GENETIC MAPPING IN SACCHAROMYCES
IV. MAPPING OF TEMPERATURE-SENSITIVE GENES AND USE OF DISOMIC STRAINS IN LOCALIZING GENES[1]

R. K. MORTIMER[2] AND D. C. HAWTHORNE

Division of Medical Physics, Donner Laboratory, University of California, Berkeley 94720, and Department of Genetics, University of Washington, Seattle 98105

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ABSTRACT

Through use of tetrad, random spore, trisomic, and mitotic analysis procedures a large number of genes, including 48 new genetic markers, were studied for their locations on the genetic maps of the yeast *Saccharomyces cerevisiae*. Eighteen new centromere linked genes were discovered and all but one was located on various ones of the 16 previously-established chromosomes. Five fragments of linked genes were also assigned to chromosomes; four were located on known chromosomes while the fifth determined one arm of a new chromosome. The experiments indicate that seventeen is likely to be the haploid chromosome number in this yeast. Most chromosomes have been established by genetic means to be metacentric and their genetic lengths vary from 5 cM to approximately 400 cM. Functionally-related sets of genes generally were found to be dispersed over the genome.

PREVIOUS genetic mapping studies in the yeast *Saccharomyces cerevisiae* have established the location of over 100 genes on sixteen chromosomes and six fragments (HAWTHORNE and MORTIMER 1960, 1968; LINDEGREN *et al.* 1962; HWANG, LINDEGREN and LINDEGREN 1963, 1964; MORTIMER and HAWTHORNE 1966). The sixteen chromosomes were each defined by independently-segregating sets of one or more centromere-linked genes whereas the fragments represented groups of linked genes not associated with a centromere. A seventeenth chromosome has since been proposed by RESNICK (1969). Additional chromosomes are implied from the linkage studies of LINDEGREN and coworkers (LINDEGREN *et al.* 1962; SHULT, DESBOROUGH and LINDEGREN 1962; HWANG and LINDEGREN 1966; SHULT, LINDEGREN and LINDEGREN 1967). These authors contend that several genes originally assigned to one of the 16 chromosomes are in reality located on separate chromosomes which assort preferentially with respect to certain other chromosomes. Thus, some uncertainty attaches to the number of chromosomes that comprise the yeast genome as determined by genetic mapping studies. Cytological investigations also have, so far, failed to yield a consistent estimate of the

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TABLE 1

Second division segregation frequencies of 48 genes in Saccharomyces cerevisiae

Gene	Division segregation			Source†
	First	Second	Percent second	
cdc1	10	30	75	L. HARTWELL
cdc2	26	75	74	L. HARTWELL
cdc4	59	11	16*	L. HARTWELL
cdc5	52	21	29*	L. HARTWELL
cdc6	12	22	65	L. HARTWELL
cdc7	15	2	12*	L. HARTWELL
cdc8	29	29	50*	L. HARTWELL
cdc9	9	18	67	L. HARTWELL
cdc10	24	0	0*	L. HARTWELL
cdc11	29	33	53*	L. HARTWELL
cdc13	12	30	72	L. HARTWELL
cdc14	70	71	50*	L. HARTWELL
cdc15	26	4	13*	C. MCLAUGHLIN
cly2	46	60	57*	L. HARTWELL
cly3	35	10	22*	L. HARTWELL
cly4	9	28	76	L. HARTWELL
cly5	7	22	76	L. HARTWELL
cly6	11	17	61	L. HARTWELL
cly7	32	60	65	L. HARTWELL
cly8	10	23	70	L. HARTWELL
cly9	16	3	16*	L. HARTWELL
let1	18	1	5*	.....
mes1	3	13	81	L. HARTWELL
nul3	20	44	69	.....
pet14	17	45	73	.....
pet18	23	10	29*	.....
pet19	98	208	68	.....
prt1	19	24	56	L. HARTWELL
prt2	13	38	74	L. HARTWELL
prt3	18	43	71	L. HARTWELL
rad2	115	237	68	M. RESNICK
rad18	13	39	75	M. RESNICK
rad52	78	39	33*	M. RESNICK
rad55	26	61	70	.....
rna1	5	11	69	L. HARTWELL
rna2	105	11	10*	L. HARTWELL
rna3	38	51	58	L. HARTWELL
rna4	10	19	66	L. HARTWELL
rna5	5	15	75	L. HARTWELL
rna8	13	25	66	L. HARTWELL
rna11	66	36	35*	L. HARTWELL
SUP30	99	6	6*	.....
SUP51	189	10	5*	.....
SUP61	21	49	70	.....
SUP71	39	2	5*	.....
SUP(his2)	12	3	20*	.....
tsm134	8	11	58	L. HARTWELL
tsm437	16	31	66	L. HARTWELL

* Percent second division segregation significantly less than 66.7 ($P < 0.05$).
† If no source is indicated, the corresponding strain was isolated by the authors.

chromosome number in yeast (MATILE, MOOR and ROBINOW 1969). Because of these uncertainties and because adequate genetic maps are an important framework for a number of genetic and molecular studies, the present extension of our previous mapping work was undertaken.

The specific objectives of this study were to (a) assign the fragments to established or new chromosomes, (b) map as many as possible of a set of conditional mutants (HARTWELL 1967) and previously identified but unmapped genes, and (c) resolve some of the uncertainties in the earlier maps.

MATERIALS AND METHODS

The new genetic markers used in these studies are listed in Table 1. Also included in this table are the centromere linkage data and sources for the various markers. A major source of new genes was a set of 35 temperature-sensitive lethals characterized by HARTWELL and coworkers (HARTWELL 1967, 1971; HARTWELL and MCLAUGHLIN 1968; HARTWELL, MCLAUGHLIN and WARNER 1970; HARTWELL, CULOTTI and REID 1970; CULOTTI and HARTWELL 1971). These mutants have been shown to affect protein synthesis (*ils, mes, prt*), RNA synthesis (*rna*), the cell division cycle (*cdc*), and cell lysis (*cly*). Other genes used in these experiments are described in our previous publications (HAWTHORNE and MORTIMER 1960, 1968; MORTIMER and HAWTHORNE 1966, 1969) or, for the radiation sensitivity genes (*rad*), in the article by GAME and Cox (1971). The gene symbols, in addition to those defined above or in text, are those proposed at the IVth International Yeast Genetics Conference, Osaka, (VON BORSTEL 1969).

To describe all the stocks used in this study would be very difficult and of limited value. Approximately 300 hybrids were constructed throughout the course of the experiments and some had quite complicated genotypes. To illustrate the types of stocks used, the genotypes of some of the principal strains are described below:

A364A *a ade1 ade2 gal1 lys2 tyr1 his7 ura1*
(Parent of all temperature-sensitive mutants)

X3103–14C *a ade1 gal1 leu2 trp1 ura3 leu1 arg4 met14 lys7 pet17* (centromere tester)

X3104–8C *a leu2 his2 his6 lys1 ilv3 met14 pet8 tyr7 ade2 rad2* (centromere tester)

X3144–11A *a leu2 trp1 arg9 his6 ilv3 met14 pet8 pet19 rad2* (centromere tester)

Z140–51C *a* $\frac{arg4\text{–}2 \quad + \quad + \quad CUP1 \quad leu1 \quad trp1 \quad trp5}{+ \quad arg4\text{–}17 \quad thr1 \quad cup1}$ *his5 ade2* (chromosome VIII disome)

The media and procedures for maintenance of stocks, construction and sporulation of hybrids, dissection of asci, and scoring of genetic characters have been described (JOHNSTON and MORTIMER 1959, HAWTHORNE and MORTIMER 1960, 1968; MORTIMER and HAWTHORNE 1966, 1969; HARTWELL 1967, HARTWELL, CULOTTI and REID 1970; HARTWELL, MCLAUGHLIN and WARNER 1970).

To place genes on the genetic maps of yeast, four different approaches were employed: tetrad analysis, random spore analysis, mitotic mapping, and trisomic analysis. The investigation of a new gene started with a tetrad analysis: firstly, to insure that we were dealing with a single locus, i.e. to verify the 2:2 segregation of the phenotype in question; and secondly, to see if the locus showed centromere linkage. If there was an indication of centromere linkage, i.e. a second division segregation frequency significantly less than 67%, the gene was paired with the markers for the 16 centromeres for a second series of tetrad analyses. With the multiply-marked test stocks constructed for this purpose, only one or two crosses were needed to place a centromere-linked gene on one of the 16 chromosomes. If the new gene did not show centromere linkage, it was

incorporated into hybrids designed for either random spore or mitotic recombination studies to detect linkage with an assortment of markers dispersed over the genome.

For the random spore analyses, the hybrids were heterozygous for an ochre-specific nonsense suppressor, an ochre allele of *can1* (canavanine resistance), and miscellaneous markers. Asci were treated with "glusulase" (Endo Laboratories, Garden City, N.Y.), sonicated, and the resultant suspension of spores and diploid cells was plated on synthetic medium containing canavanine. Because canavanine resistance was suppressible, only those spores that lacked the suppressor but carried the canavanine resistance gene were able to grow on this medium. Clones derived from these spores were then tested for the phenotypes of the other heterozygous markers. Linkage of a gene to the suppressor or to the canavanine resistance gene was detected as a departure from 1:1 segregation of this gene in the selected spore population. Linkage between two unselected markers was reflected as an excess of parental over recombinant segregants.

In the mitotic recombination analyses, new genes were screened for location on the chromosome arms that carried *ade2* (fragment 1 and XV-R), *ade4* (unmapped), *ade5* (VII-L), *ade6* (VII-R), and *ade8* (fragment 2 and IV-R). The adenine genes affect the pigmentation of the vegetative colony (EPHRUSSI, HOTTINGUER and TAVLITSKI 1949; ROMAN 1956). Homozygous *ade2* confers a red pigmentation, whereas additional homozygosity of *ade4*, *ade5*, *ade6*, or *ade8* blocks the formation of the pigment. The hybrids, therefore, were either heterozygous for *ade2* or homozygous for *ade2* and heterozygous for *ade4*, *ade5*, *ade6*, or *ade8*. Mitotic crossing over between the heterozygous signal marker and its centromere followed by appropriate centromere disjunction will result in a red-white sectored colony (ROMAN 1956, 1963). Both the red and white sectors from a number of sectored colonies were recovered and their phenotypes determined. Coincident sectoring for the new gene indicated it was on the same chromosome arm as the signal marker. X-rays (10 Krad) were used to induce the sectoring because of their relative specificity for the induction of mitotic crossing over (NAKAI and MORTIMER 1969).

A selective system for obtaining mitotic recombinants was used in the attempt to locate the ochre-specific suppressors *SUP2*, *SUP5*, and *SUP8*. The hybrids were homozygous for the ochre alleles *can1-53* and *leu2-1*, and heterozygous for the suppressor and for markers in repulsion on the various chromosome arms. Because of the presence of the suppressor the hybrid was sensitive to canavanine. Resistant clones isolated after irradiation lacked the suppressor, presumably because of mitotic recombination; the lack of the suppressor was confirmed by checking the phenotype for leucine. The phenotypes for the other markers were also determined, and if a recessive gene was expressed, it was assumed that its dominant allele was located on the same chromosome arm as the suppressor.

Trisomic analysis was used extensively in locating the fragments and, to a lesser extent, in assigning unmapped genes to particular chromosomes. Strains singly disomic for the chromosomes I, II, III, VI, VII, or VIII, or the unidentified chromosomes bearing *tyr6* (fragment 2), *ura4*, and *met4* were available. Some of these strains had been selected for other studies, while the rest were simply chance products found during previous tetrad analyses. Crosses of stocks bearing unmapped genes to these disomic strains were expected to yield asci with 4+:0—, 3+:1—, and 2+:2— ratisos for any gene located on the trisomic chromosome. In practice, hybrids were constructed with at least one known marker on the trisomic chromosome in duplex configuration (+/+/—) in order to verify the transmission of the extra chromosome. With some of the less stable disomes, the simultaneous loss of the new input marker and the signal marker for the disome provided evidence for their being on the same chromosome. The three cases involving disomes for unmapped genes required only one or two crosses to the centromere marker stocks to identify the chromosome in the disomic state. However, the crosses involving new genes and the known singly disomic stocks were seldom rewarding. An approach that proved much more efficient involved the use of multiply disomic progeny derived from triploid meioses. From 10% to 15% of the spores from triploid yeast form visible colonies and these have been shown to be disomic for from 1 to 5 chromosomes or else near diploid in constitution (PARRY and COX 1971). Crosses of the near haploid segregants with multiply marked haploids were of sufficient fertility to enable tetrad analysis to be performed. If a marker for a particular chromosome gave aberrant tetrad ratios while an unmapped gene or a marker on a given fragment segregated 2:2, or *vice*

versa, that gene or fragment was excluded from the disomic chromosome. Analysis of a number of such multiply trisomic crosses served to exclude each of the fragments from all, or all but one, of the 16 chromosomes.

RESULTS

A. *Localization of genes*

1. *Centromere-linked genes:* The numbers of first and second division segregations of the set of 48 genes which were given special emphasis in the present investigation are presented in Table 1. Similar data for the other genes used in this study are included in our earlier publications (HAWTHORNE and MORTIMER 1960, 1968; MORTIMER and HAWTHORNE 1966). Of the genes listed in Table 1, 20 show significant centromere linkage (SDS frequency less than 2/3). Two of these genes, *SUP30* and *SUP51*, were previously shown to be near the centromere of chromosome X (HAWTHORNE and MORTIMER 1968). Strains bearing the other 18 centromere markers, *cdc4*, *cdc5*, *cdc7*, *cdc8*, *cdc10*, *cdc11*, *cdc14*, *cdc15*, *let1*, *pet18*, *cly2*, *cly3*, *cly9*, *rad52*, *rna2*, *rna11*, *SUP71*, and *sup(his2)*, were each crossed to a set of strains that carried markers near the centromeres of up to 10 of the 16 chromosomes described in our earlier study (HAWTHORNE and MORTIMER 1968). At most, analysis of two such crosses served to locate a centromere-linked gene. The tetrad data that establish linkage of 17 of these genes to one of the established centromere genes are presented in Table 2. The detailed assignment of these genes to specific locations on the various chromosomes will be presented in a later section. Only one gene in this set of centromere-linked genes was not located. This gene, *cly2*, which should be within 30 cM of its centromere on

TABLE 2

Ascus-type ratios that determine linkage of the new centromere-linked genes with genes near the centromeres of the established linkage groups

Gene pair	Chromosome	Ascus type PD	NPD	T
cdc4-his2	VI	15	2	21
cdc5-lys7	XIII	20	3	32
cdc7-trp1	IV	15	0	2
cdc8-ilv3	X	21	0	20
cdc10-α	III	14	0	10
cdc11-ilv3	X	26	1	32
cdc14-his2	VI	43	0	7
cdc15-ade1	I	30	0	0
cly3-his2	VI	17	0	11
cly9-α	III	14	0	3
let1-ade1	I	11	0	1
pet18-leu2	III	42	0	6
rad52-lys7	XIII	18	2	40
rna2-pet8	XIV	73	0	9
rna11-trp1	IV	40	0	27
sup (his2)-asp5	XII	11	0	2
SUP71-ura3	V	32	0	10

TABLE 3

Ascus-type ratios (PD: NPD: T) for cly2 *in combination with centromere markers on the established linkage groups*

Centromere marker	Chromosome	Ascus type PD	NPD	T
ade1	I	19	19	44
gal1	II	25	13	65
leu2	III	11	10	34
trp1	IV	20	20	52
ura3	V	5	5	10
his2	VI	1	2	10
leu1	VII	11	12	26
arg4	VIII	10	12	25
his6	IX	10	6	36
ilv3	X	7	6	33
met14	XI	14	4	28
asp5	XII	11	11	32
lys7	XIII	8	17	29
pet8	XIV	13	4	33
pet17	XV	6	7	27
tyr7	XVI	3	4	6

the basis of its SDS frequency fails to show the degree of linkage to any of the centromere markers of chromosomes I to XVI that would be expected if it was located on one of these chromosomes (Table 3). This implies either that *cly2* marks the centromere of a new chromosome or that the data that indicate that this gene is centromere-linked are statistically significant ($0.02 < P < 0.05$) only as a result of expected sampling fluctuations. Study of additional crosses would be required to discriminate between these possibilities.

Five additional centromere-linked genes have been identified and located by other investigators: *ils1* on chromosome II (McLAUGHLIN and HARTWELL 1969), *leu*^{R} and *ilv2* on chromosome VII (MAGEE, in press), *rad1* = *uvs1* on chromosome XVI (RESNICK 1969; GAME and COX 1971), and *rad5* = *uvs10* = *rev2* on chrosmosome XII (SNOW 1967; LEMONTT 1971; GAME and COX 1971). These genes will also be added to the map.

2. *Random spore analysis:* Random spore analysis, carried out according to the method described above (MATERIALS AND METHODS), was used principally to test for linkage of genes to the eight tyrosine-inserting suppressors (HAWTHORNE and MORTIMER 1968; GILMORE, STEWART and SHERMAN 1971). Five of these genes had been mapped: *SUP6* and *SUP11* on CHR. VI, *SUP4* and *SUP7* on CHR. X, and *SUP3* on CHR. XV. *SUP2* and *SUP8* were located on the right arms of CHR. IV and CHR. XIII, respectively, as part of this study (see following section), and *SUP5* is unmapped. Several combinations of suppressors with the temperature-sensitive lethals were tested: *cdc1, 2, 3, 6, 9,* and *13 v.s. SUP2, 3, 4, 5, 6, 7, 8,* and *11*; *prt1, 2, 3, rna1, 2, 3, 4, 5, 8,* and *11 v.s. SUP5* and *8*; *cdc7, 8, 10, 12, cly3, 4,* and *8 v.s. SUP6*. Miscellaneous genes in these crosses were also examined for linkage to the selected markers or to the conditional lethals. A minimum of 40

TABLE 4

Random spore analysis

Gene pair	Spore genotype	
	Parental	Recombinant
can1-ura3	578	308
can1-prt3	69	11
can1-cdc1	34	6
SUP11-cdc4	101	19
SUP6-cly3	41	13
SUP8-rna1	82	38
SUP5-pet17	448	335

spore clones was analyzed for each diploid. Linkage of the unselected markers to either the suppressor or the canavanine gene could be revealed by these tests and seven cases of linkage were found in the 138 combinations tested (Table 4): *can1-ura3*, *can1-prt3*, *can1-cdc1*, *SUP11-cdc4*, *SUP6-cly3*, *SUP8-rna1*, and *SUP5-pet17*. Two of these linkages had already been predicted from the tetrad analysis results. Both *cdc4* and *cly3* had been located on CHR. VI on the basis of their linkage to *his2* (Table 2) and the two suppressors were already known to be on this chromosome (HAWTHORNE and MORTIMER 1968). The linkage of *ura3* (CHR. V-L) to *can1* was also confirmed by tetrad analysis (Table 8). The remaining linkages have not as yet been confirmed by other methods.

3. *Mitotic segregation analysis:* Most of the conditional mutants that failed to show centromere linkage were screened for mitotic linkage to *ade2*, *ade4*, *ade5*, *ade6*, and *ade8* as described in MATERIALS AND METHODS. Four combinations showed mitotic linkage: *prt1-ade2*, *tsm437-ade5*, *rna3-ade8*, and *cly8-ade6* (Table 5). With the exception of *prt1-ade2*, these linkages have been confirmed by tetrad analysis (Table 8).

We expected that mitotic analysis would be more efficient for the location of the suppressors, *SUP2*, *SUP5*, and *SUP8*, since it was possible to select for loss of the suppressor and then screen these mitotic segregants for simultaneous homozygosity of markers on the various chromosome arms (see MATERIALS AND METHODS). Only one cross (involving *met8* on CHR. II-R, *ade5,7* on CHR. VII-L, *lys1* on CHR. IX-R, and *tyr6* on CHR. IV-R) was required to locate *SUP2* on the right arm of chromosome IV. This cross gave 12 *tyr6/tyr6* homozygotes from a sample of 32 suppressor-less recombinants. Eight crosses with markers covering twenty chromosome arms were analyzed before *SUP8* was located on CHR. XIII-R. A sample of 83 suppressor-less recombinants included 16 that were *lys7/lys7* homozygotes. Twelve crosses with heterozygous markers on 28 arms have been examined without success in the attempt to locate *SUP5* (Table 6). Random spore results (Table 4) suggest that this suppressor may be on chromosome XV. However, there is no concomitant sectoring of this suppressor with either the right arm (*ade2*) or the left arm (*SUP3A*) markers to support this observation.

Miscellaneous examples in which two genes were placed on the same chromo-

*Mitotic segregation analysis: concomitant segregations of temperature-sensitive lethals with adenine genes**

Locus	Selected marker *ade2*	*ade4*	*ade5*	*ade6*	*ade8*
cdc1	0/11	0/3	0/11	0/1	0/23
2	0/3	0/1	0/7	0/1	0/117
3	0/4	...	...	0/3	0/28
4	...	0/5	...	...	...
6	0/11	0/6	0/11	...	0/25
8	0/4	0/7	0/17	0/4	0/17
9	0/5	0/1	0/6	0/3	1/28
10	...	0/6	...	...	...
11	0/4	0/7	0/5	0/2	...
13	0/6	0/13	1/6	0/3	1/32
cly1	0/12	...	0/2	0/4	0/27
2	0/8	0/15	0/9	0/4	0/23
4	0/9	...	1/6	0/3	0/31
5	0/7	0/8	0/7	0/2	0/34
6	0/6	0/4	0/9	0/6	0/29
7	0/6	0/3	0/6	0/7	0/28
8	0/6	0/9	0/3	52/71	0/25
prt1	11/23	...	0/10	0/3	1/26
2	1/17	0/14	0/16	0/5	0/28
3	0/17	1/34	0/10	0/9	0/34
tsm437	0/12	0/6	5/17	0/11	...
rna1	0/17	0/4	1/12	0/8	...
2	...	0/13	...	...	...
3	1/18	...	0/8	0/6	10/18
4	0/28	0/7	...	0/13	...
5	...	0/6	0/4	0/6	0/28
8	1/30	0/8	0/3	0/6	0/21
11	0/24	0/12	0/5	0/7	0/114

* The ratios represent the number of concomitant segregation of the mutant locus and the selected marker divided by the total number of sectored colonies tested.

some through mitotic analysis are *met4-lys10*, and *prt2-met2*. Trisomic analyses as well as tetrad analysis have indicated that these 4 genes as well as *pha2* and *pet2* lie on the same chromosome (see 4a below). The meiotic data established the sequence *pha2-met2-pet2-prt2* but the centromere, *met4* and *lys10* could not be placed in this sequence. The mitotic data, however, place *met4* and *lys10* on the arm opposite from the above cluster with *met4* distal: *met4* and *lys10* sectored independently with respect to these genes, and of 89 colonies sectored for *met4* only 22 were also sectored for *lys10*. For the other arm, the order *c-prt2-pet2-met2-pha2*, determined by mitotic analysis, is the preferred one. The set of fragment 4 genes, *SUC2*, *his5*, and *lys11* were placed on CHR. IX by analysis of disomic strains. Mitotic analysis revealed that this group of genes was on the *his6* rather than the *lys1* arm of this chromosome. Auxotrophic sectors from a *his6 lys1 suc/HIS6 LYS1 SUC2* diploid were tested; 7 *lys1/lys1* sectors were all

TABLE 6

Mitotic segregation analysis: concomitant segregations of chromosome markers with SUP5 *and* SUP8

Marker	Chromosome	Selected marker *SUP5*	*SUP8*
ade1	I-R	1/44	....
tyr1	II-R	1/111	0/31
ils1	II-L	0/40	0/5
thr4	III-R	0/44	0/112
his4	III-L	0/140	0/309
tyr6	IV-R	0/194	0/85
ilv1	V-R	2/327	2/223
ura3	V-L	0/116	....
met10	VI-R	0/40	0/5
ade6	VII-R	0/177	0/138
ade5,7	VII-L	2/160	0/112
lys1	IX-R	0/34	0/85
his5	IX-L	0/111	0/31
ilv3	X-R	2/77	....
SUP7	X-L	0/57	1/58
ura1	XI-R	0/40	0/117
met1	XI-L	0/116	....
gal2	XII-R	2/121	0/112
lys7	XIII-R	2/76	16/83
lys9	XIV-R	1/58	2/54
ade2	XV-R	2/150	1/85
SUP3A	XV-L	0/108	....
tyr7	XVI-R	1/106	0/112
met2	XVII-R	2/44*	....
met4	XVII-L	0/140	0/197
gal5	Frag. 6	1/116	....

* Could be either *met2* or *met8* homozygous.

sucrose fermenters while 3 of 4 *his6/his6* sectors were sucrose non-fermenters (*suc/suc*).

A surprising observation arose in a similar analysis with chromosome IV in the attempt to locate the fragment 2 genes, *aro1*, *hom2*, etc. with respect to *trp1*, *gal3*, and the centromere. Since *trp1* and *gal3* are tightly centromere-linked a strong selection system was deemed necessary. In this case, *gal3/gal3* homozygotes were selected on galactose-ethanol medium from a *gal3 gal10 trp1 aro1/+ gal10 + +* diploid. Homozygosity for *gal10* results in poisoning by the galactose and this is relieved by homozygosity for *gal3* (DOUGLAS and HAWTHORNE 1964). A sample of 108 *gal3/gal3* isolates included 35 that were *trp1/trp1*, 70 that were *trp1 aro1/trp1 aro1*, and 3 that were *aro1/aro1*. We surmise that homozygosity for *gal3* can arise either by mitotic crossing over or by gene conversion. Those clones homozygous for *aro1* can be explained by crossovers either in the centromere–*trp1* or the *trp1–gal3* regions. The remaining 79 clones must involve conversion for *gal3* or for *gal3* and *trp1*.

4. *Disomic and aneuploid analyses:*

a. *Singly disomic strains:* Strains which have arisen as meiotic products of diploids, and which have been identified to be disomic for a particular chromosome, were initially assumed to be haploid for the remaining chromosomes. However, it was found that such strains have a tendency to either lose the extra chromosome or to become disomic for additional chromosomes. To contend with this possible source of error, the hybrids were constructed, when possible, with a signal marker in duplex configuration on the trisomic chromosome and with a number of markers on the other chromosomes which had been introduced by the haploid parent. Irregular segregations for the signal marker coupled with 2:2 segregations for the markers on the other chromosomes established that the chromosomal constitution of the hybrid was as expected. Irregular segregation of an unmapped gene from the haploid parent indicated that it was on the same chromosome as the signal gene.

Two classes of singly disomic strains were available: (1) those disomic for one of the established chromosomes, and (2) those disomic for one of the fragments or for the chromosome bearing an unmapped gene. The first class included strains disomic for chromosomes I, II, III, VI, VII, or VIII, while the second class represented the unidentified chromosomes bearing *tyr6* (fragment 2), *ura4*, and *met4*.

The first class of disomic strains was used in crosses with unmapped genes or with genes on the fragments. However, because of its low efficiency in locating genes this approach was used in only a limited fashion. The chromosome I disome was crossed to a strain bearing genes on fragments 3, 4, and 5. The gene *ade1* located on chromosome I showed trisomic segregation, whereas the fragment markers *met2* (F3), *his5* (F4), and *trp3* (F5) all segregated 2:2. The results exclude these three fragments from chromosome I. In a similar fashion, these three fragments were excluded from chromosome VI. The chromosome VIII disome, which was heterozygous for *thr1* on this chromosome, was crossed to strains carrying *cdc1* to *cdc13* and *cly1* to *cly8*. In all 21 crosses *thr1* segregated as expected from a trisome, whereas the temperature-sensitive mutants segregated 2:2 in all but one cross which involved *cdc12*. Accordingly, all of the above set of genes except *cdc12* are excluded from this chromosome. Tetrad analysis results that confirm the location of *cdc12* on chromosome VIII are presented in a following section.

The second class of singly disomic strains proved to be more useful in mapping. These three strains were disomic for the chromosomes carrying *tyr6* (fragment 2), *met4* and *ura4*, respectively. In each, the markers gene was heterozygous. Each strain was crossed to a set of strains that collectively carried genes on the 16 established chromosomes. Trisomic segregations for the unmapped gene and for one of the chromosome markers established the location of the gene on that chromosome. In this fashion, *tyr6* (fragment 2) was assigned to chromosome IV and *ura4* to chromosome XII. However, in crosses involving the *met4* disome, the markers for the sixteen chromosomes segregated normally. These results exclude *met4* from chromosome I to XVI and indicate a seventeenth chromosome. However, the fragment 3 gene *met2* gave trisomic ratios in crosses with the *met4* disome.

b. *Multiply disomic strains:* To associate the remaining fragments with established chromosomes, multiply disomic strains derived from meioses of triploids were crossed to strains carrying genes on a number of chromosomes as well as on many of the fragments. Irregular segregation of a chromosome marker in a cross in which a fragment marker segregated 2:2, or *vice versa*, excluded the fragment from that chromosome. Because of the multiply trisomic nature of these crosses, coincident aneuploid segregations for a chromosome marker and a fragment marker provided a less definite indication of association. The results of a number of such crosses are summarized in Table 7. Fragment 1 was excluded from all except chromosome XV. In no cross was either this fragment or chromosome XV trisomic. The proximal gene, *ade2*, on fragment 1 was checked for linkage to the gene *pet17* which marks the centromere of chromosome XV and positive linkage was established (Table 8). The assignment of fragment 2 to chromosome IV was described above. Fragment 3 marked by *met2* was independently excluded from chromosomes I to XVI. This independent result was another reason for the assignment of genes of this fragment and *met4-lys10* to chromosome XVII. Fragment 4, marked by *his5*, was excluded from all but chromosome IX. In four crosses, coincident trisomy for *his5* and the chromosome IX genes, *his6* and/or *lys1*, was observed, providing a strong indication that fragment 4 and chromo-

TABLE 7

Disome exclusion results

	ade2 F1	*met2* F3	*his5* F4	*met1-ura1* F5	*rad2-mes1* F8	*met4-lys10*
I	X* (1)	X (2)	X (2)	X (1)	.. ...	X (1)
II	X (3)	X (5)	X (3)	X (3)	.. ...	X (2)
III	X (2)	X (2)	X (4)	X (2)	.. ...	X (3)
IV	X (1)	X (1)	X (2)	X (1)	.. ...	X (2)
V	X (1)	X (1)	X (1)	X (1)	.. ...	X (2)
VI	X (1)	X (1)	X (2)	X (2)	.. ...	X (1)
VII	X (4)	X (2)	X (6)	X (4)	.. ...	X (2)
VIII	X (4)	X (3)	X (5)	X (5)	.. ...	X (1)
IX	X (4)	X (4)	0† (4)	X (3)	X (1)	X (2)
X	X (2)	X (2)	X (4)	X (2)	.. ...	X (1)
XI	X (2)	X (4)	X (2)	0 (4)	X (2)	X (1)
XII	X (1)	X (4)	X (1)	X (3)	.. ...	X (1)
XIII	X (1)	X (1)	X (1)	X (2)	.. ...	X (1)
XIV	X (3)	X (1)	X (4)	X (1)	X (1)	X (1)
XV	.. ...	X (5)	X (2)	X (2)	.. ...	X (3)
XVI	X (2)	X (2)	X (4)	X (1)	.. ...	X (1)
F1	.. ...	.. ...	.. ...	.. ...	.. ...	.. ...
F2	.. ...	.. ...	.. ...	.. ...	.. ...	.. ...
F3	X (4)	.. ...	X (2)	X (3)	.. ...	0 (3)
F4	X (2)	X (1)	.. ...	.. ...	.. ...	X (1)
F5	X (2)	X (2)	X (2)	.. ...	X (1)	X (2)

* X indicates that either the fragment marker or chromosome marker, but not both, gave trisomic segregations. Number in parentheses is the number of crosses in which exclusion was observed.

† 0 indicates co-disomy of fragment and chromosome markers. Number in parentheses is the number of crosses in which co-disomy was observed.

TABLE 8

Tetrad data indicating gene to gene linkage

Linkage group	Gene pair	PD	NPD	T	Reference
I	*ade1-cdc15*	30	0	0	
	let1-ade1	11	0	1	
II	*pet9-ils1*	30	0	23	McLAUGHLIN and HARTWELL 1969
	gal1-tsm134	8	0	11	
	lys2-tsm134	11	0	8	
	glc1-lys2	22	0	14	PRINGLE 1972, pers. com.
	glc1-tyr1	28	0	8	PRINGLE 1972, pers. com.
	his7-rna5	15	0	5	
III	*α-cdc10*	14	0	10	
	α-pet18	42	0	6	
	leu2-pet18	22	0	10	
	α-cly9	14	0	3	
	α-rad18	29	1	22	
	α-SUP61	35	3	53	
IV	*trp1-cdc2*	23	3	75	
	trp1-cdc7	15	0	2	
	trp1-nul3	14	6	44	
	trp1-rad55	24	2	61	
	trp1-rna11	60	0	30	
	aro1-nul3	52	0	31	
	aro1-rad55	8	3	7	
	aro1-rna11	7	9	22	
	rad55-rna11	6	0	16	
	aro1-hom2	57	0	5	
	hom2-pet14	19	0	40	
	SUP35-pet14	20	0	20	
	pet14-SUP2	33	0	27	
	SUP2-trp4	17	0	46	
	asp1-trp4	136	1	68	JONES and MORTIMER 1970
	trp4-ade8	31	1	29	
	ade8-rna3	23	2	36	
V	*ura3-SUP71*	32	0	10	
	ura3-can1	31	5	60	
	his1-SUP20	37	0	1	
VI	*his2-cdc4*	15	2	21	
	cdc4-SUP11	13	0	3	
	his2-cly3	17	0	11	
	his2-cdc14	43	0	7	
	his2-SUP6	42	0	8	
	cdc14-SUP6	46	0	4	
	SUP6-met10	52	0	3	
VII	*cyh2-tsm437*	25	0	0	
	ade6-cly8	27	0	17	
	leu1-cly8	10	2	25	

TABLE 8—Continued

Linkage group	Gene pair	PD	NPD	T	Reference
	leu1-leu^R	31	0	8	MAGEE 1973
	leu^R-ilv2	29	0	8	MAGEE 1973
VIII	*arg4-SUP31*	8	0	7	
	pet3-cdc12	7	0	4	
	thr1-cdc12	3	0	9	
IX	*his6-his5*	14	7	40	
	his6-SUC2	17	17	63	
X	*ilv3-SUP30*	58	0	15	
	ilv3-SUP51	59	0	18	
	SUP30-SUP51	42	0	0	
	ilv3-cdc8	21	0	20	
	ilv3-cdc11	26	1	32	
	cdc8-cdc11	17	0	10	
	cdc8-SUP4	17	0	0	
XI	*met14-met1*	38	5	66	
	met14-SUP25	17	2	34	
	met14-ura1	44	48	182	
	met1-SUP25	97	0	17	
	SUP25-MAL4	40	0	15	
	met1-MAL4	71	3	59	
XII	*asp5-rev2*	80	0	7	
	asp5-sup(his2)	11	0	2	
XIII	*lys7-cdc5*	20	3	32	
	lys7-rad52	18	2	40	
XIV	*pet8-rna2*	73	0	9	
	pet8-lys9	23	3	40	
	lys9-rna2	27	3	36	
XV	*pet17-ade2*	64	10	158	
	ade2-prt1	8	4	19	
XVI	*tyr7-rad1*	16	3	19	RESNICK 1969
XVII	*pet2-prt2*	33	0	0	
	pet2-met2	96	0	55	
	met2-pha2	57	4	85	
	pet2-pha2	48	8	95	
F6	*gal5-SUP50*	17	0	1	
	gal5-thi1	55	0	8	DOUGLAS and HAWTHORNE 1964
F8	*mes1-rad2*	14	0	3	
F9	*i-gal-SUP5*	20	0	0	DOUGLAS and HAWTHORNE 1972

some IX are associated. This was confirmed by a mitotic segregation analysis as described in the preceding section. Fragment 5 marked by *met1*, *ura1* and *trp3* was excluded from all but chromosome XI. In three crosses, *met14*, located on chromosome XI, and the fragment 5 genes, showed coincident trisomy. This observation was confirmed by a tetrad analysis that demonstrated linkage between *met14* and *met1* (see below).

B. *Chromosome maps*

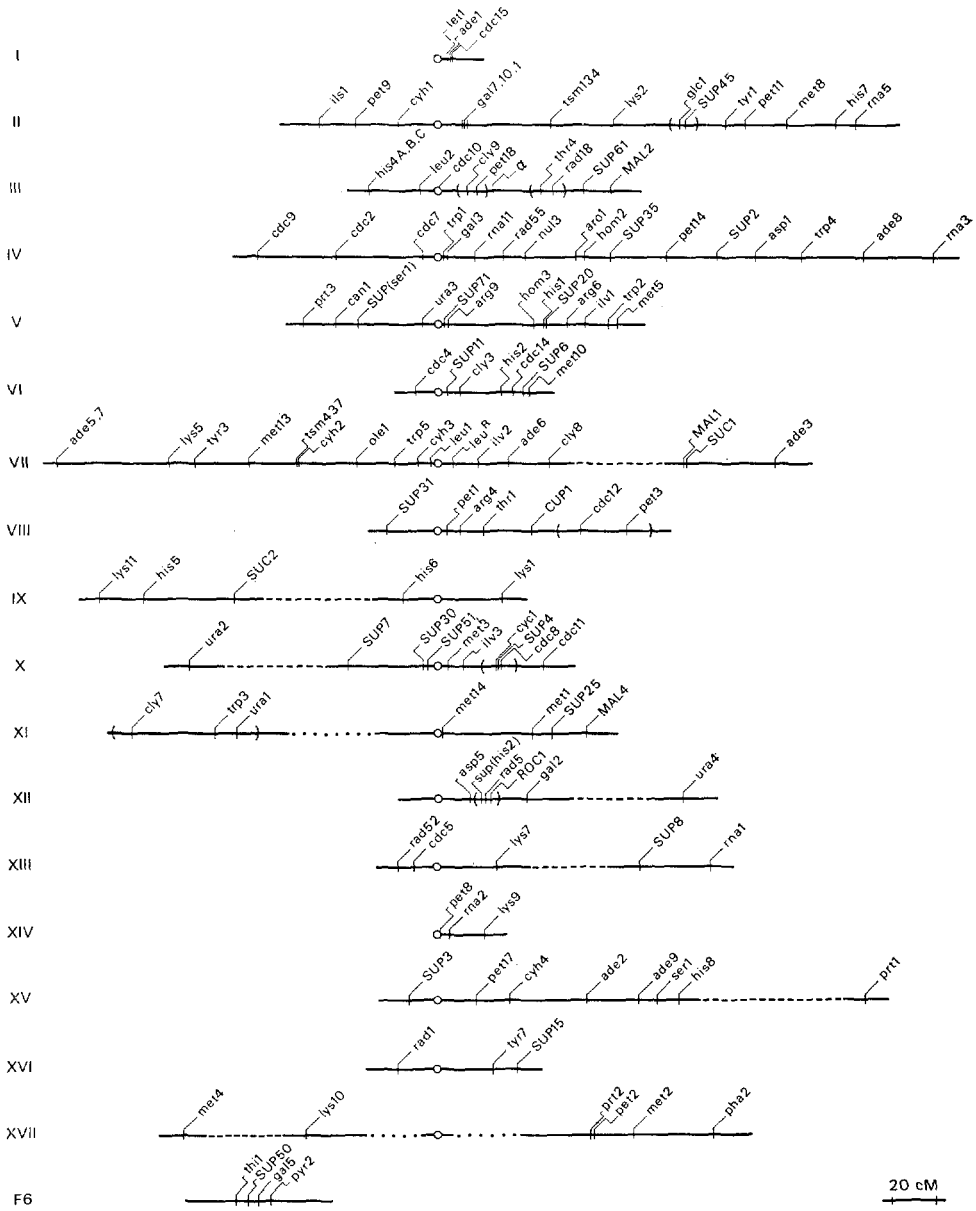
The genetic maps of *Saccharomyces cerevisiae* presented in Figure 1 represent an extension of our previous maps with the inclusion of the above results. Distances were calculated according to standard methods, and uncertainties in sequence, where they exist, are indicated. The assignment of genes to specific locations on the various chromosomes is presented below:

CHR. I: Two new genes have been placed on chromosome I—*cdc15* and *let1*. Because of the close linkages involved, their sequence is unknown except that *let1* is proximal to *ade1* or on the other arm since the one tetratype ascus was FDS for *let1* and SDS for *ade1*; no exchanges have been obtained between *ade1* and *cdc15*.

CHR. II: The four genes added to chromosome II are *ils1*, *tsm134*, *glc1*, and *rna5*. McLAUGHLIN and HARTWELL (1969) have located *ils1* distal to *pet9* on the left arm of this chromosome. On the right arm, the temperature-sensitive lethal *tsm134* was positioned between *gal1* and *lys2* on the basis of a tetrad analysis involving these three genes (Table 8). The glycogen storage gene, *glc1* has been positioned by PRINGLE (1972) about midway between *lys2* and *tyr1* but its position relative to *SUP45*, also located in this interval, is unknown. The distal gene on this chromosome arm is *rna5*. Its position was assigned from an examination of its segregation relative to *his7* and *tyr1*.

CHR. III: The addition of five new genes to chromosome III presents us with additional uncertain sequences. Only FDS tetrads have been obtained for *cdc10*, so it could be on either the right or left arm. The genes *cly9* and *pet18* are on the right arm proximal to α; they have not been tested against one another. The radiation sensitivity gene, *rad18* (*uxs1*) is distal to α about the same distance as *thr4* but has not been ordered with respect to this gene. *SUP61* is located distal to these two genes but has not been ordered relative to *MAL2* which is in the same region. On the basis of its distance from α, *SUP61* is tentatively placed proximal to *MAL2*.

CHR. IV: Besides the genes formerly assigned to fragment 2, ten additional genes have been located on chromosome IV. The fragment 2 genes were placed on the right arm distal to *trp1* and *gal3* (see section 3) in the sequence *aro1-hom2-SUP35-trp4-ade8*. Tetrad analyses allowed the placement of *nul3* proximal to *aro1*. Similarly, the genes *pet14*, *SUP2*, and *asp1* were located in that order between *SUP35* and *trp4*, and *rna3* was found to be distal to *ade8*. The three temperature sensitive mutants *cdc7*, *rna11*, and *cdc2* show progressively less linkage to the centromere marker, *trp1*. Neither *rna11* or *cdc2* show meiotic linkage to the proximal marker of fragment 2, *aro1* (Table 8). The cell division



GENETIC MAP OF SACCHAROMYCES

FIGURE 1.—Linkages established by tetrad or random spore analysis are represented by solid lines; those determined by mitotic and trisomic analysis are indicated by dashed and dotted lines, respectively. The sequence of genes within parentheses has not been determined relative to outside markers.

mutant *cdc7* is close to *trp1* and in a cross involving these two genes and *rna11* one crossover ascus favored the sequence *cdc7*–centromere–*trp1*–*rna11*. The gene *rad55* which confers sensitivity to X-rays also is loosely linked to *trp1*. It is not linked to *cdc2* but is linked to *rna11*. In contrast to *cdc2*, *rad55* sectors mitotically with *ade8*. Of 173 colonies sectored for *ade8*, eleven were also sectored for X-ray sensitivity conferred by homozygosity for *rad55*. All 11 colonies sectored for *rad55* were also sectored for *aro1* but 22 additional *aro1* sectors did not involve *rad55*. This establishes that *rad55* is in the *trp1*–*aro1* interval on the right arm of CHR. IV. Because of its failure to sector with *ade8*, *cdc2* is placed on the left arm. Meiotic analysis favors placement of *rna11* between *trp1* and *rad55* and its failure to sector with *ade8* can be explained by its relative proximity to the centromere on this exceptionally long arm.

CHR. V: A number of uncertainties arise in the attempt to position the new genes on CHR. V. The canavanine resistance gene, *can1*, was located on the left arm of this chromosome on the basis of its linkage to *ura3* and its lack of linkage to markers on the right arm. However, its position relative to *SUP(ser1)* is uncertain because these two genes have not been studied in the same cross. It is tentatively placed distal to *SUP(ser1)* because of its greater map distance from *ura3* (Table 8, MORTIMER and HAWTHORNE 1966). The temperature-sensitive lethal *prt3* showed linkage to *can1* by random spore analysis (Table 4). Analysis of the segregation of these two genes relative to *ura3* led to the conclusion that *prt3* is distal to *can1*. Thus the tentative left arm sequence is centromere–*SUP(ser1)*–*can1*–*prt3*. The position of *cdc1* in this sequence is unknown since only random spore linkage of this gene to *can1* has been determined (Table 4). Additions to the right arm of CHR. V involve a centromere-linked UGA suppressor, *SUP71* and the weak ochre suppressor *SUP20* formerly located on fragment 1 (now XV-R). The position of *SUP71* with respect to *arg9* has not been determined. There was only one exchange in 38 tetrads between *SUP20* and *his1* and in this tetratype ascus *SUP20* and *ilv1* were in a parental ditype array. Thus *SUP20* is placed distal to *his1*. The gene *met5* had been mapped distal to *trp2* in another study (LINDEGREN *et al.* 1962).

CHR. VI: Three genes have been added to the chromosome VI map—*cdc4*, *cly3*, and *cdc14*. *cdc4* was placed on a new left arm of this chromosome from an examination of its second division segregation frequency and its segregation relative to *SUP11* and *his2* (Tables 1, 2 and 8). On the right arm, *cly3* has been located between *SUP11* and *his2* as a consequence of its distances from the centromere and *his2*. The cell-division-cycle mutant *cdc14* was located between *his2* and *SUP6*, and *met10* was positioned distal to this suppressor from a tetrad analysis of a cross heterozygous for all four genes. This analysis was complicated by the high frequency of gene conversion encountered in the *his2*–*SUP6* interval (HURST, FOGEL and MORTIMER 1972). Of 62 complete tetrads analyzed, only 36 segregated 2:2 for all four genes. Assuming the sequence centromere–*his2*–(I)–*cdc14*–(II)–*SUP6*–(III)–*met10*, two of these 36 asci are explained by a crossover in region I, two by a crossover in region II, one by a crossover in III, and one by a three-strand double in regions I and II. Any other sequence would require additional multiple crossovers to explain the findings.

CHR. VII: Two temperature-sensitive lethals have been added to the map of this chromosome. *tsm437* showed mitotic linkage to *ade5* and subsequently was shown to be tightly linked to *cyh2*, also located on the left arm of VII (Tables 5 and 8). No exchanges were observed between these two genes in 25 asci. Distal to *ade6* on the right arm is *cly8*. Again both mitotic and meiotic results support this positioning (Tables 5 and 8). Two other genes, *leu*^{R} and *ilv2*, have been located by MAGEE (1972) in the centromere–*ade6* region in the sequence centromere–*leu*^{R}–*ilv2*–*ade6*. The arrangement of the other genes on this chromosome remains as described in our earlier publication (HAWTHORNE and MORTIMER 1968).

CHR. VIII: Although one of the incentives for initiating the present study was to map in greater detail the region around *arg4* because of previous work on this gene (FOGEL and MORTIMER 1969; HURST, FOGEL and MORTIMER 1972) only one additional gene was located on this chromosome and it was far removed from *arg4*. The cell cycle mutant *cdc12* was located on VIII by trisomic analysis and has been shown to exhibit linkage to *pet3* (Table 8). However the order of these two genes relative to the centromere was not determined.

CHR. IX: Fragment 4, which contains the genes *SUC2*, *his5*, and *lys11*, was located on the left arm of this chromosome by trisomic and mitotic analyses (sections 3 and 4, Table 7). Our earlier results (MORTIMER and HAWTHORNE 1966) indicated that *SUC2* was the proximal gene of fragment 4. Thus the left arm sequence is centromere–*his6*–*SUC2*–*his5*–*lys11*. Significant meiotic linkage between *his6* and *SUC2* (formerly *SUC1*), however, does not exist. The ascus type ratios for these two genes are 17:17:63 (HAWTHORNE and MORTIMER 1960).

CHR. X: Two cell division cycle mutants, *cdc8* and *cdc11*, have been placed on the right arm of this chromosome; *cdc8* is closely linked to *SUP4* while *cdc11* is distal. Also closely linked to *SUP4* and *cdc8* is the structural gene for iso-l-cytochrome-c, *cyc1* (SHERMAN, personal communication). On the left arm, *SUP30* and *SUP51* are closely linked with no reciprocal exchanges in 42 tetrads. In one ascus, *SUP30* segregated 3:1 while *SUP51* segregated 2:2; hence these two suppressors are not alleles. The tentative order of genes, based on SDS frequencies, is centromere–*SUP51*–*SUP30*. The gene *ura2* was assigned to chromosome X by trisomic analysis (LACROUTE, personal communication). This gene sectors concomitantly (9/14) with *SUP7* located on the left arm of this chromosome. Because *ura2* is not centromere-linked it is placed distal to *SUP7*.

CHR. XI: Trisomic analysis (Table 7) was used to locate fragment 5 on this chromosome which was formerly identified only by the centromere-linked gene, *met14*. The standard markers for this fragment, *met1* and *ura1*, were examined for linkage to *met14* and the *met14-met1* combination showed significant meiotic linkage. Analysis of a cross heterozygous for *met14*, *met1*, *SUP25*, and *MAL4* revealed that these four genes were arranged in that sequence (Table 8). A second cross yielded one exchange in 45 tetrads between the centromere and *met14*. In this ascus, *met14* and *met1* were parental ditype. Hence *met14* is placed on the right arm in the sequence centromere–*met14*–*met1*–*SUP25*–*MAL4*. No linkage between *met14* and *ura1* was revealed. Mitotic analysis showed that *met1* and *ura1* sector independently and, hence, *ura1* and the genes *trp3* and *cly7*

which are linked to *ura1* have been assigned to the left arm of CHR. XI. However, the sequence of these three genes relative to each other or to the centromere is unknown, with the exception that *cly7* is not located between *trp3* and *ura1*.

CHR. XII: Two new centromere-linked genes, *sup(his2)* and *rad5* are located on chromosome XII. *rad5* (formerly *uvs-10* and *rev2*) was located by LEMONTT (1971), while *sup(his2)* was mapped during the present study. This recessive suppressor, isolated as part of this study, may be an allele of one of the recessive missense suppressors described by GORMAN and GORMAN (1971). Both *sup(his2)* and *rad5* show close linkage to *asp5* and both are placed distal to this gene from an examination of exchanges in asci from crosses heterozygous for the two gene combinations. However, the sequence of *sup(his2)*, *rad5*, and *ROC1*, which are all located in the *asp5–gal2* interval, is unknown. Analysis of a disome for the chromosome that carries *ura4* revealed that this gene is also on CHR. XII (section 4). Mitotic sectors for *ura4* were recovered from a cross heterozygous for both *gal2* and *ura4*. Of 100 colonies sectored for *ura4*, 7 were sectored for *gal2*. This places *ura4* distal to *gal2*.

CHR. XIII: The two new genes on the left arm of this chromosome, *cdc5* and *rad52* (*xs1*), have not been included in the same cross; the tentative order *rad52–cdc5*–centromere is based on SDS frequencies (Table 1). The location of *SUP8* distal to *lys7* on the right arm was by a mitotic analysis (section 3, Table 6). Random spore analysis indicated linkage between *rna1* and *SUP8* (Table 4).

CHR. XIV: Only one additional gene was positioned on this chromosome. The temperature-sensitive lethal *rna2* was found to be linked to *pet8*. Analysis of a cross heterozygous for *pet8*, *rna2*, and *lys9* revealed that *rna2* is in the middle. Of a sample of 53 asci, 7 had an exchange between *pet8* and *rna2*; 5 of these 7 asci were in a PD array for *rna2* relative to *lys9*, the other two asci had an additional exchange between *rna2* and *lys9*. Because exchanges between centromere XIV and *pet8* are rare, it is unknown whether *pet8* is on the same side of the centromere as the other two genes.

CHR. XV: Disomic studies assigned the genes of the former fragment 1 to chromosome XV and they were placed on the right arm by the linkage between *pet17* and *ade2*. A mitotic analysis placed *prt1* on the right arm also (Table 5); the tetrad data for *prt1* against *ade2* indicate that it is distal to and far removed from *ade2*; it has not been tested for linkage with the other markers on this arm. Random spore analysis suggested linkage of *SUP5* to *pet17* (Table 4). However, mitotic analysis failed to reveal sectoring of this suppressor with either *ade2* (XV-R) or *SUP3A* (XV-L) and hence *SUP5* was not placed on this chromosome. One other revision from our previous map places *SUP20* on CHR. V (see above). The tetrad data of this suppressor with *ade2* now stands at 22 PD:7 NPD:58 T.

CHR. XVI: No new genes were found to be on this chromosome. However, we have added to the map the gene *rad1* (*uvs1*) which was located by RESNICK (1969).

CHR. XVII: The gene *rad2* (*uvs9*) was proposed by RESNICK (1969) to mark the centromere of this chromosome. This gene was included in a number of crosses involving centromere-linked genes and the original evidence that it was centromere linked, and hence established a new chromosome, could not be con-

firmed. In 352 asci, the percent SDS for *rad2* was 67.3 (Table 1). This chromosome number has been reassigned to the sequence of genes *met4–lys10*–centromere–*prt2–pet2–met2–pha2* described in the above section on mitotic segregation analysis (section 3). An uncertainty in this sequence is in order of *pet2* and *prt2* relative to the other genes. In 33 tetrads, no exchanges were observed between these two genes. It is possible that *cly2* is linked to the centromere of this chromosome (Table 3) but no data are available to establish this point.

Fragment 6: None of the mutants in this linkage group result in a clear recessive phenotype and hence disomic studies to assign this fragment to a centromere were precluded. There is close linkage between *SUP50* and *gal5*, formerly placed on fragment 8 (MORTIMER and HAWTHORNE 1966). Although it has not been tested, it is likely that the thiamine genes designated *thi1* and *thi2* are alleles or identical; therefore another linkage group will be assigned to fragment 8.

Fragment 7: The closely linked genes controlling the fermentation of sucrose *SUC3*, maltose *MAL3*, and alpha-methyl glucoside *MGL2* (MORTIMER and HAWTHORNE 1966) were not included in this investigation.

Fragment 8: The methionyl synthetase mutant *mes1* was found to be linked to *rad2* (*uvs9*) but neither gene has been associated with an established chromosome.

Fragment 9: The repressor locus for galactose utilization *i-gal* is tightly linked to *SUP5* (DOUGLAS and HAWTHORNE 1972). This suppressor has been excluded from most of the chromosome arms by the results of the mitotic analyses (Table 6).

DISCUSSION

These experiments establish that seventeen is the minimum number of chromosomes in the haploid genome of the yeast *Saccharomyces cerevisiae*. Sixteen of these chromosomes had been characterized in previous mapping studies and their independence was confirmed in numerous crosses involving either tetrad or trisomic analysis. Twenty-one new centromere-linked genes were examined and all except one were located on various one of these sixteen chromosomes. The seventeenth chromosome was identified by trisomic analysis and no markers were found near its centromere, although it is possible that the unassigned centromere gene is on this chromosome. Of five fragments of linked genes studied, four were assigned to certain of the established chromosomes and the fifth constitutes one arm of the new chromosome. We believe that seventeen is likely to be the haploid chromosome number for the following reason. There are now 56 genes classified as centromere markers. The number of genes per centromere, assuming 17 chromosomes, is 3.5. The number of unmarked centromeres, assuming the centromere genes are randomly distributed over the centromeres, would be 0.6. Since the centromere of chromosome XVII is unmarked, no additional chromosomes need to be proposed.

Of necessity, the linkage maps (Figure 1) represent a composite of the results of innumerable tetrad analyses as well as mitotic segregation and trisomic studies. In this compilation of the data, we excluded the results from those hybrids in which heterozygosity for chromosome rearrangements, inversions and transloca-

tions were indicated by the viability patterns for the four ascospores. We are reasonably confident that our own summations of the data were obtained from cross-fertile hybrids. From results obtained with specific hybrids, SHULT, DESBOROUGH and LINDEGREN (1962) and SHULT, LINDEGREN and LINDEGREN (1967) contend that we erred in placing *CUP1* on chromosome VIII and *leu1* on chromosome VII (HAWTHORNE and MORTIMER 1960). In the first instance, they found a hybrid displaying "reverse linkage" for *CUP1* and *arg4* with 1 PD, 18 NPD, and 21 T asci. In the second case, a series of hybrids was obtained in which *leu1* failed to show linkage with *trp5* and *ade6* while these markers on opposite arms of chromosome VII retained their linkage with one another. They proposed that the linkages normally seen are the consequences of affinity or preferential segregation of non-homologous centromeres. We will not offer another interpretation of their results but simply reiterate that the original assignment of these loci to chromosomes VIII and VII has been confirmed by the trisomic studies during this investigation and by mitotic recombination experiments with the markers on chromosome VII (NAKAI and MORTIMER 1969).

The genetic lengths of the various chromosomes in *S. cerevisiae* differ greatly. Chromosomes I, VI, and XIV all are relatively short (< 50cM) whereas four chromosomes, II, IV, VII, and XV, are exceptionally long (> 250cM). An approach that permits comparison of the genetic lengths with the physical lengths of the different chromosomes has recently been developed and initial results indicate a good correlation (PETES and FANGMAN 1972; BLAMIRE *et al.* 1972). An interesting exception involves chromosome I with a genetic length of only 5 cM. This chromosome was found to be of intermediate physical size and the basis of this discrepancy was found to be due to the presence of a majority of the ribosomal RNA cistrons on this chromosome (FINKELSTEIN, BLAMIRE and MARMUR 1972). The average size of a yeast chromosome has been estimated, from the haploid DNA content and chromosome number, to be about 7×10^8 daltons of DNA (PETES and FANGMAN 1972), a value about one-fourth that of *Escherichia coli*. The physical separation studies are consistent with this estimate and with the assumption that each chromosome is a single DNA molecule.

Locating genes on the genetic maps of yeast is inherently difficult because of the large number of chromosomes and extreme genetic lengths of some of these chromosomes. It can be estimated that the likelihood that two genes selected at random would show significant linkage is in the order of 0.01. However, through use of the various tactics described in this report, it has been possible to locate a relatively high percentage of the genes selected for study. For example, 24 of the 35 temperature-sensitive lethals were mapped and a comparable fraction applies to the other genes included in this study and in our earlier experiments.

As found previously with regard to genes coding the enzymes comprising a biosynthetic pathway, functionally related genes generally are not clustered on the same chromosome but are dispersed over the genome. Thirteen cell division cycle mutants have been mapped (HARTWELL *et al.* 1973) and they are located on ten different chromosomes. Similarly, the five *rna* genes that were mapped are dispersed. Nevertheless, it is still possible that the genetic location of certain genes is critical since periodic synthesis of different gene products has been

reported as being correlated to the positions of the genes along the chromosome (TAURO, HALVORSON and EPSTEIN 1968). In particular, this consideration may have bearing on the location of the cell division cycle mutants.

Several broad categories of genes, such as those controlling vitamin biosynthesis, antibiotic sensitivity, and radiation sensitivity, have received only limited attention for mapping purposes. Additional genes that control critical cellular functions could be studied in cold sensitive as well as heat sensitive mutants or suppressible lethals. For any substantial extension of the linkage maps, one will probably need to employ these new sources of markers.

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