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Mapping of the *Saccharomyces cerevisiae CDC3, CDC25,* and *CDC42* Genes to Chromosome XII by Chromosome Blotting and Tetrad Analysis

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CDC3, CDC25 and *CDC42* were localized to chromosome XII by hybridizing the cloned genes to Southern blots of chromosomes separated by orthogonal-field-alternation gel electrophoresis. Meiotic tetrad analyses further localized these genes to the region distal to the *RDN1* locus on the right arm of the chromosome. The *STE11* gene, which had previously been mapped to chromosome XII (Chaleff and Tatchell, 1985), was found to be tightly linked to *ILV5*. The data suggest a map order of *CEN12-RDN1-CDC42-(CDC25-CDC3)-(ILV5-STE11)-URA4*. Certain oddities of the data set raise the possibility that there may be constraints on the patterns of recombination in this region of chromosome XII.

KEY WORDS—Cell cycle genes; genetic mapping; *Saccharomyces*; OFAGE.

INTRODUCTION

The *Saccharomyces cerevisiae CDC3* and *CDC42* genes are involved in the morphogenetic processes of the cell cycle (Hartwell, 1971; Pringle and Hartwell, 1981; A. Adams, C. Evans, B. Sloat and J. Pringle, manuscript in preparation). The *CDC25* gene appears to be involved in the control of growth via the *RAS*/cAMP/protein kinase system (Pringle and Hartwell, 1981; Robinson *et al.*, 1987). All three genes were identified using temperature-sensitive (*ts*) lethal mutations and have since been isolated from yeast genomic-DNA libraries by plasmid complementation of these mutations (Robinson *et al.*, 1987, and references cited therein; B. Haarer, D. Johnson and J. Pringle, unpublished results). However, these genes have heretofore evaded genetic mapping, despite several efforts using a variety of techniques.

Recent advances in the separation of large DNA molecules using orthogonal-field-alternation gel electrophoresis (OFAGE) (Schwartz and Cantor, 1984; Carle and Olson, 1984, 1985) and field-inversion gel electrophoresis (FIGE) (Carle *et al.*, 1986) have allowed the resolution of whole yeast chromosomes as discrete DNA bands on agarose gels. This has made it possible to determine the chromosomal location of a cloned gene by DNA–DNA (Southern) hybridization to nitrocellulose blots containing the separated chromosomes. We report here the use of this approach together with conventional meiotic linkage analyses to locate *CDC3, CDC25* and *CDC42* on the right arm of chromosome XII. In addition, these analyses allowed us to map more precisely the *STE11* gene, which had previously been shown to map to chromosome XII (Chaleff and Tatchell, 1985).

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MATERIALS AND METHODS

Strains and genetic methods

The haploid *Saccharomyces cerevisiae* strains used in this study are described in Table 1; crosses involving these strains are described in Tables 1, 3, 4 and 5. Standard techniques were used for complementation and meiotic linkage analyses and for

Table 1. *Saccharomyces cerevisiae* strains used in this study.

Strain	Relevant genotype	Source or reference
AB972	α *trp1*	Sandmeyer and Olson (1982)
JC302-26B	α *ura3 his4 leu2 ras2*	Cannon *et al.* (1986)
C276-4A	**a** *gal2* (prototrophic)	Wilkinson and Pringle (1974)
C276-4B	α *gal2* (prototrophic)	Wilkinson and Pringle (1974)
TD4	**a** *ura3 his4 leu2 trp1 gal2*	G. Fink
DC5	**a** *his3 leu2 gal2*	Broach *et al.* (1979)
JPDC5D-1A	α *his3 leu2 gal2*	J. Pringle[a]
104BD4-1B	**a** *cdc3-1*	A. Adams[b]
104BD4-3D	α *cdc3-1*	A. Adams[b]
SLTD3-6B	**a** *cdc3-1 his3 leu2 trp1 gal2*	S. Lillie[c]
321	**a** *cdc25-1*	Hartwell *et al.* (1973)
BR205-2	**a** *cdc25-2*	L. Hartwell
LR426	α *CDC25:URA3 ura3 leu2 met3*	L. Robinson[d]
LR683	**a** *CDC25:URA3 ura3 leu2 met3*	L. Robinson[e]
JPT163BD5-4D	α *cdc42-1 gal2*	A. Adams[f]
C82-1785	α *ura4 asp5 ilv5-1 GAL2*	YGSC[g]
C82-1857	**a** *ura4 arg1 asp5 ilv5-1 met1 gal2*	YGSC[g]
AH229T9	**a** *his4 leu2 gal2 RDN1::LEU2*	Petes (1980)
BE286	**a** *car2*	T. Petes
XCO26C	α *ste11*^{ts} *his3 his4 leu2 trp1*	Chaleff and Tatchell (1985)
CJMD1-2C	**a** *cdc3-1 arg1*	This study[h]
CJMD1-17D	α *ura4 asp5 ilv5-1*	This study[h]
CJMD12-18D	α *cdc3-1 ura4 his3 ilv5-1 leu2 trp1*	This study[i]
DJTD1-20B	α *cdc42-1 ura3 his4 leu2 gal2*	This study[j]
DJTD1-24C	α *cdc42-1 trp1 gal2*	This study[j]
DJMD1-8A	**a** *cdc42-1 met1 gal2*	This study[k]
DJMD2-7C	α *cdc42-1 ura3 his4 leu2 gal2 RDN1::LEU2*	This study[l]
DJMD3-10A	α *cdc3-1 cdc42-1 his4 leu2 gal2 RDN1::LEU2*	This study[m]
DJMD3-36D	**a** *cdc3-1 cdc42-1 ura3 his3 leu2 gal2 RDN1::LEU2*	This study[m]
DJMD4-30B	**a** *ura4 asp5 his3 ilv5-1 leu2 GAL2*	This study[n]
DJMD9-16B	**a** *ura4 ilv5-1*	This study[o]
DJMD14-6D	α *cdc3-1 ste11*^{ts} *his3 his4 leu2 trp1 gal2*	This study[p]
638-3A	**a** *ura3 (ura4?) ilv5 leu2*	This study[q]
640-27D	α *cdc3-1 CDC25:URA3 ura3 leu2*	This study[r]
657-8A	**a** *cdc3-1 CDC25:URA3 ura3 ilv5-1 leu2*	This study[s]
644-14A	α *ste11*^{ts} *ura3 leu2*	This study[t]
LH6727-8C	α *ura3 his3 leu2 mif1::HIS3*	This study[u]

[a]A segregant from the cross of DC5 to C276-4B.
[b]Segregants from the fourth 'backcross' of mutant 104 (Hartwell, 1971) to C276-4A and C276-4B (see Adams and Pringle, 1984).
[c]A segregant from the cross of TD4 to an α *cdc3* segregant from the cross of 104BD4-1B to JPDC5D-1A.
[d]A segregant from the cross of LR400 to LR469-9C (Robinson *et al.*, 1987).
[e]A segregant from the cross of LR426 to a sibling spore from the same tetrad.
[f]A segregant from the fifth backcross of JPT163 (A. Adams, C. Evans, B. Sloat and J. Pringle, manuscript in preparation) to C276-4A and C276-4B.
[g]Yeast Genetics Stock Center, Berkeley, CA 94720.
[h]Segregants from the cross of 104BD4-3D to C82-1857.
[i]A segregant from the cross of CJMD1-17D to SLTD3-6B.
[j]Segregants from the cross of JPT163BD5-4D to TD4.
[k]A segregant from the cross of DJTD1-24C to C82-1857.
[l]A segregant from the cross of DJTD1-20B to AH229T9.
[m]Segregants from the cross of DJMD2-7C to SLTD3-6B.
[n]A segregant from the cross of DC5 to C82-1785.
[o]A segregant from the cross of BE286 to C82-1785.
[p]A segregant from the cross of XCO26C to SLTD3-6B.
[q]A segregant from the cross of DJMD4-30B to LRA13 [a segregant from the same cross as LR599 (Robinson *et al.*, 1987)].
[r]A segregant from the cross of LR683 to a segregant from the cross of DJMD3-10A to a segregant from the cross of LRA13 (*cf.* note q) to TX2.545.2-4C (Tatchell *et al.*, 1984).
[s]A segregant from the cross of 638-3A to 640-27D.
[t]A segregant from the cross of XCO26C to LR599 (Robinson *et al.*, 1987).
[u]A segregant from the diploid obtained by transforming strain 4539 with the *mif1:HIS3* DNA fragment (Meeks-Wagner *et al.*, 1986).

scoring auxotrophic and fermentation markers (Sherman *et al.*, 1982). All strains were grown routinely at 24°C. The *ts*-lethal *cdc3*, *cdc25* and *cdc42* markers were scored by replicating segregants to YEPD plates at the restrictive temperature (36°C). In crosses involving more than one *ts*-lethal marker, each marker was scored by complementation. Segregants were replicated onto a mixed lawn of MAT**a** and MAT**α** *cdc*^{ts} strains on a YEPD plate and these replicas were then incubated at 36°C. Linkage of markers to the *CDC25* gene was also determined by scoring the segregation of the *CDC25:URA3* marker (the *URA3* gene inserted adjacent to *CDC25*; Robinson *et al.*, 1987) in a *ura3/ura3* background. Similarly, linkage of markers to the *RDN1* locus was evaluated by scoring the segregation of the *RDN1::LEU2* marker (the *LEU2* gene inserted within the *RDN1* cluster; Petes, 1980) in a *leu2/leu2* background. In some crosses in which both *RDN1::LEU2* and *ilv5* were segregating, the *RDN1::LEU2* marker could not be scored reliably by simply replicating to leucine-free medium (see Discussion). In these cases, the *RDN1::LEU2* genotypes were scored by replicating segregants onto a mixed lawn of *MAT***a** *ILV5 leu2* and MAT**α** *ILV5 leu2* testers. The *ste11*^{ts} marker was scored by its inability to yield complementing diploids by mating at 36°C (Chaleff and Tatchell, 1985). Because of the underestimation of map distances over 35 centiMorgans (cM) using Perkins equation ($Xp = (100/2) \times [(T + 6N)/(P + N + T)]$, we have extrapolated the map distances where necessary using Figure 2 of Mortimer and Schild (1980).

OFAGE and Southern blots

Chromosomal DNA samples were prepared from yeast strain AB972 or JC302-26B as described previously (Carle and Olson, 1985) and OFAGE was performed using the procedure described previously (Carle and Olson, 1984) or a modification thereof. In the modified procedure, nearly homogeneous electric fields intersecting at an angle of 115° were used. The 1% agarose gel was run in 0·5 × TBE buffer (Maniatis *et al.*, 1982) for 23 h at 13°C, with a switching interval of 70 s; the instantaneous voltage gradient in the gel was 5·8 V/cm. The gels were then blotted to nitrocellulose paper and hybridized to ^{32}P-labelled probes using standard techniques (Maniatis *et al.*, 1982). The *CDC25* probe was pL110 (Robinson *et al.*, 1987). The *CDC3* and *CDC42* probes were pBR322 into which fragments containing *CDC3* and *CDC42* had been separately cloned (B. Haarer, D. Johnson and J. Pringle, unpublished results). The *SUP2* probe was the lambda clone λPM1405 (Carle and Olson, 1985), and the *PPR1* probe was from F. Lacroute (Liljelund *et al.*, 1984).

RESULTS

Under the modified electrophoretic conditions used in this study, the top two bands in the OFAGE gel (Figure 1A) correspond to chromosomes IV and XII, respectively, as evidenced by the hybridization of the *SUP2* probe (chromosome IV) to the top band and the *PPR1* probe (chromosome XII) to the next band (Figure 1B). Both the *CDC3* and *CDC42* probes clearly hybridized to the DNA band corresponding to chromosome XII (Figure 1B). OFAGE blots using a *CDC25* probe and the original electrophoretic systems of Carle and Olson (1984, 1985), in which chromosome XII was not resolved, showed no hybridization to any band but strong hybridization to the well (data not shown); this suggested that this gene was also on chromosome XII.

cdc3, *cdc25* and *cdc42* were then tested for linkage to known chromosome XII markers (Figure 2). Significant linkage was detected between *cdc42* and *RDN1* (Table 2, line 5; Tables 3 and 4, columns 5). As *cdc42* showed no linkage to the centromere-proximal markers *asp5* and *gal2* (Table 2, lines 1 and 2; Table 4, columns 1 and 2), it is presumably centromere distal to *RDN1*. Significant linkage was also detected between *cdc42* and *cdc3* (Table 2, line 6; Tables 3 and 4, columns 6). As *cdc3* displayed little or no linkage to *RDN1*, *gal2*, or *asp5* (Table 2, lines 7, 4 and 3; Table 3, column 7; Table 4, columns 7, 4 and 3), it is presumably centromere distal to *cdc42*. [The apparent linkage between *cdc3* and *asp5* in cross VIII (Table 4) is one of the oddities of the data set—see Discussion.] The putative order *RDN1-cdc42-cdc3* is also consistent with the results of crosses I, II and III (Table 3) and VIII (Table 4, including the three-spore tetrads) taken as three-point crosses for these markers.

Strong linkage was detected between *cdc3* and *cdc25* (Table 2, line 8; Table 3, column 8). Similar results were obtained whether *CDC25* was marked by the *cdc25-1*^{ts} allele from strain 321 (Table 3, cross IV), the *cdc25-2*^{ts} allele from strain BR205-2 (data not shown), or a *URA3* insertion adjacent to *CDC25* (Table 3, cross III). The close linkage between *cdc3* and *cdc25* implies that cross V (Table 3) can be viewed as providing additional three-point-cross support for the putative order *RDN1-*

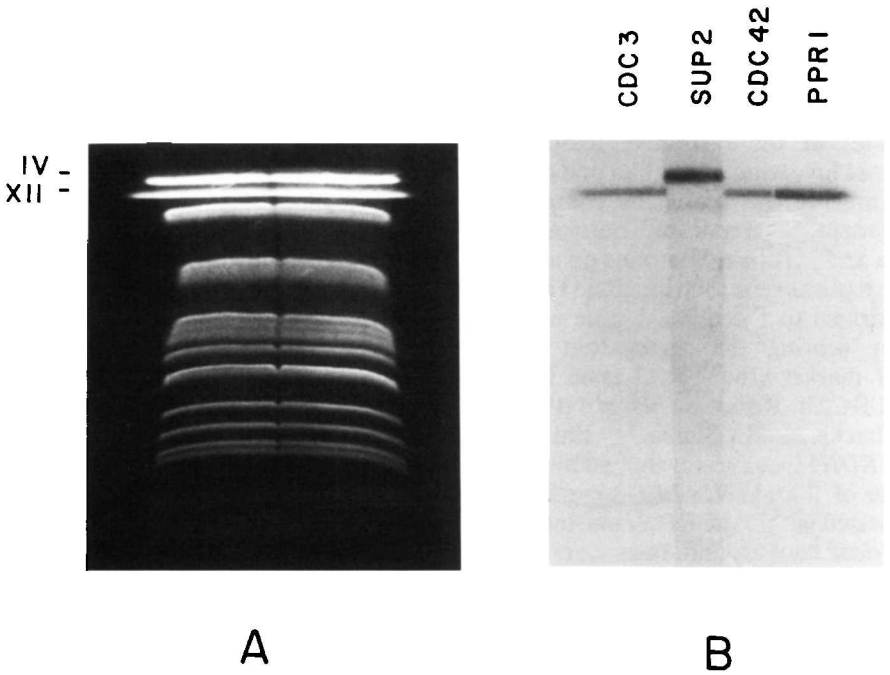

Figure 1. Physical mapping of genes *CDC3* and *CDC42* to chromosome XII. (A) Yeast chromosomal DNA molecules from strain AB972 were resolved using a modified OFAGE apparatus (see Materials and Methods) and stained with ethidium bromide. (B) DNA from the gel was transferred to a single sheet of nitrocellulose, which was then cut into four strips. These strips were separately hybridized with DNA probes (see Materials and Methods) specific for *CDC3*, *SUP2* (chromosome IV), *CDC42* and *PPR1* (chromosome XII), and then positioned in their original alignment before the autoradiogram was exposed.

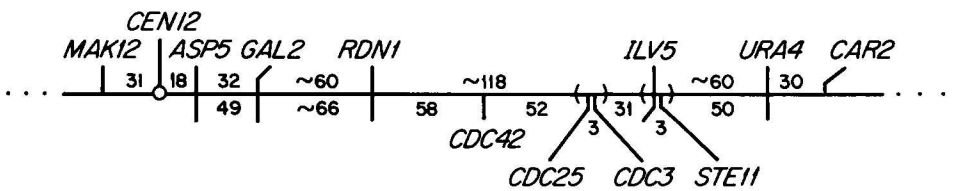

Figure 2. Genetic map of selected markers on chromosome XII. The gene order and approximate map distances (given in centiMorgans) shown above the line have been determined in previous studies. The order and distances of the genes from *MAK12* to *RDN1* were well established, whereas the order and distances of *ILV5*, *URA4* and *CAR2*, were still somewhat uncertain (Petersen *et al.*, 1983; Mortimer and Schild, 1985; see also Discussion). In addition, *STE11* had been shown to be linked (~41 cM) to *URA4* (Chaleff and Tatchell, 1985), but its linkage to *ILV5* had not been tested. The positions of *CDC42*, *CDC25*, *CDC3* and *STE11* and the approximate map distances shown below the line were determined in the present study (see text, Table 2, and notes to Table 4). The parentheses indicate pairs of markers whose order is still somewhat uncertain (see text).

CDC42-(*CDC25*-*CDC3*). However, *RDN1* and *cdc42* were too distant (Table 2, lines 6, 7, 9 and 10; Table 3, columns 6, 7, 9 and 10; Table 4, columns 6 and 7) to be useful as third markers for determining the relative order of *cdc25* and *cdc3*. This issue is addressed further below.

ilv5 displayed significant linkage to *cdc3* (Table 2, line 11; Tables 3 and 4, columns 11) and to *cdc25* (Table 2, line 12) but not to *cdc42* (Table 2, line 13; Table 4, column 13). Thus, *ilv5* must be centromere distal to *cdc3*. The putative order *cdc42-cdc3-ilv5* is also consistent with the results of cross VIII (Table 4, including the three-spore tetrads) taken as a three-point cross for these markers. It should be noted that the apparent linkage of *ilv5* to *RDN1* (Table 2, line 14; Tables 3 and 4, columns 14) is inconsistent with

Table 2. Aggregate linkage data for chromosome XII markers[a].

	Marker pair	Tetrad type (P:N:T)	Map distance[b]
1.	*asp5* vs. *cdc42*	34:35:151	Unlinked
2.	*gal2* vs. *cdc42*	42:33:143	Unlinked[c]
3.	*asp5* vs. *cdc3*	34:17:155	78[d]
4.	*gal2* vs. *cdc3*	17:22:96	Unlinked
5.	*RDN1* vs. *cdc42*	134:36:543	58
6.	*cdc42* vs. *cdc3*	204:41:482	54
7.	*RDN1* vs. *cdc3*	139:100:484	>120[e]
8.	*cdc3* vs. *cdc25*	349:0:25	3
9.	*cdc42* vs. *cdc25*	77:16:179	54
10.	*RDN1* vs. *cdc25*	30:29:114	Unlinked
11.	*ilv5* vs. *cdc3*	304:14:307	31
12.	*ilv5* vs. *cdc25*	111:5:127	32
13.	*ilv5* vs. *cdc42*	37:26:156	Unlinked[c]
14.	*ilv5* vs. *RDN1*	52:20:139	77[d]
15.	*ilv5* vs. *ste11*	249:0:16	3
16.	*ste11* vs. *cdc3*	130:6:154	33
17.	*ilv5* vs. *ura4*	149:32:289	55
18.	*ste11* vs. *ura4*	31:3:41	39
19.	*ura4* vs. *cdc3*	73:42:251	102
20.	*ura4* vs. *cdc42*	35:40:154	Unlinked

[a]For each marker pair, the table shows the number of parental ditypes (P), the number of non-parental ditypes (N), and the number of tetratypes (T). The aggregate data are from the crosses listed in Tables 3, 4 and 5 and from other crosses not presented in detail.
[b]Map distances were calculated as described in Materials and Methods.
[c]Chi-square analysis indicates that the excess of parental ditypes over non-parental ditypes is not statistically significant.
[d]The apparent linkage represents an oddity of the data set (see Discussion).
[e]Chi-square analysis indicates that the excess of parental ditypes over non-parental ditypes is statistically significant ($P = 0{\cdot}01$).

the putative map order of *RDN1-cdc42-(cdc25-cdc3)-ilv5* as just proposed. Indeed, taken as a three-point cross for *RDN1, cdc3* and *ilv5*, cross VIII indicates a map order of *RDN1-ilv5-cdc3*; taken as a three-point cross for *RDN1, cdc42* and *ilv5*, it indicates a map order of either *cdc42-RDN1-ilv5* or *RDN1-ilv5-cdc42*. However, all of the resulting possible overall map orders (*cdc42-RDN1-ilv5-cdc3, RDN1-ilv5-cdc42-cdc3,* and *RDN1-ilv5-cdc3-cdc42*) seem ruled out by various other features of the data set. Thus, the apparent linkage of *ilv5* to *RDN1* appears to be another oddity of the data set (see Discussion).

We attempted to use *ilv5* as a third marker for determining the relative order of *cdc25* and *cdc3* (Table 5). The bulk of the data, including the data from the more satisfactory cross XII (see note b, Table 5), support a map order of *cdc25-cdc3-ilv5*. However, it should be noted that this order requires us to postulate a surprisingly large number of two-strand double crossovers, a peculiarity that is only exaggerated if the alternative order of *cdc3-cdc25-ilv5* is postulated. Thus, the putative map order should be regarded as somewhat tentative.

ste11 displayed strong linkage to *ilv5* (Table 2, line 15; Table 3, column 15) and (as expected from this result) significant linkage to *cdc3* (Table 2, line 16; Table 3, column 16). We attempted to use *cdc3* as the third marker for determining the relative order of *ilv5* and *ste11*. The 187 tetrads of cross XII (Table 5) yielded 11 that were recombinant (all tetratype) between *ilv5* and *ste11*. Of these, seven were parental ditype for *cdc3* vs. *ilv5* and tetratype for *cdc3* vs. *ste11*, two were tetratype for *cdc3* vs. *ilv5* and non-parental ditype for *cdc3* vs. *ste11*, and two were tetratype for *cdc3* vs. *ilv5* and parental ditype for

Table 3. Meiotic linkage analyses of chromosome XII markers[a].

	Marker pair												
Cross[b]	5 *RDN1* vs. *cdc42* P:N:T	6 *cdc42* vs. *cdc3* P:N:T	7 *RDN1* vs. *cdc3* P:N:T	8 *cdc3* vs. *cdc25* P:N:T	9 *cdc42* vs. *cdc25* P:N:T	10 *RDN1* vs. *cdc25* P:N:T	11 *ilv5* vs. *cdc3* P:N:T	14 *ilv5* vs. *RDN1* P:N:T	15 *ilv5* vs. *ste11* P:N:T	16 *ste11* vs. *cdc3* P:N:T	17 *ilv5* vs. *ura4* P:N:T	18 *ste11* vs. *ura4* P:N:T	19 *ura4* vs. *cdc3* P:N:T
I	36:13:157	53:10:138	44:30:137										
II	19:2:55	25:2:51	20:7:50										
III	22:3:59	15:8:60	16:12:55	82:0:2	15:8:60	15:13:55							
IV		11:0:10		21:0:0	11:0:9								
V	15:5:65				17:7:63	15:16:59							
VI[c]			13:8:72				38:0:46	17:10:46			25:10:49		16:8:57
VII							32:3:35		73:0:5	33:2:37	34:4:43	31:3:41	16:7:40

[a]For each marker pair, the table shows the number of parental ditypes (P), the number of non-parental ditypes (N), and the number of tetratypes (T). All data are from tetrads with four viable spores.

[b]Parent strains for each cross were as follows (see Table 1 for genotypes): (I) DJMD2-7C × SLTD3-6B; (II) LH6727-8C × DJMD3-36D; (III) LR426 × DJMD3-36D; (IV) 321 × DJMD3-10A; (V) LR683 × DJMD2-7C; (VI) CJMD12-18D × AH229T9; (VII) DJMD9-16B × DJMD14-6D.

[c]Additional data included (P:N:T): *ura4* vs. *RDN1* (13:8:60).

Table 4. Meiotic linkage analyses of chromosome XII markers[a].

	Marker pair												
Cross[b]	1 *asp5* vs. *cdc42* P:N:T	2 *gal2* vs. *cdc42* P:N:T	3 *asp5* vs. *cdc3* P:N:T	4 *gal2* vs. *cdc3* P:N:T	5 *RDN1* vs. *cdc42* P:N:T	6 *cdc42* vs. *cdc3* P:N:T	7 *RDN1* vs. *cdc3* P:N:T	11 *ilv5* vs. *cdc3* P:N:T	13 *ilv5* vs. *cdc42* P:N:T	14 *ilv5* vs. *RDN1* P:N:T	17 *ilv5* vs. *ura4* P:N:T	19 *ura4* vs. *cdc3* P:N:T	20 *ura4* vs. *cdc42* P:N:T
VIII[c]	4:2:28	6:2:25	10:3:22	7:5:23	6:2:25	11:2:26	6:1:26	18:0:23	5:2:31	10:0:24	13:0:26	6:2:31	5:7:28
VIII[d]	20:16:67	25:19:58	16:6:80	10:17:73	12:7:71	29:7:62	17:16:65	55:3:47	16:11:68	25:10:69	27:12:59	18:17:68	15:17:71
IX[e]	10:17:56	11:12:60							16:13:57		32:1:56		15:16:55
X[f]			8:8:53					43:3:36			18:5:56	17:8:55	

[a]For each marker pair, the table shows the number of parental ditypes (P), the number of non-parental ditypes (N), and the number of tetratypes (T). All data are from tetrads with four viable spores, except as noted (note d).

[b]Parent strains for each cross were as follows (see Table 1 for genotypes): (VIII) DJMD3-10A × DJMD4-30B; (IX) DJMD1-8A × C82-1785; (X) CJMD1-2C × C82-1785.

[c]These data are from tetrads with four viable spores, which constituted only 21% of the tetrads dissected in this cross. All markers segregated 2:2 in these tetrads with only occasional exceptions. Additional data from these tetrads included (P:N:T): *asp5* vs. *gal2* (13:1:14); *gal2* vs. *RDN1* (7:3:19); *asp5* vs. *RDN1* (2:7:19); *ilv5* vs. *asp5* (13:1:21); *ilv5* vs. *gal2* (11:3:22); *ura4* vs. *asp5* (6:4:26); *ura4* vs. *gal2* (4:5:27); *ura4* vs. *RDN1* (8:3:22).

[d]These data are from tetrads with three viable spores, assuming 2:2 segregation of all markers (*cf.* note c). Additional data included (P:N:T): *asp5* vs. *gal2* (29:7:62); *gal2* vs. *RDN1* (20:6:72); *asp5* vs. *RDN1* (18:16:69); *ilv5* vs. *asp5* (18:9:76); *ilv5* vs. *gal2* (20:13:66); *ura4* vs. *asp5* (17:20:68); *ura4* vs. *gal2* (16:12:75); *ura4* vs. *RDN1* (21:16:63).

[e]Additional data included (P:N:T): *asp5* vs. *gal2* (25:3:54); *ilv5* vs. *asp5* (22:11:46); *ilv5* vs. *gal2* (11:12:61); *ura4* vs. *asp5* (18:9:55); *ura4* vs. *gal2* (12:13:59).

[f]Additional data included (P:N:T): *ilv5* vs. *asp5* (13:7:48); *ura4* vs. *asp5* (9:9:43).

Table 5. Assessment of the relative order of *CDC25* and *CDC3* by examination of individual tetrads[a].

Cross[b]	Number of tetrads recombinant between *CDC25* and *CDC3* that were:		
	P for *CDC3* vs. *ILV5* T for *CDC25* vs. *ILV5*	T for *CDC3* vs. *ILV5* P for *CDC25* vs. *ILV5*	T for *CDC3* vs. *ILV5* T for *CDC25* vs. *ILV5*
XI	2	4	2
XII	11	2	1

[a]For each cross, tetrads with four viable spores that were recombinant between *CDC25:URA3* and *cdc3*[ts] were identified (all were tetratype) and scored for tetrad type with respect to *cdc3* vs. *ilv5* and *CDC25:URA3* vs. *ilv5*. P, parental ditype; T, tetratype.
[b]Parent strains for cross XI were 638-3A and 640-27D (see Table 1). This cross was marred by poor spore viability and an unexplained difficulty in scoring the *CDC25:URA3* marker. Nonetheless, 56 tetrads with four viable spores were analyzed; eight were recombinant between *CDC25* and *CDC3*. Parent strains for cross XII were 657-8A and 644-14A (Table 1). This cross displayed good spore viability and clear 2:2 segregation of all markers. 187 tetrads with four viable spores were analyzed; 14 were recombinant between *CDC25* and *CDC3*.

cdc3 vs. *ste11*. Data obtained in cross VII (Table 3) were similar, although most of the relevant tetrads had only three viable spores. Thus, the data suggest a map order of *cdc3-ilv5-ste11*, although this order should be regarded as somewhat tentative and the apparent excess of two-strand and four-strand double crossovers is again peculiar.

ura4 displayed significant linkage to *ilv5* (Table 2, line 17; Tables 3 and 4, columns 17) and to *ste11* (Table 2, line 18; Table 3, column 18), but showed little or no linkage to *RDN1* (Table 3, note c; Table 4, notes c and d), *cdc42* (Table 2, line 20; Table 4, column 20), or *cdc3* (Table 2, line 19; Tables 3 and 4, columns 19). Thus, *ura4* must be centromere distal to *ilv5*. The putative order of *cdc3-(ilv5-ste11)-ura4* is consistent with the results of crosses VI and VII (Table 3) and VIII and X (Table 4) taken as three-point crosses for these markers. The apparently closer linkage of *ura4* to *ste11* than of *ura4* to *ilv5* (Table 2, lines 17 and 18) is consistent with the relative order suggested above for *ste11* and *ilv5*.

In summary, our data suggest an overall map order of *CEN12-RDN1-CDC42-CDC25-CDC3-ILV5-STE11-URA4* (Figure 2), with some uncertainty about the relative order of *CDC25* and *CDC3* and that of *ILV5* and *STE11*.

DISCUSSION

We have localized the *CDC3*, *CDC25*, and *CDC42* genes to the right arm of chromosome XII using a combination of molecular and classical genetic techniques. Our meiotic linkage data are consistent with the previously established map order *ASP5-GAL2-RDN1* and in reasonable agreement with previous data on the map distances over this interval (Mortimer and Schild, 1980, 1985; Table 4, notes c–e; Figure 2). Moreover, our results are consistent with the previously suggested map order *RDN1-ILV5-URA4* and in good agreement with previous data on the map distance from *ILV5* to *URA4* (Petersen *et al.*, 1983; Mortimer and Schild, 1985; see above and Figure 2). Our results do suggest that the map distance from *RDN1* to *ILV5* is even greater than that inferred from the previously available data (Petersen *et al.*, 1983; Mortimer and Schild, 1985) or, indeed, from our own data on the *RDN1-ilv5* marker pair (Table 2, line 14), which suggest a map distance of only 77 cM. However, it is clear that the map distances from *RDN1* to *CDC3* and from *ILV5* to *URA4* should be regarded only as rough estimates until additional markers have been mapped in these intervals. Finally, our results are in good agreement with the previously reported linkage of *ste11* to *ura4* (Chaleff and Tatchell, 1985). As *ura4* seems to lie between *ste11* and *car2* (Chaleff and Tatchell, 1985), our positioning of *ste11* close to *ilv5* implies that *car2* is centromere distal to *ura4* (Figure 2), as also suggested previously (Petersen *et al.*, 1983).

Our mapping of *CDC25* to chromosome XII is in disagreement with the recent report (Portillo and

Mazon, 1986) that *CDC25* is on chromosome II. The reason for this discrepancy is not clear, but it should be noted that we used two different *cdc25*^{ts} alleles, plus an adjacent *URA3* insertion, in mapping *CDC25* to chromosome XII. In addition, although *MIF1* has been reported to map distal to *GAL2* on chromosome XII (Meeks-Wagner *et al.*, 1986), we observed no linkage of the *mif1::HIS3* marker to *RDN1*, *cdc42* or *cdc3* (data not shown). These results agree with other data suggesting that *MIF1* is in fact on another chromosome (M. Brown and L. Hartwell, personal communication).

Although the order of genes shown in Figure 2 is probably correct, it is important to note the following caveats. First, the linkages of *RDN1* to *cdc42*, of *cdc42* to *cdc25* and *cdc3*, and of *ilv5* and *ste11* to *ura4* are sufficiently weak that the interpretation of the three-point cross data is not entirely unequivocal. Second, attempting to establish the order of genes by comparing map distances obtained in different crosses may be even more dangerous than usual in the present case. This danger reflects both the large map distances involved and the appearance of substantial cross-to-cross variability in recombination frequencies for given intervals (see Tables 3 and 4). Such variability might reflect variations in the severity of the suppression of recombination that occurs in the *RDN1* region (Petes, 1979) or in the extent of the region affected by this phenomenon. Third, confidence in the ordering of *cdc25* and *cdc3* and of *ilv5* and *ste11* relative to the appropriate outside markers is reduced because these outside markers are relatively far away (see Results). Finally, there are oddities or inconsistencies in our data that probably represent statistical flukes, selective inviability of spores in certain tetrad types (notably in cross VIII, Table 4), or constraints on patterns of recombination, but could conceivably mean that the actual map order is different from that implied by the bulk of the data. These oddities include the suggestions of linkage between *cdc3* and *asp5* (Table 2, line 3; Table 4, column 3) and between *ilv5* and *RDN1* (Table 2, line 14; Tables 3 and 4, columns 14), as already mentioned in Results; the suggestion of linkage between *ilv5* and *asp5* (Table 4, notes c–f; cumulative data 66P:28N:191T); and the apparent excess of two-strand (and perhaps four-strand) double crossovers observed upon analysing individual tetrads (see Results and Table 5).

These uncertainties should be resolvable as additional genes are mapped to the region centromere distal to *RDN1* on chromosome XII. Such mapping should be facilitated by the availability of OFAGE techniques that resolve chromosome XII and of the easily scorable markers now mapped to this region.

In earlier applications of large-DNA electrophoresis to the yeast chromosomes, chromosome XII failed to form a normal band (Schwartz and Cantor, 1984; Carle and Olson, 1985). Under the modified electrophoretic conditions described above, it migrates as an intense, discrete band that is well separated from the remaining chromosomes (Figure 1). Despite the fact that the electrophoretic mobility of chromosome XII exceeds that of chromosome IV, it is almost certain that chromosome XII is physically the largest chromosome (Mortimer and Schild, 1985). Whether or not the relative mobilities of chromosomes IV and XII represent an electrophoretic anomaly or simply the normal size-mobility relationship for molecules in this size range is not presently known.

An interesting question concerns the relationship between physical and genetic distances on chromosome XII. *A priori*, it might be imagined that the suppression of meiotic recombination observed within the *RDN1* cluster (Petes, 1979) also affects the surrounding regions. Alternatively, the suppression of recombination within *RDN1* might be balanced by a compensatory recombinational hot spot (Keil and Roeder, 1984; Coleman *et al.*, 1986) outside the cluster. Thus, the physical distance between *RDN1* and *CDC42* might be either much larger or much smaller than would ordinarily be suggested by the genetic map distance. Molecular cloning of the DNA between *CDC42* and the centromere-distal *RDN1* junction fragment (Zamb and Petes, 1982) should allow resolution of this issue.

In some crosses involving both the *RDN1::LEU2* and *ilv5* markers, we observed a large excess of Leu$^-$ segregants when segregants were scored simply by replication to selective plates lacking leucine. Such apparently aberrant segregation of the *RDN1::LEU2* marker was also observed by Petersen *et al.* (1983), who suggested that it was due to loss of the inserted *LEU2* gene by unequal sister chromatid exchange within the *RDN1* cluster during meiosis (Petes, 1980). However, we observed normal 2 Leu$^+$:2 Leu$^-$ segregation when the *RDN1::LEU2* marker was scored by complementation using *ILV5 leu2* tester strains. In addition, all Leu$^-$ segregants that were Leu$^+$ when scored by complementation were *ilv5*. Moreover, in other crosses, the *RDN1::LEU2* marker was seen to segregate >98% 2:2 even when scored simply by replication to plates lacking leucine. Thus, it appears that the *ilv5* mutation,

in some genetic backgrounds, can lead to leucine auxotrophy in a *LEU2* strain, presumably because the *ILV5* gene product is a common enzyme in the isoleucine-valine and leucine biosynthetic pathways (Petersen *et al.*, 1983). The exclusion of tetrads in which *RDN1::LEU2* did not appear to segregate 2:2 probably led to an underestimation of the *RDN1-ILV5* map distance by Petersen *et al.* (1983).

The use of OFAGE and Southern blot hybridization should greatly facilitate the mapping of other cloned genes, as it has for *CDC3, CDC25* and *CDC42*. Such mapping should be further facilitated by the use of restriction endonucleases with eight-base pair recognition sites to divide the yeast chromosomes into smaller, defined DNA fragments that can be separated, blotted, and hybridized as we have done here for the whole chromosomes (Carle and Olson, unpublished results).

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