A RAPID CHROMOSOME-MAPPING METHOD FOR CLONED FRAGMENTS OF YEAST DNA

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ABSTRACT

A rapid and generally applicable method is described for mapping a cloned yeast DNA segment to the chromosome(s) from which it originated. The method is based upon the recent finding that the integration into a yeast chromosome of a segment of the 2μ plasmid DNA results, in heterozygous diploids, in the specific loss of genetic information from the chromosome into which the 2μ DNA was integrated (FALCO *et al.* 1982). After verification of the accuracy of the method using several genes whose position was known in advance, the method was used to locate the yeast actin gene, which lies on the left arm of chromosome *VI*, about 50 cM distal to *CDC4*.

ALTHOUGH the genetics of the yeast *Saccharomyces cerevisiae* is very well developed, mapping to chromosomes of newly discovered genes can be a difficult and time-consuming process, particularly when the gene of interest is not centromere-linked [see MORTIMER and SCHILD (1980) for a recent review]. To facilitate meiotic mapping of genes, methods involving use of aneuploid strains (MORTIMER and HAWTHORNE 1973; WICKNER 1979) and meiotic mutants (KLAPHOLZ and ESPOSITO 1982) have been developed. These methods, although quite useful, have drawbacks that have limited their application in the general case.

Mitotic methods have also been developed for mapping genes to chromosomes. Most of these depend upon analysis of chromosome loss events: loss or homozygotization of a particular chromosome or chromosome arm is detected by the appearance of recessive phenotypes in heterozygous diploids. Strains heterozygous for alleles of the gene of interest and also for recessive markers from each of the chromosomes are constructed for the purpose of detecting the simultaneous loss of an allele of the gene of interest and one of the markers during mitotic growth. The normally low spontaneous rate of chromosome loss can be increased by chemical treatments (WOOD 1982) or mutations such as *cdc6* and *cdc14* (KAWASAKI 1979), *ch11* (LIRAS *et al.* 1978) or *rad52* (MORTIMER, CONTROPOULOU and SCHILD 1981). Again, each of these methods has drawbacks that have limited its general usefulness.

It is now possible to find, by complementation, a recombinant DNA clone

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containing virtually any yeast gene in which mutants can be found [see BOTSTEIN and DAVIS (1982) for review]. Recombinant DNA methods have, in addition, allowed the identification of interesting DNA segments corresponding to no mapped yeast gene. The classical mapping methods referred to can be applied to mapping such DNA segments, usually *via* the introduction into the chromosome of markers present on an integrating vector plasmid (HINNEN, HICKS and FINK 1978). Unfortunately, however, the instability of such integrated plasmids exacerbates the problems associated with these mapping methods.

In this paper, we describe a new general method for mapping cloned DNA segments to the chromosome(s) from which they are derived. The method is based on the properties of chromosomes into which plasmids carrying segments of the 2μ circle DNA have integrated (FALCO *et al.* 1982). The genetic principle behind the 2μ mapping method is similar in some ways to other mitotic mapping methods: loss of information from particular chromosomes is detected by the appearance of recessive phenotypes in heterozygous diploids. However, the method differs from other mitotic methods in the way in which chromosomes are destabilized and the basis on which the DNA segment of interest is associated with a chromosome. Genetic instability of chromosomal information, in the 2μ-mapping method, results from the integration of a segment of the 2μ circle DNA which, in heterozygous diploids, results in homozygotization of the chromosome arm distal to the inserted 2μ DNA or, less frequently, in homozygotization or loss of the entire chromosome (FALCO *et al.* 1982). The basis for association of the DNA segment of interest to the destabilized chromosome is the ability of the DNA segment to direct integration of a plasmid by homologous recombination with the locus from which it was derived; no phenotypic information about the cloned DNA segment is required.

To test the method, we applied it to mapping several genes whose positions were already known. To illustrate its usefulness, even in cases in which no mutants are available, we mapped to cloned actin gene to the left arm of chromosome *VI*, about 50 cM distal to *CDC4*.

MATERIALS AND METHODS

Strains: The strains used are listed in Table 1; all are derivatives of standard laboratory strains of *S. cerevisiae*.

Plasmids: All of the plasmids used here are shuttle vector derivatives of the standard *E. coli* cloning vector pBR322 capable of being selected and maintained in yeast [see BOTSTEIN and DAVIS (1982) for a review]. They are described here in relation to the unique restriction sites of that pBR322 (BOLIVAR *et al.* 1977). Plasmids pRB27 and pRB30 are plasmids described fully in the accompanying paper (FALCO, ROSE and BOTSTEIN 1983) and previously (FALCO *et al.* 1982): they each contain a 2.3-kb *Sal*I-*Xho*I fragment bearing the *LEU2* gene inserted in the *Sal*I site of pBR322, a 5.5-kb *Bam*HI fragment carrying a mutant (*ura3-3*) *URA3* gene in the *Bam*HI site of pBR322, and an *Eco*RI fragment of the 2μ circle B form in the *Eco*RI site of pBR322. In the case of pRB30 the 2μ fragment is the 2.2-kb carrying origin of replication, and in the case of pRB27 it is the 4.1-kb fragment lacking the origin. pRB55 is a derivative or YEp24 (BOTSTEIN *et al.* 1979) into which was cloned a Sau3a partial digestion fragment of yeast DNA carrying a mutant (*suc2-215*) form of the *SUC2* gene [see CARLSON and BOTSTEIN (1982) for details]. YEp24 has the same 2.2-kb *Eco*RI fragment of 2μ DNA and contains the 1.1-kb *Hind*III *URA3* fragment (BACH, LACROUTE and BOTSTEIN 1979) in the *Hind*III site of pBR322. Plasmid pSL17-9 is another YEp24

TABLE 1

List of strains

Strain	Genotype	Source
Yeast		
DBY127	α *his5-2 leu2-1 lys1-1 aro7-1 can1-100*	D. BOTSTEIN, Massachusetts Institute of Technology (MIT)
DBY689	**a** *leu2-3 leu2-112 ura3-50 can1*	M. ROSE, MIT
DBY703	α *his3 trp1 ura3-52 cir*^{0}	R. DAVIS, Stanford
DBY879	**a** *cdc6 his2 his6 his7 ade1 ade2 ade6 lys7 lys9 leu1 leu2 ura3 trp1 met2 met14 aro7 arg1 arg4 ilv3 asp5 gal1 mal suc*	G. KAWASAKI, University of (U) Washington
DBY884	α *his2 gal1 leu1 ade1 ura3 trp1 met14*	G. KAWASAKI, U. Washington
DBY885	**a** *his6 ade2 lys9 ura1 trp5 met2 arg4 mal suc*	G. KAWASAKI, U. Washington
DBY889	**a** *his7 lys7 met6 arg1 gal4*	G. KAWASAKI, U. Washington
DBY931	**a** *his4 leu2-3 leu2-112 met8-1 ura3-50 can1-101*	S. C. FALCO, MIT
DBY946	**a** *suc2-432 ura3-52*	M. CARLSON, MIT
DBY947	α *ade2-102 ura3-52*	M. CARLSON, MIT
DBY982	α *suc2-432 ura3-52 lys2 his4*	M. CARLSON, MIT
DBY1042	**a** *arg9 leu2-3 leu2-112 lys2 met8-1 ura3-50*	S. C. FALCO, MIT
DBY1043	α *his1 leu2 ura3-50*	S. C. Falco, MIT
DBY1089	**a** *leu2-3 leu2-112 can1 ura3-50* [pRB1 (*URA*$^+$ at actin)	D. SHORTLE, MIT
DBY1208	α *cdc4 his4 trp1 ura3*	N. NEFF, MIT
FY169	**a** *ade1 his1 leu2 lys1 ura3-50*	S. C. FALCO, MIT
FY7	α *leu2 can1 his5 lys1 aro7*	S. C. FALCO, MIT
FY195	**a** *spo11 ura3 ade1 his7 hom3 can1 cyh2*	S. KLAPHOLTZ, U. Chicago
FY197	**a** *lys2 trp1 ura3 ade6 arg4 spo11 met14 asp5 pet17 aro7*	S. KLAPHOLTZ, U. Chicago
Plasmids		
pRB1	*URA3 ACT1*	D. SHORTLE, MIT
pRB27	*LEU2 ura3-3* (2μ *ORI*$^-$)	M. ROSE, MIT
pRB30	*LEU2 ura3-3* (2μ *ORI*$^+$)	M. ROSE, MIT
pRB55	*URA3 suc2-215* (2μ *ORI*$^+$)	M. CARLSON, MIT
pRB148	*URA3 act1-Δ1* (2μ *ORI*$^+$)	D. SHORTLE, MIT
pSL17-9	*URA3 lys2-Δ* (2μ *ORI*$^+$)	S. C. FALCO, MIT

derivative containing a deleted form of the *LYS2* gene: the intact gene was found by complementation using the same library that yielded pRB55 (S. C., FALCO, unpublished results); a *Bgl*II to *Sal*I fragment carrying only part of the *LYS2* gene was inserted into YEp24, yielding pSL17-9. Plasmids pRB1 and pRB148 are based on YIp5 (*i.e.*, pBR322 with the 1.1-kb *Hind*III fragment carrying *URA3* inserted into the *Ava*II site; BOTSTEIN *et al.* 1979). Plasmid pRB1 is YIp5 with the *Eco*RI fragment carrying the actin gene (NG and ABELSON 1980) inserted at the *Eco*RI site. Plasmid pRB148 (SHORTLE, HABER and BOTSTEIN 1982) is pRB1 from which a *Bgl*II fragment internal to the actin gene has been removed and the 2.2-kb *ORI*$^+$ 2μ fragment added at the *Eco*RI site so that the final order is 2μ, deleted actin, *URA3*.

Growth, mating, sporulation and tetrad analysis: Formulations used for media (YEP-glucose for nonselective growth; SD with appropriate supplements for selective growth and scoring of nutritional markers) are described by SHERMAN, FINK and LAWRENCE (1974). Matings were carried out

by cross-streaking strains on YEP-glucose and replica plating to selective (SD medium plus supplements) plates. Diploids were single-colony purified on YEP-glucose. Sporulation was done on plates [2% potassium acetate, 0.1% glucose, 0.25% Bacto-yeast extract (Difco), and 1.5% Bacto-agar (Difco)]. After 3 days at 30° asci were digested in glusulase, and spores were separated by micromanipulation, grown up on YEP-glucose and replica plated onto various minimal media to ascertain phenotype(s).

DNA preparation and transformation of yeast: Plasmid DNA was made from *E. coli* strains by the CsCl equilibrium density gradient method described by DAVIS, BOTSTEIN and ROTH (1980). Transformation of yeast was done by a modification of the method of HINNEN, HICKS and FINK (1978): glusulase treatment was done in 1 M sorbitol containing 1% mercaptoethanol and buffered to pH 5.8 using 0.1 M citrate. The concentration of glusulase was 2%, and the spheroplasting treatment was 3 hr at 30°.

Measurements of marker stability in yeast: Strains were grown nonselectively, as isolated single colonies, on solid YEP-glucose medium. Single colonies were picked and diluted in water; we estimate that these colonies contained between 10^7 and 10^8 cells, corresponding to about 25 generations of growth from the single cell deposited on the plate. The dispersed, diluted cells were replated on YEP-glucose so that 100–300 colonies appeared per plate. These plates were then replica plated to minimal media containing supplements such that the various phenotypes could be scored.

RESULTS

Chromosome mapping by the 2μ method involves several steps. First, the gene of interest must be cloned onto a plasmid vector that contains a fragment of the 2μ circle DNA (*i.e.*, a YEp vector). Second, the plasmid must be integrated, *via* the homology provided by the gene of interest (as opposed to other yeast DNA on the vector) into its chromosome of origin. Third, the haploid strain with the integrated 2μ DNA must be mated with one or more strains which, among them, have recessive mutations marking all of the chromosomes or, if possible, all of the chromosome arms; the appearance of these recessive phenotypes is then followed during mitotic growth.

Properties of vectors: The only special requirement imposed by the 2μ-mapping methods upon vector plasmids is that they contain the target (located in the inverted repetition) of the 2μ site-specific recombination system (FALCO *et al.* 1982; BROACH, GUARASCIO and JAYARAM 1982). All YEp plasmids meet this requirement, since their autonomous maintenance in yeast requires the same site or the very closely linked origin of DNA replication (BEGGS 1978; STRUHL *et al.* 1979; BROACH and HICKS 1980). Of course, the vectors usually contain additional yeast DNA to provide traits selectable in yeast.

The plasmid vectors used for the experiments described here are listed in Table 1. Each is derived from the bacterial plasmid pBR322 (BOLIVAR *et al.* 1977), retains a functional ampicillin-resistance determinant (Ap^R) and bacterial origin of replication allowing selection for and maintenance of the plasmids of *E. coli*. Each plasmid also contains three segments of yeast DNA: first, a segment of the 2μ DNA which includes the inverted repetition; second, a selectable yeast gene (either *LEU2* or *URA3*); and third, the segment of yeast DNA to be mapped. It should be noted that the 2μ DNA segment in the plasmid does not always include the 2μ origin of replication (*e.g.*, pRB27).

Chromosomal integration of plasmids containing 2μ DNA: The mapping method depends upon the isolation of yeast strains in which the plasmid carrying the

2μ DNA has integrated into a chromosome by homologous recombination within the segment to be mapped. Having made the appropriate YEp plasmids, one then must find chromosomal integrants. One way of getting such integrants is to put mutations in the gene of interest in both the plasmid and the chromosome and then to select crossovers between them. A second way is to select for stable maintenance of a YEp vector in a yeast strain that lacks a resident 2μ plasmid (*cir*^{0}). This method works because all of the YEp vectors used (even those that contain the origin of replication) require the provision of diffusible replication functions by an intact resident 2μ circle. Thus, transformants grow poorly unless the vector integrates into a chromosome. A third way is to apply the observation of ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) that cleavage of a circular plasmid in a region of homology with a yeast chromosome results in directed integration of the plasmid within the region of homology. We have used all of these methods successfully. No matter which method is used, however, the primary problem is the detection of the integrants among autonomous transformants and the verification that the integration has occurred at the locus one wants to map. As will be shown, the simplest criterion for integration is the stability of plasmid maintenance relative to the autonomous mode [see also accompanying paper by FALCO, ROSE and BOTSTEIN (1983)]. The best evidence of integration at the locus of interest is a gel-transfer hybridization experiment (SOUTHERN 1975) using DNA from the gene of interest as hybridization probe (FALCO *et al.* 1982).

In most of our studies, we found integrants by selecting for crossovers between mutations on the plasmid and the chromosome (FALCO, ROSE and BOTSTEIN 1983). Therefore, many of the starting plasmids used in these experiments contain a mutant allele of the yeast gene to be mapped. Plasmids pRB27 and pRB30 carry the *ura3-3* amber mutation (ROSE 1982; FALCO *et al.* 1982); pRB55 carries the *suc2-215* amber mutation (CARLSON and BOTSTEIN 1982); pSL17-9 carries a deletion of the *LYS2* gene: it is a subclone, which contains only part of the gene.

When any of the plasmids, pRB27, pRB30, pRB55 or pSL17-9, are introduced into a yeast strain by DNA transformation (HINNEN, HICKS and FINK 1978), the transformant phenotype is unstable. When grown under conditions that do not select for presence of the plasmid, segregants that have lost the plasmid marker phenotype(s) are frequent. Results of a typical experiment are shown in Table 2: about ¼ of all cells after about 25 generations of nonselective growth have lost the plasmid marker(s). The plasmid pRB27 displayed greater instability than the other plasmids, probably because pRB27, unlike the other plasmids, lacks the 2μ origin of DNA replication.

The transformant strains in Table 2 contain different mutant forms of the same gene on the plasmid and on a chromosome, allowing selection of the recombinant phenotype in each case. Table 3 shows the stability of particular recombinants that result from carrying out this selection. These recombinants maintain the plasmid-associated selective markers as well as the selected recombinant marker stably: no segregants were observed after about 25 generations of nonselective growth. When such recombinants are subjected to meiotic anal-

TABLE 2

Stability of transformants derived from autonomously replicating plasmids

Transformant	Parent strain	Plasmid	Plasmid marker	Total no. of colonies	Phenotypes: % of total	
					Leu$^+$	Leu$^-$
YT326	DBY931	pRB27	*LEU2*$^+$	154	26	74
YT328	DBY931	pRB30	*LEU2*$^+$	250	73	27
					Ura$^+$	Ura$^-$
YT500	DBY982	pRB55	*URA3*$^+$	144	82	18
YT377	DBY1042	psL17-9	*URA3*$^+$	158	71	29

Data are from a single transformant clone in each case.

TABLE 3

Stability of recombinants in which an autonomously replicating plasmid has become integrated into a chromosome

Recombinant strain	Parent strain	Recombinant phenotype selected	Plasmid-associated phenotype	Total no. of colonies	Phenotypes: % of total			
					Ura$^+$Leu$^+$	Ura$^+$Leu$^-$	Ura$^-$Leu$^+$	Ura$^-$Leu$^-$
YT331	YT326	Ura$^+$	Leu$^+$	206	100	0	0	0
YT336	YT328	Ura$^+$	Leu$^+$	120	100	0	0	0
					Suc$^+$Ura$^+$	Suc$^+$Ura$^-$	Suc$^-$Ura$^+$	Suc$^-$Ura$^-$
YT376	YT500	Suc$^+$	Ura$^+$	59	100	0	0	0
					Lys$^+$Ura$^+$	Lys$^+$Ura$^-$	Lys$^-$Ura$^+$	Lys$^-$Ura$^-$
YT428	YT377	Lys$^+$	Ura$^+$	191	100	0	0	0

Data are from a single parental stable clone in each case; repetition with sister clones gave identical results.

ysis and gel-transfer hybridization analysis (FALCO *et al.* 1982; FALCO, ROSE and BOTSTEIN 1983; S. C. FALCO, M. ROSE and D. BOTSTEIN, unpublished results) one finds that they contain the entire plasmid integrated at the locus of the gene in which recombination was selected.

Our experience indicates that stable inheritance of the plasmid-associated marker along with the selected marker is a necessary, and usually sufficient, indication of integration of the entire plasmid vector, including the 2μ DNA sequences (FALCO, ROSE and BOTSTEIN 1983). However, cases of integration at the plasmid-associated marker locus do occur rarely, and gel-transfer experiments are required for verification of integration at the correct locus.

The other methods for integrating 2μ DNA-containing plasmid vectors (*i.e.*, using recipients lacking 2μ circles as recipients and/or cleaving the plasmid in the region of homology with the gene of interest) are discussed later in connection with mapping of the actin locus.

Using integrated YEp plasmids in chromosome mapping: We showed previously

(FALCO *et al.* 1982) that integrated YEp plasmids, although stable in haploids, caused instability of the chromosome into which the 2μ DNA has been integrated in diploids heterozygous for the integrated plasmid. This instability was most marked for the locus of integrated plasmid itself and all genetic markers centromere-distal to the integrated plasmid. However, the instability extended also to markers on the other arm of the chromosome into which the YEp plasmid had integrated, although loss of these markers was less frequent.

The use of these properties in mapping several genes (*URA3*, *SUC2* and *LYS2*) is illustrated in experiments in which strains bearing integrated 2μ plasmids were mated to strains carrying a variety of recessive markers representing all of the known chromosomes. The genotypes of the strains constructed are given in Table 4. These strains were then streaked on a nonselective medium (YEP-glucose); when colonies had grown (*i.e.*, after about 25 generations of growth) the cells in them were dispersed and replated on nonselective medium. When these colonies had grown, replica plating was carried out to determine whether any new requirements had appeared. In most cases, the marker associated with the plasmid, as well as the phenotype selected in order to ensure integration, could be scored. From the proportion of cells that had lost these phenotypes during the 25 generations of growth, the percent of segregants could be calculated. The colonies were also scored for the appearance of additional phenotypes.

The data for the case of *URA3* are shown in Table 5. As expected from previous results (FALCO *et al.* 1982) the frequency of segregation of the integrated plasmid (judged from loss of the Ura^+Leu^+ phenotype) is very high (between 13 and 53%). The centromere-distal marker (*CAN1*) is lost in virtually all of the segregants from the relevant diploids (YT406/DBY931 and YT408/DBY931). The *HIS1* allele (which is on the other arm of chromosome *V*) is lost much less frequently (from the diploids YT331/DBY1043 and YT336/DBY1043) but still at a frequency of several percent. The most important result, however, is that all of the markers on all of the other chromosomes are completely stable: of more than 800 cells (including more than 140 segregants) heterozygous for markers on every chromosome (the last two lines of Table 5) we did not observe a single case in which a marker on a chromosome other than chromosome *V* was lost. Thus, the pattern of instability clearly indicates the position of the integrated plasmid (and thus the *URA3* gene) proximal to *CAN1* on chromosome *V*. It is worth noting in Table 5 that, when the diploid is heterozygous for *CAN1* with the recessive allele *cis* to the integrated plasmid, no Can^R segregants are found, showing that the loss of information is asymmetric: information loss is limited to the chromosome that contains the integrated 2μ DNA.

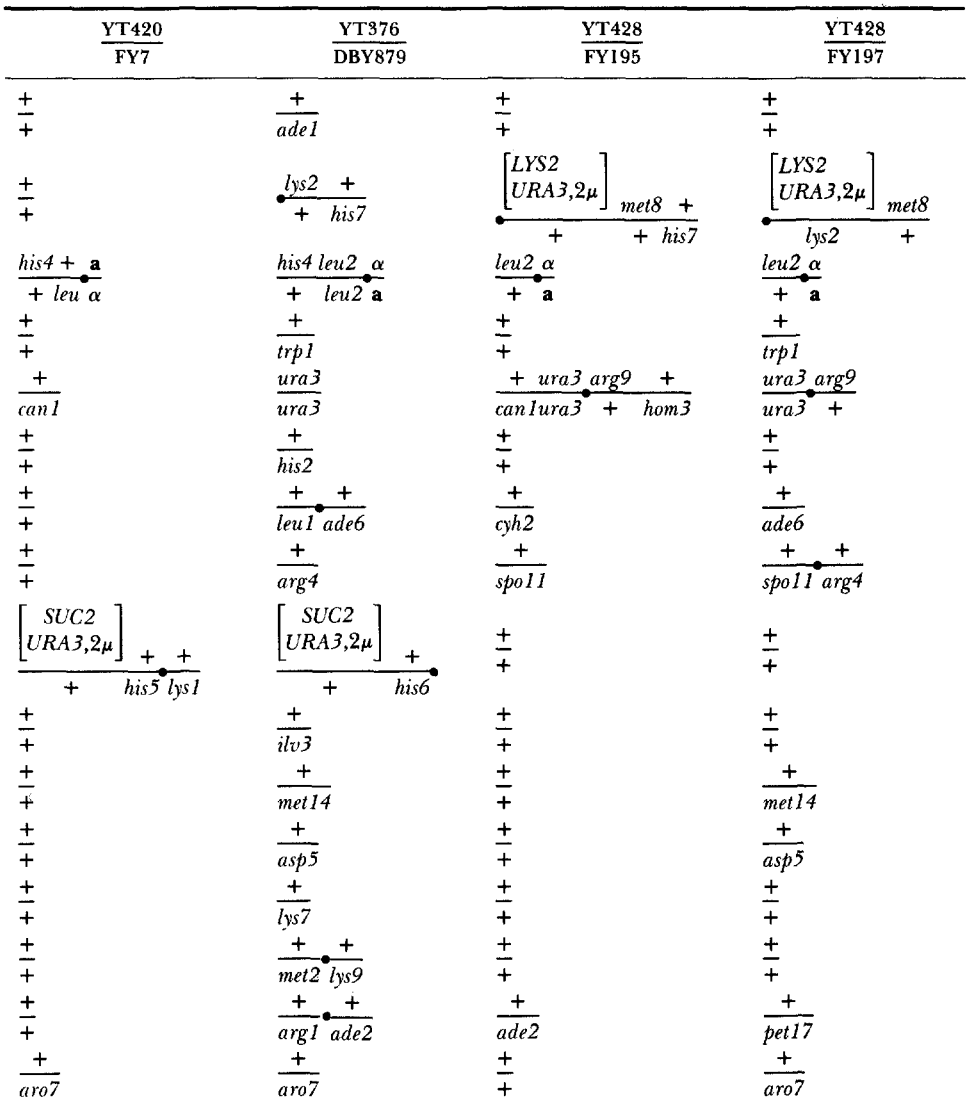
Table 6 shows a similar analysis for the *SUC2* gene. Again, the frequency of loss of chromosome *IX* markers is high, and markers on all other chromosomes seem completely stable (no cases in more than 700 colonies including more than 130 segregants). Several additional points deserve emphasis concerning the data in Table 6. First, the *SUC2* gene is distal (or on the other arm) relative to the other chromosome *IX* markers used. Nevertheless, the instability of *HIS5*

TABLE 4

Genotypes of diploids containing integrated 2μ plasmids

Chromosome	YT331 or YT336 / DBY1043	YT406 or YT408 / DBY931	YT406 or YT408 / DBY879	YT417 / FY169
I	+ / +	+ / +	+ / *ade1*	+ / *ade1*
II	*met8* / +	*lys2 met8* / + *met8*	*lys2 met8* + / + + *his7*	+ / +
III	*his4 leu2* **a** / + *leu2* α	+ *leu2* α / *his4 leu2* **a**	*leu2* α / *leu2* **a**	*his4* + α / + *leu2* **a**
IV	+ / +	+ / +	+ / *trp1*	+ / +
V	*can1* [*URA3* *LEU2,2μ*] + / + *ura3* *his1*	+ [*URA3* *LEU2,2μ*] / *can1* *ura3*	[*URA3* *LEU2,2μ*] / *ura3*	*ura3* + / *ura3* *his1*
VI	+ / +	+ / +	+ / *his2*	+ / +
VII	+ / +	+ / +	+ + / *leu1 ade6*	+ / +
VIII	+ / +	+ / +	+ / *arg4*	+ / +
IX	+ / +	+ / +	+ / *his6*	[*SUC2* *URA3,2μ*] + / + *lys1*
X	+ / +	+ / +	+ / *ilv3*	+ / +
XI	+ / +	+ / +	+ / *met14*	+ / +
XII	+ / +	+ / +	+ / *asp5*	+ / +
XIII	+ / +	+ / +	+ / *lys7*	+ / +
XIV	+ / +	+ / +	+ + / *met2 lys9*	+ / +
XV	+ / +	+ / +	+ + / *arg1 ade2*	+ / +
XVI	+ / +	+ / +	+ / *aro7*	+ / +

Strains were constructed by mating haploids carrying integrated 2μ plasmids (Table 3) with tester strains (Table 1). In some cases the integrated plasmid was crossed into another strain first. Thus, YT406 and YT408 are α *leu2 lys2 met8* derivatives containing the integrated plasmid from YT331 and YT336, respectively; YT417 is an α *his4 ura3* derivative of YT376, and YT420 is a sister spore **a** *his4* derivative of YT376.



is greater than that of *LYS1* (line 2 of Table 6), consistent with the fact that the latter marker is on the other chromosome arm. Second, the second line shows data for a case in which segregation of the plasmid-bearing locus could not directly be scored, since the strain was not chromosomally homozygous for either plasmid-associated marker; this suggests that one can map even though direct assay of segregation is not possible.

Table 7 shows partial data for mapping *LYS2*. Again, instability is limited to

TABLE 5

Mapping the URA3 *gene*

Plasmid marker: *LEU2*. Expected chromosome: *V*. Arrangement: *CAN1-URA3-CEN5-HIS1*					
			Phenotypes revealed (% of total)		
Diploid strain	Total colonies	Segregants (% of total)	Can^R	His^-	Other
YT331/DBY1043	618	36	0	8[a]	0
YT336/DBY1043	693	53	0	2[a]	0
YT406/DBY931	640	35	35	0[b]	0
YT408/DBY931	670	29	28	0[b]	0
YT406/DBY879	480	13	N/A	0[b]	0
YT408/DBY879	346	22	N/A	0[b]	0

Genotypes of diploids are given in Table 4. Diploids were grown nonselectively on YEP-glucose from single cells into colonies (about 25 generations), dispersed in water, replated on YEP-glucose and replica plated to determine growth phenotypes. Data are summed from more than one parental clone. Segregants are given as percent Ura^- among the total. N/A indicates that relevant marker was not heterozygous in the starting diploid strain.

[a] Further tests of genotype showed that *HIS1* (and not *HIS4*) was lost from these strains.

[b] The *his* marker in these strains is not *his1*.

TABLE 6

Mapping the SUC2 *gene*

Plasmid Marker: *URA3*. Expected chromosome: *IX*. Arrangement: *SUC2-HIS5-HIS6-CEN9-LYS1*						
			Phenotypes revealed (% of total)			
Diploid strain	Total colonies	Segregants (% of total)	His^-Lys^+	His^-Lys^-	His^+Lys^-	Other
YT417/FY169	488	28	N/A	N/A	3	0
YT420/FY7	437	NT[a]	7	3	0	0
YT376/DBY879	719	19	3	N/A	N/A	0

Genotypes of strains are given in Table 4. The procedure was the same as described in Table 5. Segregants were determined as percent Ura^- among total. Data are summed from more than one parental clone. N/A indicates strain was not heterozygous for *his5* or *his6* or *lys1*.

[a] Segregants could not be determined directly since one haploid parent was $URA3^+SUC2^+$.

the chromosome (*II*) on which the gene maps. All of the segregants from the diploid YT428/FY195 are his^-, consistent with the fact that *his7* is centromere-distal to *lys2*. It is worth recalling that, in this case, integration of the plasmid was obtained by using only a fragment of the *LYS2* gene to recombine with the *LYS2* DNA on the chromosome.

Mapping the yeast actin gene: To demonstrate the utility of chromosome mapping based on the properties of chromosomally integrated 2μ DNA, we

TABLE 7

Mapping the LYS2 *gene*

Plasmid Marker: *URA3*. Expected Chromosome: *II*. Arrangement: *HIS7-LYS2-CEN2*				
			Phenotypes revealed (% of total)	
Diploid strain	Total colonies	Segregants (% of total)	His$^-$	Other
YT428/FY195	160	12	12	0
YT428/FY197	253	16	N/A	0

Genotype of the strain is given in Table 4. Segregants were determined as percent Ura$^-$ or Ura$^-$Lys$^-$ among total. Data are summed from more than one parental clone. N/A indicates that the diploid was not heterozygous for *his7*.

mapped the actin gene of yeast. The molecular cloning of the yeast actin gene has been described (NG and ABELSON 1980; GALLWITZ and SURES 1980), and it has been shown to be a single gene essential for yeast viability (SHORTLE, HABER and BOTSTEIN 1982). For the purpose of mapping, we used the plasmid pRB148 which is derived from the shuttle vector YEp24 (BOTSTEIN *et al.* 1979). The plasmid contains an internal deletion (constructed *in vitro*) of the actin gene because multiple copies of the intact actin gene are apparently deleterious in some (if not all) yeast strains (D. SHORTLE, unpublished result). The plasmid still contains ample homology to the actin locus to allow homologous recombination.

Since we did not, at this time, have any chromosomal actin mutants, we could not use recombination to select for integration of pRB148. Instead, we turned to alternate methods that allow the recovery of integrants of normally autonomously replicating YEp plasmids. We obtained integrants by transforming a strain lacking the endogenous 2μ circle (*cir*^{0}). Under these circumstances YEp24 and its derivatives replicate poorly (even when they have the origin of replication) due to a lack of 2μ-encoded functions (BROACH and HICKS 1980). Consequently, the transformants are extremely unstable. By growing such transformants a few generations nonselectively followed by reimposition of selection, one finds stable derivatives that grow well, *i.e.*, large colonies lacking the "Nibbled" appearance characteristic of unstable transformants on selective plates. These derivatives usually are integrants of the entire plasmid into a chromosome. Such integrants must be mated to 2μ circle-bearing (*cir*$^+$) strains in order for the chromosome instability characteristic of diploids heterozygous for an integrated YEp plasmid to be manifested, because this instability depends upon site-specific interactions between the integrated and autonomous 2μ DNA (FALCO *et al.* 1982).

Another method for obtaining chromosomal integrants takes advantage of the discovery by ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) that cleavage of a circular plasmid within a yeast DNA segment greatly stimulates integrative recombination in the region of the cleavage. This observation extends to plasmids containing 2μ DNA. If one cuts within the DNA from the gene to be

mapped, one directs integration to the locus of interest. We found that directed integration by this method has not worked in every case; the reasons for this are not entirely clear and may reflect dependence of this method on details of the genetic background of the strains or upon the extent of homology left after restriction enzyme cleavage.

To obtain chromosomal integration of 2μ DNA at the actin locus, we transformed a *cir*^{0} strain (DBY703) with plasmid pRB148 in two ways: using intact circular plasmid and using plasmid linearized by cleavage with *Bgl*II, which cleaves within the actin gene itself. We obtained a low frequency of Ura$^+$ transformants in both cases, suggesting that even the circular form could not replicate autonomously, despite the fact that it carries the 2μ origin of DNA replication. All of the transformants were stably Ura$^+$ (*i.e.*, no segregants in more than 150 colonies after 25 generations of nonselective growth), indicating that the plasmids were, in each case, stably integrated. The failure to find any autonomous transformants is not a general property: there are reasons to believe that the actin gene, at high copy number, may be lethal (D. SHORTLE and D. BOTSTEIN, unpublished observation).

To find the site of chromosomal integration, the transformants YT432 (from intact pRB148 DNA) and YT451 (from cleaved pRB148 DNA) were mated with the tester strain DBY879, which carries recessive markers on every chromosome (see Tables 1 and 4). Several independent diploid clones were grown nonselectively and dispersed in water, replated nonselectively and replica plated to detect Ura$^-$ segregants as well as any additional auxotrophy which might be revealed. As shown in Table 8, most segregants required only uracil, but others (a few percent of total segregants) required an additional nutrient. Further analysis revealed that the only additional requirement in each case was histidine. The tester strain (DBY879) carries three different histidine mutations, *his2*, *his6* and *his7*, which mark chromosomes *VI*, *IX* and *II*, respectively. To determine which mutation was responsible, the aforementioned analysis was repeated by mating YT432 and tester strains carrying only single *his* mutations: as shown in Table 8, only diploids made with *his2* strains became unstably prototrophic for histidine. Complementation analysis [after allowing mitotic homozygotization at the *MAT* locus (data not shown)] confirmed that the histidine requirement in the original segregants from the diploid YT432/DBY879) was due to a *his2* lesion. Similar experiments with strain YT435 gave identical results. From these data we concluded that the actin locus is on chromosome *VI*. Since the appearance of the *his2* phenotype was restricted to a minority of the *Ura*$^-$ segregants, it would appear that the actin locus is not proximal to *HIS2*, in accord with the observation of D. SHORTLE (unpublished results) that plasmid markers integrated at the actin locus do not show centromere linkage.

To prove that the actin locus is indeed located on chromosome *VI*, and to determine more precisely where on this chromosome the actin gene resides, we performed standard tetrad analysis. Since we had no mutations in the actin gene, we integrated the *URA3* gene at the actin locus using actin homology (HINNEN, HICKS and FINK 1978) in a strain that was chromosomally *ura3*$^-$; an integrating plasmid was used that contained no 2μ DNA. Table 9 shows tetrad

TABLE 8

Mapping the actin gene

Plasmid marker: *URA3*. Expected chromosome: unknown

Diploid strain	Total colonies	Segregants (% of total)	Phenotypes revealed (% of total)	
			His⁻	Other
YT432 / DBY879	520	48	2	0
YT435 / DBY879	651	33	1	0
YT432 / DBY884(*his2*)	417	24	6	0
YT432 / DBY885(*his6*)	451	NT[a]	0	0
YT432 / DBY889(*his7*)	420	NT[a]	0	0

Genotypes of strains: YT432 and YT435 are stable transformants of DBY703 (Table 1) with plasmid pRB148. DBY879 (Table 1) contains recessive markers on all of the chromosomes including *his2*, *his6* and *his7*. Procedure was as described in the legend to Table 5 and the text. Segregants are given as percent Ura⁻ among total.

[a] NT. Segregants could not be scored since DBY885 and DBY889 (Table 1) are *URA3*⁺.

TABLE 9

Meiotic mapping of the actin gene

Gene pair	PD	NPD	T
URA3 (at actin)-*CDC4*	15	3	52
URA3 (at actin)-*LEU2*	16	13	44
CDC4-LEU2	20	26	21

Cross: DBY1089 / DBY1208 (Table 1)

Genotype: $\frac{\mathbf{a}\ leu2\ +}{\alpha\ +\ his4}$ $\frac{can1\ ura3}{+\ ura3}$ $\frac{+}{cdc4}$ $\frac{[\text{pRB1}(URA3^{+})\text{ at actin}]}{ACT1^{+}}$

pRB1 is the actin *Eco*RI fragment inserted into the *Eco*RI site of YIp5 (BOTSTEIN *et al.* 1979). The calculated distance ACT1-CDC4 is 50 cM.

data which indicate that the actin locus (for which we propose the gene name *ACT1*) is about 50 cM from the *cdc4* mutation we used. Since it is not centromere linked and not linked at all to *CDC26*, which lies about 25 cM from *CDC4* on the other arm of chromosome *VI*, we conclude that *ACT1* lies 50 cM distal to *CDC4* on the left arm of chromosome *VI*. This extends the genetic map of chromosome *VI* about 25 cM.

DISCUSSION

The experiments described in this paper and in two other reports on this subject (FALCO *et al.* 1982; FALCO, ROSE and BOTSTEIN 1983) show that inte-

gration into a chromosome of the inverted repetition of the 2μ circle DNA results in stability of the plasmid-borne phenotypes in haploids but also a very particular instability in diploids heterozygous for the integrated plasmid. The latter instability is useful in mitotic chromosome mapping because of the following properties:

1. The information on the integrated plasmid itself is frequently lost (10 to 50% segregants after 25 generations of unselected growth).

2. The information distal to the point of integration is lost essentially every time the plasmid information is lost.

3. The information proximal to the point of integration and on the other chromosome arm is lost less frequently but still at a frequency of the order of 1% after 25 generations of unselected growth.

4. The information on chromosomes other than the one into which the plasmid integrated is never lost (we observed no instance in more than 3000 colonies of appearance of a recessive marker associated with another chromosome).

These properties allow one to determine not only the chromosome into which the plasmid has integrated, but also to make possible inferences about the relationships among the point of integration, the markers used and the centromere. It is possible that there may be quantitative relationships (for example, a marker proximal to the point of integration may become homozygous more frequently when nearer to the point of plasmid integration), but these will require more data to establish.

The mapping method can be broken down into steps as follows:

1. construction of a plasmid vector containing the DNA segment to be mapped, a selectable marker (*e.g.*, *URA3* or *LEU2*), and a segment of the 2μ DNA which includes the inverted repetition. This is not a difficult requirement to meet, since many libraries of yeast genes are made on YEp vectors to begin with, and because any easily subcloned fragment of a gene of interest in a YEp vector will suffice to carry out mapping.

2. transformation of a haploid yeast strain with the plasmid and isolation of transformants (or derivatives of transformants) in which the plasmid has integrated into a chromosome by recombination within the segment to be mapped. Some methods lead to integration preferentially at the site of interest, as opposed to integration at the marker locus. These include use of deletions of rearrangements at the marker locus to inhibit integration therein, selection for recombination between mutations on the chromosome and the plasmid at the locus of interest and cleavage of the plasmid within the desired region of homology to direct integration. Stability of the transformed phenotype has turned out to be a good primary criterion for integration. It is, nevertheless, a good practice to isolate several independent integrants and, if any ambiguity arises, to undertake gel-transfer hybridization (SOUTHERN 1975) experiments to demonstrate both the fact and site of integration.

3. mating the haploid strains containing integrated plasmids with suitable tester strains carrying recessive markers. Among them, the strains should have markers on each chromosome and, if possible, each chromosome arm. Although we used strain DBY879, we do not recommend this strain in general

since it has several genes causing the same nutritional requirement: these can revert unnoticed. This property also requires extra experiments to determine which gene is responsible for the observed phenotype. We recommend, instead, the set of strains recently described by KLAPHOLTZ and ESPOSITO (1982) for use in the *spo11* mapping method. These strains not only have a unique gene for each phenotype, they also carry *ura3* mutations, making it easy to use YEp24 and YIp5 derivatives in mapping.

The mapping method is applicable to all fragments of yeast DNA, regardless of what is known (or not known) about their function. The only property required of a DNA fragment is that it have enough homology with a region of a chromosome to allow integrative recombination. Since any gene can be cloned by complementation for which a mutation with a scorable phenotype exists, the method should be applicable to any yeast gene. Recently, several additional genes have been located using this method in several laboratories including mapping of *TUB2* to chromosome *6L* (J. THOMAS, unpublished result); reconfirmation of the locations of *HXK1* (*6R*) and *HXK2* (*7L*) (J. SWAN, unpublished results); mapping of *AAS1* (*4R*), *AAS2* (*11R*) and *AAS3* (*5R*) (A. HINNEBUSH, personal communication); and confirmation of the cloning of *PRB1* by showing integration at *5L* (M. AYNARDI and E. JONES, personal communication).

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