIALS AND METHODS).

The basis for nearly all the maps can be inferred by combining the data in Tables 3 and 4 with the constraints imposed by the linking probes shown in Figure 7. Cases in which other sources of information contributed to the construction of particular maps are noted in the legend to Figure 7.

Chromosome sizes: In Table 5, we summarize our best overall estimates of the sizes of the yeast chromosomes. For 11 of the 16 chromosomes, estimates are available from *Not*I data, *Sfi*I data, and data on the whole chromosomal DNA molecule. For these 11 chromosomes, the agreement between the three esti-

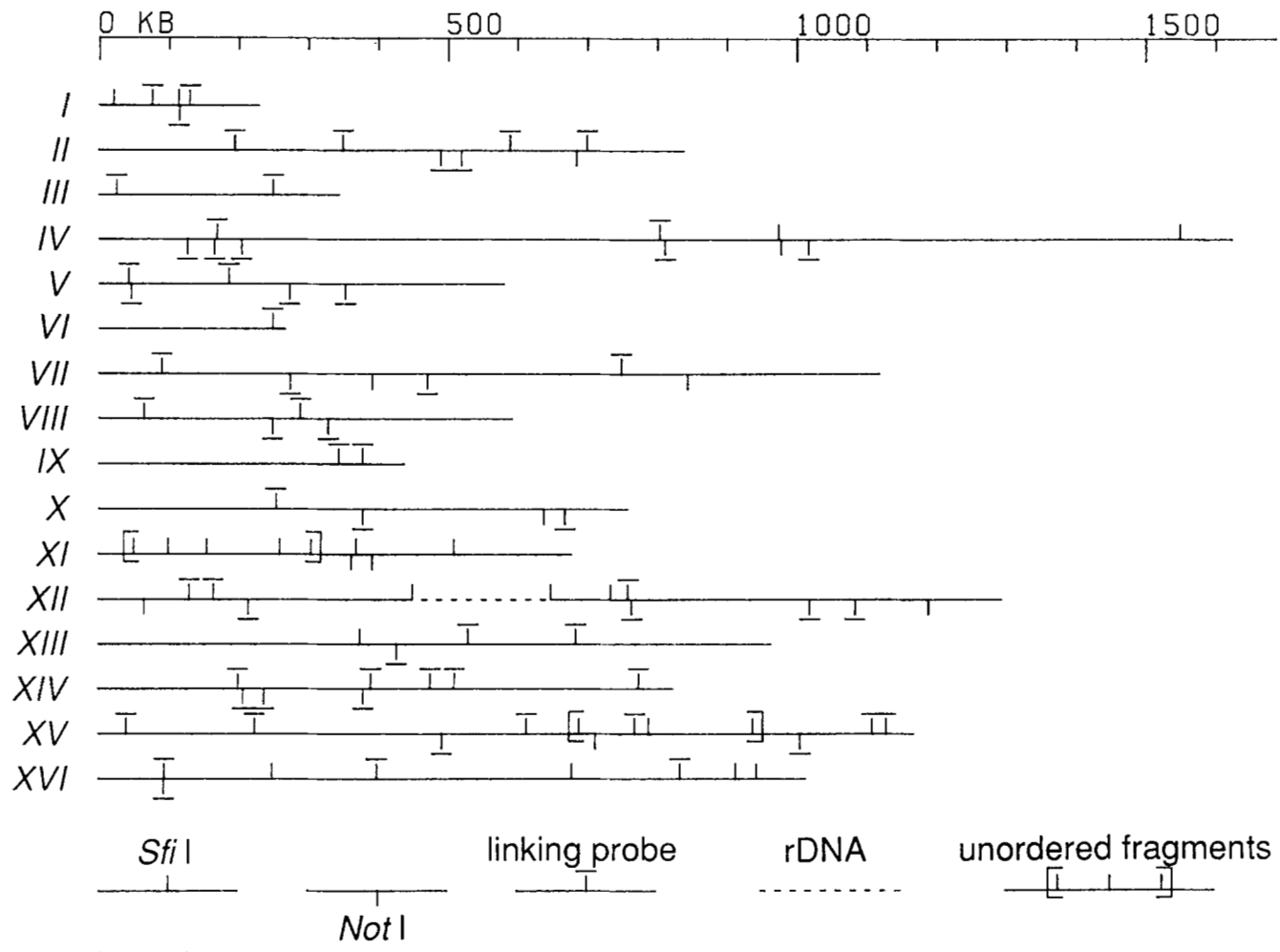

FIGURE 7.—*Not*I/*Sfi*I restriction map of the 16 physically defined chromosomes of AB972. A tick mark above the line represents a *Sfi*I site, a tick mark below the line a *Not*I site. A horizontal bar over a restriction site indicates that a linking probe was identified for that site. The order of restriction fragments enclosed in brackets has yet to be determined. The dashed line for chromosome *XII* represents rDNA and has been estimated to be 1.2–1.6 Mb in size (G. CARLE and M. OLSON, unpublished results). Notes concerning the final form of the map: *Chromosome I*, the relative order of 1S01.5-2 and 1S01.5-3 was not determined by our data; the order shown is based on the results of KABACK, STEENSMA and DE JONGE (1989). *Chromosome IX*, The data shown do not allow left-right orientation of the physical map relative to the genetic map. However, the order shown is supported by the analysis of overlapping lambda clones which indicates that *SUC2* is adjacent to an X sequence with no intervening *Sfi*I sites (M. OLSON, unpublished). *Chromosome XI*, we cannot exclude the possibility that one or more of the small *Sfi*I fragments maps between 1S11.8-7 and 1S11.8-8 since the phasing of the *Sfi*I and *Not*I maps has not been unambiguously determined. However, preliminary data on *Sfi*I/*Not*I linking probes for chromosome *XI* favor the map as drawn. *Chromosome XII*, the map shows a 65-kb *Not*I fragment at the left end of the chromosome and a 105-kb *Not*I fragment at the right end of the chromosome. We cannot exclude the possibility that the positions of these two fragments should be switched. However, the relative phase of the *Sfi*I and *Not*I maps is constrained by the *Sfi*I/*Not*I linking probe λPM4016 connecting 1S12.7-6/-7, as well as 1N12.7-3/-4: the map, as drawn, minimizes the discrepancy between the amount of DNA distal to this linking probe, as estimated by adding up the sizes of the *Not*I fragments, on the one hand, or those of the *Sfi*I fragments, on the other. *Chromosome XVI*, the adjacency of 1S16.8-2/-3 was established by probing *Sfi*I partial digests with the linking probes λPM6168 (1S16.8-1/-2) and λPM2742 (1S16.8-3/-4).

mates is excellent: the worst case is the 6% range between the highest and the lowest estimates for chromosome *XVI*. The observed additivity in fragment sizes supports the validity of the sizing method.

For five chromosomes (*III*, *VI*, *IX*, *IV* and *XII*), entries are missing from one of the three data columns in Table 5. In chromosomes *III*, *VI* and *IX*, the omissions arise because they are not cleaved by *Not*I. Chromosome *IV* could not be sized as an intact molecule because it migrates behind the resolved region of the lambda ladder. Lastly, chromosome *XII* poses a special problem because of its large size and the presence of the rDNA as a long tandem repeat. The intact chromosomal DNA molecule cannot be sized against lambda ladders directly, and even the *Not*I fragment that spans the rDNA is too large to size directly. However, the rDNA repeat contains a *Sfi*I site and the *Sfi*I data allow the chromosome to be separated into rDNA and non-rDNA components. The length of the rDNA is quite variable between strains and even between different isolates of the same strain, but typical values are 1–2 Mb, giving a chromosome size of 2–3 Mb (G. CARLE and M. V. OLSON, unpublished results). The overall estimate of the yeast genome size

TABLE 3

Yeast *Sfi*I fragments

Chromosome *I* (245 kb)		
1S01.5-5	100	X, $C_{1\text{-}3}A$, *ADE1*
1S01.5-2	65	*CDC19*
1S01.5-3	40	*CDC19*
1S01.5-1	20	X, $C_{1\text{-}3}A$
1S01.5-4	20	
Chromosome *II* (840 kb)		
1S02.5-3	240	*LYS2, PHO3/PHO5, SUP45*
1S02.5-1	195	X, Y, *ILS1*
1S02.5-2	155	*GAL1-10*
1S02.5-5	140	X, Y
1S02.5-4	110	X, $C_{1\text{-}3}A$, *AOG*
Chromosome *III* (350 kb)		
1S03.3-2	225	*HIS4, SUP61*
1S03.3-3	95	X, *SUP61*
1S03.3-1	30	X
Chromosome *IV* (1625 kb)		
1S04.5-2	635	*RAD55, TRP1*
1S04.5-4	575	*CDC40, SNF1, SUP2*
1S04.5-1	170	X, *HO*
1S04.5-3	170	*ADR1, SIR4*
1S04.5-5	75	X, Y
Chromosome *V* (585 kb)		
1S05.3-3	395	X, Y, *CHO1, HIS1, RAD3, SPT2, SUP19, TRP2*
1S05.3-2	145	*CEN5, CYC7, URA3*
1S05.3-1	45	Y, *CAN1*
Chromosome *VI* (270 kb)		
1S06.2-1	250	X, Y, *ACT1, PHO4*
1S06.2-2	20	$C_{1\text{-}3}A$, *HXK1*
Chromosome *VII* (1120 kb)		
1S07.3-2	660	*CYH2, LEU1, RAD6, RAD54, TRP5*
1S07.3-3	370	X, Y, *MES1*
1S07.3-1	90	X, *HXK2*
Chromosome *VIII* (595 kb)		
1S08.3-3	305	X, *CDC12*
1S08.3-2	225	*ARG4, PUT2, CUP1*
1S08.3-1	65	X, Y
Chromosome *IX* (440 kb)		
1S09.3-1	345	X, Y, *SUC2, SUP17*
1S09.3-3	60	X
1S09.3-2	35	
Chromosome *X* (760 kb)		
1S10.2-2	505	X, *CDC11, CEN10*
1S10.2-1	255	X, Y, *URA2*
Chromosome *XI* (680 kb)		
1S11.8-8	170	X, $C_{1\text{-}3}A$, *SIR1*
1S11.8-7	140	*CEN11*
1S11.8-4	105	
1S11.8-6	65	
1S11.8-3	55	
1S11.8-1	50	X, $C_{1\text{-}3}A$
1S11.8-2	50	*TRP3, URA1*
1S11.8-5	45	
Chromosome *XII* (1095 kb + rDNA)		
1S12.7-7	535	X, Y, *CDC3, CDC42, EDR1, SIR3, SST2*
1S12.7-3	285	*GAL2, PEP3, rDNA*
1S12.7-1	130	X, Y
1S12.7-5	85	pZ1 (rDNA distal junction)
1S12.7-2	35	*PPR1*
1S12.7-6	25	
1S12.7-4	9	*RDN1*
Chromosome *XIII* (965 kb)		
1S13.4-1	375	X, *GAL80*
1S13.4-4	280	X, *SUP8*
1S13.4-2	155	
1S13.4-3	155	
Chromosome *XIV* (825 kb)		
1S14.6-5	265	*CEN14*
1S14.6-1	200	X, Y, $C_{1\text{-}3}A$, *KEX2, RAD50*
1S14.6-2	190	*KEX2*
1S14.6-3	85	*RAS2, TOP2*
1S14.6-6	50	X, $C_{1\text{-}3}A$
1S14.6-4	35	*TOP2*
Chromosome *XV* (1170 kb)		
1S15.10-3	390	*RAS1, SUP3*
1S15.10-2	185	*ADH1*
1S15.10-8	170	*CPA1*
1S15.10-7	150	
1S15.10-5	80	
1S15.10-4	75	
1S15.10-1	40	X, Y
1S15.10-10	40	X, Y, *PHR1*
1S15.10-6	20	*HIS3*
1S15.10-9	20	
Chromosome *XVI* (1015 kb)		
1S16.8-4	280	*RAD1*
1S16.8-2	155	
1S16.8-5	155	*SUP16*
1S16.8-3	150	
1S16.8-1	95	X, Y, *GAL4*
1S16.8-6	80	
1S16.8-8	70	X, Y
1S16.8-7	30	

Comprehensive list of *Sfi*I restriction fragments of AB972. To facilitate identification, fragments were assigned identification numbers. For example, 1S01.5-4 represents version 1 (1) of the map, and *Sfi*I restriction fragment (S) on chromosome *I* (01) which has 5 *Sfi*I fragments and the specific fragment is the 4th fragment counting from the left end of the chromosome (5-4).

is (12.5 Mb + rDNA), in excellent agreement with the value of 15 Mb inferred from the reassociation kinetics of denatured yeast DNA (LAUER, ROBERTS and KLOTZ 1977).

DISCUSSION

In this report, we present a nearly complete physical map of the *S. cerevisiae* genome. Pulsed-field gel electrophoresis has been used to separate 77 *Sfi*I fragments and 54 *Not*I fragments. All the *Not*I sites and all but 5 of the *Sfi*I sites were assigned to map positions; the unmapped *Sfi*I sites lie in two clusters that were regionally localized on chromosomes *XI* and *XV*. As discussed below, the omission of small (<20–30 kb) *Sfi*I and *Not*I fragments from the analysis is likely to have resulted in undetected gaps in each map; however, the amount of unmapped DNA is estimated to be less than 1% for each enzyme and the probability that any DNA is missing from both maps is low.

TABLE 4

Yeast *Not*I fragments

Chromosome *I* (240 kb)		
1N01.2-1	120	
1N01.2-2	120	
Chromosome *II* (840 kb)		
1N02.4-1	490	X, Y, *GAL1-10, ILS1, LYS2, PHO3/PHO5*
1N02.4-3	165	*SUP45*
1N02.4-4	155	X, Y
1N02.4-2	30	
Chromosome *III* (350 kb)		
1N03.1-1	350	
Chromosome *IV* (1660 kb)		
1N04.7-4	620	*TRP1*
1N04.7-7	620	X, Y, *CDC40, SNF1*
1N04.7-5	170	*ADR1, SIR4*
1N04.7-1	130	X, *HO*
1N04.7-2	40	
1N04.7-3	40	
1N04.7-6	40	*SUP2*
Chromosome *V* (595 kb)		
1N05.4-2	230	*CHO1, CYC7, URA3*
1N05.4-4	230	X, Y, *RAD3, SPT2*
1N05.4-3	80	*SUP19, TRP2*
1N05.4-1	55	Y, *CAN1*
Chromosome *VI* (270 kb)		
1N06.1-1	270	
Chromosome *VII* (1140 kb)		
1N07.5-4	380	*LEU1*
1N07.5-1	280	*HXK2, RAD54*
1N07.5-5	280	*MES1*
1N07.5-2	120	*CYH2*
1N07.5-3	80	*RAD6, TRP5*
Chromosome *VIII* (595 kb)		
1N08.3-3	265	X, *CDC12*
1N08.3-1	250	X, Y, *ARG4, CUP1, PUT2*
1N08.3-2	80	
Chromosome *IX* (460)		
1N09.1-1	460	
Chromosome *X* (760 kb)		
1N10.4-1	380	X, Y, *URA2*
1N10.4-2	260	*CDC11, CEN10*
1N10.4-4	90	X
1N10.4-3	30	
Chromosome *XI* (675 kb)		
1N11.3-1	360	X, *URA1*
1N11.3-3	285	X, *CEN11, SIR1*
1N11.3-2	30	
Chromosome *XII* (?)		
1N12.7-3	?	*GAL2, PEP3, RDN1*
1N12.7-4	255	*CDC3, CDC42, EDR1*
1N12.7-2	150	*PPR1*
1N12.7-6	105	
1N12.7-7	105	X, Y
1N12.7-1	65	X, Y
1N12.7-5	65	*SST2*
Chromosome *XIII* (950 kb)		
1N13.2-2	530	X, *SUP8*
1N13.2-1	420	X, *GAL80*
Chromosome *XIV* (815 kb)		
1N14.4-4	440	X, $C_{1\text{-}3}A$, *CEN14, RAS2, TOP2*
1N14.4-1	205	X, Y, $C_{1\text{-}3}A$, *KEX2, RAD50*
1N14.4-3	140	
1N14.4-2	30	
Chromosome *XV* (1115 kb)		
1N15.4-1	470	X, Y, *ADH1, SUP3*
1N15.4-3	280	*CPA1, HIS3*
1N15.4-2	210	*RAS1*
1N15.4-4	155	X, Y, *PHR1*
Chromosome *XVI* (975 kb)		
1N16.2-2	885	X, *RAD1*
1N16.2-1	90	X, *GAL4*

Comprehensive list of *Not*I restriction fragments of AB972. To facilitate identification, fragments were assigned identification numbers as described in the legend to Table 3.

The mapping methodology relied heavily on the ability to separate the intact chromosomal DNA molecules in a first electrophoretic dimension before attempting the analysis of the *Sfi*I and *Not*I fragmentation patterns. Most of the physical map was worked out by identifying the *Sfi*I and *Not*I fragments that hybridize to probes prepared from genetically mapped, cloned genes, and assuming the colinearity of the physical and genetic maps. Also useful were *Sfi*I and *Not*I linking probes that were used to demonstrate the adjacency of particular pairs of fragments, as well as probes to telomeric and subtelomeric repeat sequences that allowed identification of the terminal fragments.

While we encountered no inconsistencies between the physical and genetic maps, this observation has limited significance because so much of the physical map was based on genetically inferred gene orders. Nonetheless, the use of telomere-associated probes to anchor 32 of the *Sfi*I fragments and 22 of the *Not*I fragments to the ends of the chromosomes, as well as the employment of 61 linking probes, did provide a substantial number of cross checks. The major limitation on applying these checks is the low resolution of the physical map compared to the genetic map (approximately 110 kb *vs.* 15–20 kb). For example, our data would not have allowed us to detect the most recently corrected major error in the genetic map, which involved the orientation of the rightmost third of the chromosome *VII* map (CELENZA and CARLSON 1985).

We did, however, obtain low resolution information about the physical location of 74 genetic markers. Chromosome-by-chromosome comparison of the physical and genetic maps suggests that the known genes in yeast are uniformly distributed across the

TABLE 5

Sizes of the yeast chromosomes

Chromosome	Intact chromosome (kb)[a]	Sum of *Sfi*I fragment sizes (kb)	Sum of *Not*I fragment sizes (kb)	Best estimate (kb)[b]	Range (%)
I	240	245 (5)[c]	240 (2)[c]	240	2.1
II	830	840 (5)	840 (4)	840	1.2
III	350	350 (3)		350	0.0
IV		1,625 (5)	1,660 (7)	1,640	2.1
V	585	585 (3)	595 (4)	590	1.7
VI	285	270 (2)		280	5.4
VII	1,105	1,120 (3)	1,140 (5)	1,120	3.1
VIII	585	595 (3)	595 (3)	590	1.7
IX	445	440 (3)		440	1.1
X	750	760 (2)	760 (4)	755	1.3
XI	690	680 (8)	675 (3)	680	2.2
XII		1,095 + rDNA (6)		1,095 + rDNA	
XIII	930	965 (4)	950 (2)	950	3.7
XIV	790	825 (6)	815 (4)	810	4.3
XV	1.105	1,170 (10)	1,115 (4)	1,130	5.8
XVI	955	1,015 (8)	975 (2)	980	6.1
Total		12,580 + rDNA		12,490 + rDNA	

Estimated physical sizes of AB972 chromosomes. All sizes are rounded to 5 kb.
[a] The intact chromosome size was derived by comparing the mobility of AB972 chromosome to lambda oligomers.
[b] The best estimate is the numerical average of the intact, *Sfi*I and *Not*I size of the chromosome.
[c] The number in parentheses is the apparent number of restriction fragments generated by a complete digestion of the chromosome.

genome and that genetic distance is remarkably proportional to physical distance. No substantial regions were detected that are unpopulated with known genes or of strikingly disproportionate lengths on the physical and genetic maps.

The *Sfi*I/*Not*I map should assist the mapping of single-copy probes to regions of chromosomes. As discussed below, the average distance between landmarks on the map is 110 kb, which typically corresponds to a genetic distance of 40 cM. Therefore, in many instances, it is possible to localize the origins of a cloned gene sufficiently well by hybridization to make it possible to place the gene on the genetic map in a single cross. Assignments of single-copy probes to the *Sfi*I/*Not*I map can be carried out by the method illustrated in Figure 5. Although band transplantation played an essential role in constructing the *Sfi*I/*Not*I map, this step need not be repeated when assigning new probes to map intervals. These assignments can be made more simply with filter strips such as those employed in Figure 5A. Since single-copy probes rarely cross a *Sfi*I or *Not*I site, they are expected to hybridize to a single chromosome, a single *Sfi*I fragment, and a single *Not*I fragment. The combination of a chromosome assignment and the sizes of the hybridizing *Sfi*I and *Not*I fragments always provides enough information to allow an unambiguous map assignment. Indeed, even a band assignment made for a strain such as AB972 (in which chromosomes *V* & *VIII*, *XIII* & *XVI*, and *VII* & *XV*, are not ordinarily resolved), in combination with the sizes of the hybridizing *Sfi*I and *Not*I fragments, provides adequate information to map a new probe.

TABLE 6

Distribution of X and Y sequences at telomeres

	AB972		Alternate strains[a]		
Chromosome	Left arm	Right arm	Left arm	Right arm	Strain
I	X –	X –			
II	X Y	X Y			
III	X –	X –			
IV	X –	X Y			
V	– Y	X Y	X Y	X Y	A364a
VI	X Y	– –			
VII	X –	X Y			
VIII	X Y	X –	X Y	X Y	A364a
IX	X Y	X –			
X	X Y	X –			
XI	X –	X –			
XII	X Y	X Y			
XIII	X –	X –	X –	X Y	YNN281
XIV	X Y	X –			
XV	X Y	X Y			
XVI	X Y	X Y	X Y	X Y	YNN281

[a] Data about the distribution of X and Y sequences in strains other than AB972 were limited to chromosomes *V* and *VIII* in A364a and *XIII* and *XVI* in YNN281.

The map would also allow more refined studies of the distribution of repeated sequences in the yeast genome. In the course of the mapping, we have already acquired an overview of the distribution of the subtelomeric X and Y sequences, which is summarized in Table 6. These data are inconsistent with any simple hypothesis that the X or Y sequences play essential

cis-acting roles in chromosome structure. Telomeres are found in AB972 with all four possible combinations of the presence or absence of X or Y. Most notable is the apparent absence of either sequence on the right arm of chromosome *VI*. However, given that the end of the X sequence that is distal to the telomere is poorly conserved from telomere to telomere (CHAN and TYE 1983b), the absence of sequences that hybridize to any given probe does not necessarily imply that no portion of an X sequence is present. There are no chromosomes that lack X, but there are four that lack Y (*I*, *III*, *XI* and *XIII*). The strain-to-strain variation is also notable; it presumably reflects the rapid shuffling of these sequences from telomere-to-telomere, probably by way of short terminal translocations. Although this process appears to be rapid on an evolutionary time scale, the distribution of subtelomeric repeats in a particular genetic background may be relatively stable. In this regard, comparison of the data in Table 6 with those of LOUIS and HABER (1990) are of interest. These authors determined the distribution of Y sequences in the strain YP1, which is described as having been "derived from a strain backcrossed to S288C at least ten times." While the method employed by LOUIS and HABER did not allow the left and right telomeres of each chromosome to be distinguished, there is a striking similarity between the number of telomeres bearing Y sequences on the different chromosomes in YP1 and AB972: *I* (0 (LOUIS and HABER 1990), 0 (this work)); *VI* (1, 1); *III* (0, 0); *IX* (1, 1); *V* & *VIII* (4, 3); *XI* (0, 0); *X* (1, 1); *XIV* (1, 1); *II* (2, 2); *XIII* (1, 0); *XVI* (2, 2); *VII* & *XV* (2, 3); *XII* (2, 2); *IV* (2, 1). The picture that emerges is that the shuffling of subtelomeric sequences occurs on a laboratory time scale but the distribution of these sequences is not randomized in the course of normal genetic manipulations. Particularly significant is the history of YP1, which suggests that this conclusion holds during both the meiotic and mitotic phases of the life cycle.

Given the expanding role of physical maps in genetics, the yeast *Sfi*I/*Not*I map is of some interest as a data structure. For example, there is no clear precedent for defining the resolution of such maps. Resolution is most simply defined as the length of a map divided by the number of intervals between landmarks. However, for this definition to be useful, it must be coupled to some criterion for the randomness with which the sites are distributed.

In the case of the yeast map, the landmarks are *Sfi*I sites, *Not*I sites, and chromosome ends. Since most applications of the map are likely to employ single digests, the most relevant statistical question is the extent to which the sizes of the single-digest fragments conform to the exponential distribution expected for randomly positioned sites. A minor complication in analyzing the observed distributions is that the number of very small fragments is not known. Although we detected fragments as small as 20 kb, a more conservative detection threshold would be 30 kb. The 69 *Sfi*I fragments that are ≥30 kb (counting only one of the two 30-kb fragments in this class) had an average size of 180 kb. It can be shown that if only fragments with sizes larger than the threshold S_T are sampled from an exponential distribution, the true average fragment size, S_{ave}, is related to the mean size of the sampled fragments, $S_{ave/samp}$, by the relationship $S_{ave} = S_{ave/samp} - S_T$. Consequently, we estimate the true average size of a *Sfi*I fragment as 150 kb (180 − 30 kb). Furthermore, given that S_{ave} = 150 kb and 69 fragments ≥ 30 kb were detected, one can calculate the expected total number of fragments to be 84 from the equation:

number of fragments in the interval S_1 (0 kb) to

$$S_2 \text{ (30 kb)} = (e^{-S_1/S_{ave}} - e^{-S_2/S_{ave}})N_{tot}.$$

If the 69 fragments ≥30 kb are grouped into 10 deciles for a χ^2 analysis, the occupancies of these ten equally populated groups show excellent conformity to a model in which 84 fragments are sampled from an exponential distribution with an average fragment size of 150 kb. The χ^2 value is 8.9 (with 8 degrees of freedom, $P = 0.35$ for $\chi^2 > 8.9$). Since the predicted number of fragments ≤30 kb is 15 and we detected 8, the expected number of fragments that were missed is 7.

Analysis of the *Not*I digest yields similar results: excluding the fragment that spans the rDNA cluster, there is a good fit to a model in which 59 fragments are sampled from an exponential distribution with an average fragment size of 208 kb ($\chi^2 = 8.0$, $P = 0.43$ for a value of $\chi^2 > 8.0$). Since only 53 fragments were detected, the expected number of missed small fragments is 6. The estimate that 7 *Sfi*I and 6 *Not*I fragments of ≤30 kb were missed suggests that somewhat less than 100 kb of DNA are likely to be missing from each map. However, this hypothetical missing DNA is expected to be scattered in the genome and there is little likelihood that any given segment is missing from both the *Sfi*I and the *Not*I map. Not all the linking probes that were placed on the map were analyzed for the possible presence of closely spaced *Sfi*I or *Not*I sites. One example of two closely spaced *Sfi*I sites, which we mapped as a single site, was discovered by DNA sequence analysis of the *PRB*1 gene near the left end of chromosome *V* (MOEHLE *et al.* 1987; GenBank Accession No. M18097). The leftmost *Sfi*I site on our map is actually two sites separated by only 1816 bp. Both sites are present in the linking probe λPM5917. Clones that contain a fragment that is on the map along with only part of a small fragment that was missed would have been excluded from the

set of linking probes since they would not have hybridized to two fragments in the size range that was analyzed.

It should be noted that in the above analyses, many of the "*Sfi*I fragments" and "*Not*I fragments" are actually bounded on one or both ends by a telomere rather than a restriction site. Nonetheless, the essential point is that the landmarks detected by each single digestion, which are a combination of restriction sites and chromosome ends, are randomly spaced. Particularly since the *Sfi*I and *Not*I recognition sites are completely comprised of G/C base pairs, the distribution of these sites provides some insight into the organization of yeast DNA. In mammalian DNA, there is considerable evidence for clustering of the sites for enzymes such as *Sfi*I and *Not*I in G/C-rich "islands," a phenomenon that has been attributed to the effects of DNA methylation (BIRD 1987). We find no evidence for such clustering in yeast, which lacks known cytosine methylation.

Returning to the question of resolution, the simplest measure would be the average distance between mapped intervals. For a genome containing N_c linear chromosomes, the number of intervals in a map produced with n enzymes, each of which cleaves N_i times, is given by the equation $N_{\text{intervals}} = N_c + \Sigma N_i$. For the yeast *Sfi*I/*Not*I map (counting only the two outermost *Sfi*I sites in the rDNA), $N_c = 16$, $N_{SfiI} = 61$, and $N_{NotI} = 38$ (counting only the sites actually mapped, not the total number of sites estimated to be present); consequently, $N_{\text{intervals}} = 115$. Since the size of the non-rDNA component of the genome was estimated to be 12.5 Mb, the average spacing between intervals is 109 kb. We would propose that the resolution of large-scale physical maps be calculated in this way as long as the mapped landmarks are distributed randomly. When this criterion is not met, more sophisticated measures of resolution will be required.

We conclude with a few comments about the relationship between the physical map presented here and longer range efforts to map the yeast genome at higher resolution. In the context of our previous formulation of genomic mapping, the *Sfi*I and *Not*I mapping follows the "top-down" paradigm (OLSON *et al.* 1986). In top-down mapping, one starts with intact chromosomal DNA and works toward higher resolution, while seeking to maintain continuity at each stage. Thus, in yeast, the development of an electrophoretic karyotype was the first phase of top-down mapping, and *Sfi*I and *Not*I mapping is the second phase. Further progress requires the completion of a "bottom-up" map based on the construction of high-resolution maps of local regions from data on sets of overlapping clones. A project of this type, based on overlapping lambda clones is underway (OLSON *et al.* 1986). The most difficult task in bottom-up mapping is the establishment of long range continuity. The continuous, low resolution map presented here is already playing a critical role in this effort. High resolution map fragments that include *Sfi*I or *Not*I sites are being positioned and oriented precisely on the low resolution map, while map fragments that lack these sites are being assigned to a *Sfi*I/*Not*I map interval. Thus, the map described here allows a systematic regional approach to final closure of the high resolution map.

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