INOSITOL MUTANTS OF *SACCHAROMYCES CEREVISIAE*: MAPPING THE *ino1* LOCUS AND CHARACTERIZING ALLELES OF THE *ino1*, *ino2* AND *ino4* LOCI

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ABSTRACT

An extensive genetic analysis of inositol auxotrophic mutants of yeast is reported. The analysis includes newly isolated mutants, as well as those previously reported (CULBERTSON and HENRY 1975). Approximately 70% of all inositol auxotrophs isolated are shown to be alleles of the *ino1* locus, the structural gene for inositol-1-phosphate synthase, the major enzyme involved in inositol biosynthesis. Alleles of two other loci, *ino2* and *ino4*, comprise 9% of total mutants, with the remainder representing unique loci or complementation groups. The *ino1* locus was mapped by trisomic analysis with an $n + 1$ disomic strain constructed with complementing alleles at this locus. The *ino1* locus is shown to be located between *ura2* (11.1 cM) and *cdc6* (21.8 cM) on chromosome *X*. An extended map of chromosome *X* of yeast is presented. Unlike most yeast loci, but similar to the *his1* locus, the *ino1* locus lacks allelic representatives that are suppressible by known suppressors. This finding suggests that premature termination of translation of the *ino1* gene product may be incompatible with cell viability.

INOSITOL is an essential component of the phospholipids of eukaryotic organisms, including yeast. Its synthesis involves internal cyclization of glucose-6-phosphate to inositol-1-phosphate with subsequent dephosphorylation. Inositol auxotrophs have been isolated in a variety of fungal microorganisms and, historically, were among the first mutants described by BEADLE and TATUM (1945). Inositol auxotrophs of *Saccharomyces cerevisiae* were isolated and described by CULBERTSON and HENRY (1975). These mutants represented ten unlinked loci, of which none is centromere-linked. More than half of the mutants, however, were alleles of *ino1*, a locus at which a complex pattern of interallelic complementation is observed. Subsequently, it was shown that mutants representing all ten loci lacked inositol-1-phosphate synthase, the enzyme that is responsible for the synthesis of inositol-1-phosphate (CULBERTSON, DONAHUE and HENRY 1976b). Furthermore, it has been shown that wild-type inositol-1-phosphate synthase activity is subject to some 50-fold repression when 50 μM inositol is present in the growth medium (CULBERTSON, DONAHUE and HENRY

1976a). The enzyme inositol-1-phosphate synthase is a large multimer (240,000 daltons) consisting of identical 62,000 dalton subunits. It catalyzes the conversion of glucose-6-phosphate to inositol-1- phosphate, a reaction that is postulated to involve three partial reactions (EISENBERG 1978). Immunological analysis of inositol mutants has established that the *ino1* locus is the structural gene of the 62,000 dalton subunit of this enzyme (DONAHUE and HENRY 1981). The role of other *ino* loci is not clear, although mutations at the *ino2* and *ino4* loci have been shown to confer pleiotrophic defects in phospholipid metabolism (HENRY *et al.* 1981).

In the present study, we have extended the genetic characterization of inositol auxotrophs of yeast. Additional mutants have been isolated, genetically characterized and compared with the original auxotrophs in an attempt to saturate the complementaton map. Furthermore, an in-depth analysis of the primary loci (*ino1*, *ino2* and *ino4*) for nonsense suppressibility has been performed. Finally, the *ino1* gene, the structural locus for structural locus for inositol-1-phosphate synthase, was mapped by trisomic analysis. The construction of an $n + 1$ disomic strain, using complementing alleles at this locus, is reported.

MATERIALS AND METHODS

Strains: The genotypes of the strains used for genetic studies of inositol auxotrophs are presented in Table 1. All are heterothallic, haploid laboratory strains of *S. cerevisiae*. Parent strains of inositol mutants were "wild-type" either *ade5* **a**; *ade5* α or 398 **a**. Genetic procedures and media have been described previously (CULBERTSON and HENRY 1975). Temperature-sensitive (ts) strains were grown at 23°, while the phenotype was tested at the restrictive temperature, 37°.

Isolation and characterization of inositol-requiring mutants: Inositol auxotrophs were induced with ethyl methansulofnate (EMS), as previously described (CULBERTSON and HENRY 1975), or with ultraviolet irradiation (2.5 J mm^{-2} sec^{-1}). The screening employed in detecting inositol mutants has been described previously (CULBERTSON and HENRY 1975). Newly isolated mutants were crossed to strains P49 or S5-*lys*^{2} (Table 1). Only those inositol auxotrophs that showed a $2+:2^-$ segregation were further analyzed. Inositol-requiring segregants were selected such that the **a** maters contained an *ade1* or *ade5* marker, while α maters had the *lys2* marker.

Complementation analysis of these mutants was then performed as previously described (CULBERTSON and HENRY 1975). The **a** and α segregants of the newly isolated mutants were used in crosses among themselves and with previously characterized mutants. The complementation of *ade* and *lys* markers provided an internal control for mating.

Nonsense suppression: Inositol-requiring mutants at the *ino1*, *ino2* and *ino4* loci that segregated $2+:2^-$ when crossed to wild-type yeast strains were examined by tetrad analysis for nonsense suppressibility in crosses to known tRNA nonsense suppressor strains, FM6 α (ochre: UAA), FM11 **a** (amber:UAG), and A5364-8D3 α or A5364-8D4 **a** (opal:UGA). Crosses that showed aberrant segregation of the inositol-requiring phenotype ($4+:0^-$, $3+:1^-$), as well as the normal $2+:2^-$, provided preliminary evidence of a nonsense-suppressible allele. Segregants assumed to contain both the *ino*$^-$ allele and the suppressor were then crossed to either strain 398 **a** or 398 α, both of which contain known ochre, amber and opal mutations (Table 1). Tetrad analysis of this cross provided confirmation of the suppression of the *ino* allele. An *ino* allele was judged not to be suppressible if the inositol-requiring phenotype segregated $2+:2^-$ in at least 8 four-spore tetrads and if the suppressor was determined to be present in an auxotrophic spore. The segregation of the suppressor was monitored by using appropriate suppressible markers segregating in each cross. In instances where poor spore viability made this goal impractical, evidence of lack of suppression was based upon identification of several Ino$^-$ spores in which the

TABLE 1

Strains of Saccharomyces cerevisiae *used for genetic studies*

Strain	Genetic markers (chromosome assignment)	Source of strains
"wild type"*	**a** *ade5*(7)	Laboratory strains
"wild type"*	α *ade5*(7)	Laboratory strains
S5-*ade1*	**a** *ade1*(1)	Laboratory strains
S5-*ade1*	α *ade1*(1)	Laboratory strains
S5-*lys2*	**a** *lys2*(2)	Laboratory strains
398	**a** *arg4–17* $_{(UAA)}$(8), *lys1–1* $_{(UAA)}$(9), *met8–1* $_{(UAG)}$(2), *trp1–1* $_{(UAG)}$(4), *leu2–2* $_{(UGA)}$(3)	G. FINK
399	α *arg4–17* $_{(UAA)}$(8), *lys1–1* $_{(UAA)}$(9), *met8–1* $_{(UAG)}$(2), *trp1–1* $_{(UAG)}$(4), *leu2–2* $_{(UGA)}$(3)	G. FINK
A5364–8D4	**a** *ade5*(7), *leu2* $_{(UGA)}$(3), *SUP* $_{(UGA)}$	G. FINK
A5364–8D3	α *lys2*(2), *leu2* $_{(UGA)}$(3), *SUP* $_{(UGA)}$	G. FINK
FM6	α *ade2–1* $_{(UAA)}$(15), *lys1–1* $_{(UAA)}$(9), *can1–100* $_{(UAA)}$(5), *SUPIV–1* $_{(UAA)}$(10)	G. FINK
FM11	**a** *ade2–1* $_{(UAA)}$(15), *lys1–1* $_{(UAA)}$(9), *met8–1* $_{(UAG)}$(2), *his4–580* $_{(UAG)}$(3), *SUPIV–3* $_{(ts\text{-}UGA)}$(10)	G. FINK
612	α *met3*(10), *leu2–1*(3), K+R+	R. WICKNER
1117	α *his1*(5), *ura1*(11), *ade2*(15), *lys1*(9), *rna1–1*(13), [KIL-k]	R. WICKNER
1118	**a** *his1*(5), *ura1*(11), *ade2*(15), *lys1*(9), *rna1–1*(13), [KIL-k]	R. WICKNER
1119	α *pet8*(14), *ilv3*(10), *leu1*(7), *his2*(6), *his6*(9), *met2*(17), [KIL-k]	R. WICKNER
1125	α *ura2*(10), *ade2*(15), *lys1*(9), *his1*(5), *his7*(2), *rna1–1*(13), [KIL-k]	R. WICKNER
1136	α *ura2*(10), *his6*(9), *thr1*(8), *arg4*(8), *met1*(11), K+	R. WICKNER
1220	α *gal1*(2), *asp5*(12), *ade1*(1), *trp1*(4), *pet17*(15), *aro7*(16), *his2*(6), *his6*(9), *cdc14*(6), [KIL-k]	R. WICKNER
1230	**a** *pet8*(14), *met2*(17), *arg1*, *his7*(2), [KIL-k]	R. WICKNER
1232	α *pet8*(14), *met* **a**(17), *arg1*, *his7*(2), [KIL-k]	R. WICKNER
1267	**a** *gal1*(2), *pet17*(15), *arg4*(8), *aro7*(16), *his2*(16), *his6*(9), *trp1*(4), *ade1*(1), *asp5*(12), *cdc14*(6), [KIL-k]	R. WICKNER
1268	α *gal1*(2), *pet17*(15), *arg4*(8), *aro7*(16), *his2*(6), *his6*(9), *trp1*(4), *ade1*(1), *asp5*(12), *cdc14*(6), [KIL-k]	R. WICKNER
AN33	α *arg1*, *thr1*(8), [KIL-o]	R. WICKNER
S288C	α wild type	Univ. Calif., Berkeley, Yeast Genetics Stock Center
P49	α *lys2*(2)	Univ. Calif., Berkeley, Yeast Genetics Stock Center
S856C	**a** *ade1*(1), *lys2*(2)	Univ. Calif., Berkeley, Yeast Genetics Stock Center
N361–9A	*cdc6 his4* mal GAL suc α *arg3* **a** and α	G. KAWASAKI F. MESSENGUY

* Tested for absence of suppressors by crossing to strains 398 **a** or 399 α.

suppressor was known to be present. In rare cases, a single 3+:1- or 1+:3- segregation of the inositol-requiring phenotype was observed (less than 1% overall, presumably due to gene conversion). Nevertheless, in such instances special care was taken to show that several segregants retained the Ino- phenotype in the presence of the suppressor.

In order to broaden the spectrum of tRNA suppressors capable of suppressing *ino* alleles, a second screening procedure was used. For this study, strain 398 **a** (Table 1) was employed, which has two ochre mutations (*lys1* and *arg4*), two amber mutations (*trp1* and *met8*) and one opal mutation (*leu2*). Inositol-requiring mutants were induced in this strain with either EMS or UV. Lawns of each mutant were made on YEPD plates and replica plated to arginine or lysine dropout medium, methionine or tryptophan dropout medium, and leucine and inositol dropout media. Revertants were picked and tested for co-reversion of other markers. Co-reversion of the Ino- phenotype with any suppressible marker was taken as preliminary evidence for a suppressible *ino* allele. Putative nonsense-suppressible, inositol-requiring mutants were crossed to known suppressor-carrying strains to confirm the co-reversion data. In addition, the suppressed version of the mutation isolated by co-reversion was crossed to a strain marked with known nonsense suppressible alleles to confirm reversion by tRNA suppression.

Mapping studies: Previous analysis of the *ino* genes of yeast showed none to be centromere-linked (CULBERTSON and HENRY, 1975). In order to map the *ino1* locus, a strain disomic for the chromosome bearing the *ino1* locus was constructed, using methods similar to those previously described by MORTIMER and HAWTHORNE (1973) and SHAFFER *et al.* (1971). The disomic strain construction was facilitated by the existence of interallelic complementation at the *ino1* locus (CULBERTSON and HENRY 1975). Two *ino1* mutants, MC14 (**a** *ino1-14, ade1, can*^{S}) and MC28 (*ino1-28, lys2, can*^{R}), which complement vigorously at 23° and 30°, were crossed. Diploids selected on minimal medium with inositol were cloned, retested and sporulated. Random spores were prepared by treatment with glusulase and subsequent sonication. The spores were plated out on canavanine medium [arginine dropout medium containing canavanine (60 mg/l)], and the plates were incubated at 30° until colonies appeared. In diploids heterozygous at the *can* locus (*can*^{R}/*can*^{S}), sensitivity to canavanine is dominant to canavanine resistance. Therefore, canavanine medium selects against the growth of diploids and *Can*^{S} spores. Canavanine-resistant colonies were tested for the *ino1*, *ade1* and *lys2* markers, as well as mating type. These strains were then crossed to wild-type strains, and tetrads were analyzed for segregation of the Ino- phenotype. The spores were also allele tested for the presence of the original *ino1* alleles. In this fashion, a strain disomic for the chromosome bearing *ino1* was selected. The heteroallelic presumptive *ino1* disomic strain, selected as described above, was crossed to strains carrying known mapped markers for every chromosome (strains shown in Table 1). Tetrads were examined for aberrant segregation patterns of the mapped gene. Each spore colony was allele tested for the original *ino1-14* and *ino1-28* alleles to confirm the stability of the disome.

RESULTS

Isolation of ino- *mutants*: A series of forty inositol requiring mutants were isolated after EMS mutagenesis of the "wild-type" strain (*ino*+ *ade5* **a**). Of these, 38 mutants showed 2:2 segregation in crosses and were retained for complementation analysis. Strain 398**a**, which contains a number of nonsense-suppressible markers, was mutagenized both by EMS and ultraviolet irradiation. From the EMS mutagenesis, 22 inositol-requiring mutants were selected; from the UV mutagenesis four additional mutants were obtained. These mutants were used in complementation analysis.

Complementation analysis: The distribution of newly isolated mutants into complementation groups is compared to the original series of inositol auxotrophs in Table 2. The results of each successive mutagenesis are quite similar. In each case, *ino1* mutants predominate, representing approximately 70% of the total. In

TABLE 2

Distribution of ino *mutants into complementation groups*

	ino1	*ino2*	*ino3*	*ino4*	*ino5*	*ino6*	*ino7*	*ino8*	*ino9*	*ino10*	New complementation groups*
Mutagenesis of strain *ade5* **a** with EMS	26	5	0	2	0	0	0	0	0	0	5
Mutagenesis of strain 398 **a** with EMS	16	1	0	1	0	0	0	0	0	0	5
Mutagenesis of strain 398 **a** with UV	1	2	0	0	0	0	0	0	0	0	1
Original series of mutants isolated by EMS mutagenesis (CULBERTSON and HENRY 1975)	36	2	1	7	1	1	1	1	1	1	—
Total/locus	79	10	1	10	1	1	1	1	1	1	11
% of total *ino* mutants	*ino1* 68%	*ino2* 9%		*ino4* 9%			all others 14%				

* New complementation groups are defined by mutants complementing representatives of *ino1–ino10*, originally described by CULBERTSON and HENRY (1975).

each mutagenesis, new alleles of *ino2* and *ino4* are obtained; however, new representatives of *ino3* and *ino5–ino10* were not isolated. Instead, in each case, new complemetation groups represented by only a single mutant were discovered. These mutants have not been tested for independent assortment in pairwise crosses with representatives of all other *ino* loci. Therefore, they are not assigned locus designations. The five new complementation groups obtained during EMS mutagenesis of strain *ade5* **a** in the present study were found to complement each other, as well as representatives of the original 10 groups. The six mutants described as representing new complementation groups from mutagenesis of strain 398 **a** have not been so exhaustively tested. Some were not outcrossed from the original 398 **a** strain in which they were isolated and thus have not been complementation tested among themselves. Therefore, it cannot be said with certainty that they represent single gene defects. Nonetheless, it is clear that mutations at many more than the 10 genes identified by CULBERTSON and HENRY (1975) give rise to the Ino$^-$ phenotype. However, only the *ino1*, *ino2* and *ino4* loci are represented by more than a single allele, and these three loci have received the most attention in the genetic studies described here, as well as in biochemical studies described elsewhere (DONAHUE and HENRY 1981).

Characterization of ino1, ino2 *and* ino4 *alleles for nonsense suppression*: The majority of *ino1* alleles were tested for nonsense suppression in crosses to UAA, UAG and UGA suppressor-bearing strains. The data are summarized in Table 3. None of the *ino1* alleles tested gave any evidence of suppressibility in these crosses. In contrast, two *ino2* alleles, *ino2–2* and *ino2–21*, were demonstrated to be suppressible in crosses with the same suppressor strains. Allele *ino2–2* was found to be an amber mutation, while allele *ino2–21* is an ochre mutation. Although the

TABLE 3

Crosses of ino1 *alleles with suppressor-bearing strains*

	Suppressor strains		
	FM6 (UAA)	FM11 (UAG)	A5364 (UGA)
Number of *ino1* alleles crossed to suppressor bearing strains	56	53	53
Number of *ino1* alleles exhibiting suppression as assessed by tetrad analysis of these crosses	0	0	0
Suppressible markers used to monitor suppressor segregation	*lys1* (UAA) *ade2* (UAA)	*his4* (UAG)	*leu2* (UGA)

The criteria used to ascertain suppression of the Ino⁻ phenotype is detailed in the MATERIALS AND METHODS section.

suppressible *ino2* mutants were derived from the same parent strain as the majority of the *ino1* mutants tested above, we made certain that no suppressor was present in the original parent strains. Both *ade5* **a** and *ade5* α were crossed to strains 398 **a** and 398 α bearing amber, ochre and opal mutations. No aberrant segregation of suppressible alleles was observed in tetrad analysis of these crosses.

We recognize that some suppressible mutations might not be detected in the crosses reported above, due to lack of efficient suppression by the specific suppressor used (CHATTOO *et al.* 1979). Therefore, we used a second method for detecting suppressibility that does not rely on the action of a single suppressor. This method is the co-reversion procedure described in the MATERIALS AND METHODS section. The procedure could be carried out only on those *ino*⁻ mutants that were isolated in the 398 **a** strain, since it requires the simultaneous presence of a large number of suppressible markers. The mutants tested were the 27 mutants isolated in the 398 **a** parent. As shown in Table 2, 17 of these mutants are *ino1* alleles. Of these, 15 showed no co-reversion with any suppressible marker. Mutant 398-U26, an *ino1* allele isolated after UV mutagenesis, produced a single spontaneous inositol revertant (out of three obtained) that appeared to have lost the UAA markers *arg 4–17* (UAA) and *lys1–1* (UAA) as well, However, in crosses, the revertant did not segregate the Ino⁻ phenotype. Furthermore, in a larger sample of 10 spontaneous revertants of the Arg⁻ phenotype, none had co-reverted for the Ino⁻ phenotype; whereas seven out of the 10 had co-reverted for the Lys⁻ phenotype. Furthermore, in crosses with UAA suppressor-bearing strain FM6, the *ino1–398 U26* allele showed no signs of suppressibility. Similar results were obtained with *ino1* mutant MC90, which was isolated after EMS mutagenesis. Of nine Ino⁺ revertants selected, all appeared to have simultaneously co-reverted for the Arg⁻ and Lys⁻ phenotype as well. One of the nine revertants had co-reverted for all UAG and UGA markers as well. When several of the Ino⁺ revertants were crossed to the "wild-type" strain carrying the *ade5* marker, the Ino⁻ phenotype did not segregate; whereas, the suppressible (Arg⁻ and Lys⁻) phenotypes did. In

addition, of 28 revertants of the Arg$^-$ phenotype tested, none had reverted for the Ino$^-$ phenotype; whereas, 26 had become Lys$^+$. Furthermore, strain MC90 did not respond to the UAA suppressor in a cross with strain FM6. Therefore, we concluded that *ino1* mutants MC90 and 398-U26 are not demonstrably suppressible. The reversion pattern of these two mutants, although aberrant, does not appear to be attributable to nonsense suppression, and it was not further investigated. In contrast, mutants MC120, an EMS-induced *ino4* allele, and 398–U101, an *ino2* allele produced by UV irradiation, both showed co-reversion patterns consistent with UAA mutations, in screening large numbers of revertants selected against either the Arg or the Ino phenotypes. These two mutants have not been further tested.

Mapping of the ino1 *locus*: Because primary *ino* loci are not centromere-linked (CULBERTSON and HENRY 1975), a method of mapping the *ino1* locus based on the selection of an aneuploid derivative of a heteroallelic, complementing *ino1* diploid was employed. The selection procedure for the putative *ino1–14/ino-128* $n+1$ haploid is described in the MATERIALS AND METHODS. Of 6000 haploid colonies screened, five were isolated that were *Ino*$^+$ and gave a strong mating response. All five were crossed to the standard laboratory haploid strains (**a** or α) carry the marker *ade5*, and tetrad analysis of the diploid was performed. Only one of the five strains segregated Ino$^-$ spores of both the *ino1–14* and *ino1–28* genotype. The segregation of Ino$^-$ and Ino$^+$ phenotypes shown in Table 4 is consistent with a $2n+1$ diploid of $+/-/-$ genotype, given two complementing alleles and no centromere linkage (CULBERTSON and HENRY 1973). All other markers in the cross, including mating type, segregated 2$^+$:2$^-$. The original disomic strain, designated Idl-1, has the genotype *lys2 ino1–14/ino1–28*. From among the tetrads shown in Table 4, a second haploid disomic strain of genotype **a** *ino1–14/ino1–28 ade5* was selected. These two strains were used in crossed with strains bearing markers for all 17 chromosomes.

The trisomic mapping analysis of the Ino$^+$ disomic strains crossed to strains bearing genetic markers for all the chromosomes of the yeast genome is shown in

TABLE 4

Observed and expected frequencies of ascal classes derived from crosses of an n + *1*, —/—, *disomic haploid containing complementing* ino1 *alleles to a normal prototrophic haploid*

	Segregation of Ino$^-$ phenotype ascal classes			
	4$^+$:0$^-$	3$^+$:1$^-$	2$^+$:2$^-$	1$^+$:3$^-$
Number of asci observed in original cross	2	7	10	1
Observed frequency (%)	10.0	35.0	50.0	5.0
Total number of asci observed in trisomic mapping study	2	33	45	8
Observed frequency (%)	2.3	37.5	51.1	9.1
Expected frequency (%)	13.3	33.3	46.7	6.7

Table 5. All chromosomes, except chromosome *X*, were ascertained to exist in single copy on the basis of normal $2^+:2^-$ segregation patterns for known mapped genetic loci. Three chromosome-*X* markers, *ilv3*, *met3* and *ura2*, showed aberrant segregation patterns in three independent crosses. Tetrads from all crosses were analyzed for the segregation of the inositol-requiring phenotype, and auxotrophic spores were analyzed for the segregation of the inositol-requiring phenotype, and auxotrophic spores were allele tested for the *ino1–14* and *ino1–28* alleles to confirm the presence of the aneuploid chromosome. In all cases, aberrant segregation of the inositol auxotrophy was observed. The total number of tetrad classes observed is compiled in Table 4, and expected frequencies of classes are compared. The observed frequencies of ascal classes do not precisely match the theoretical values. These values may partially reflect some instability of the $n + 1$ haploid or the $2n + 1$ diploid.

The disomic strains tend to lose the extra chromosome when maintained under nonselective conditions. In more than 90% of all the haploid Ino^- segregants, the chromosome lost was the one bearing the *ino1–14* allele. The relatively high rate of loss of the disomic chromosome may well explain (or partially explain) the excess of $2^+:2^-$ tetrads and the deficiency of $4^+:0^-$ tetrads reported in Table 4. However, when selective pressure is maintained (*i.e.*, the strain is kept on inositol-free medium), the disomic strain is sufficiently stable for most genetic manipulations. The occasional revertant or recombinant Ino^+ clones can be distinguished from *ino1–14/ino1–28* disomic clones because the Ino^+ phenotype of the complementing alleles is temperature sensitive. At 35°, the disomic *ino1–14/ino1–28*

TABLE 5

Crosses of the n+1 ino1-14/ino1-28 *disomic strains to chromosome marker strains*

Chromosome	Marker(s)	Tetrads (+:−)			
		2:2	4:0	3:1	1:3
I	*ade1*	10	0	0	0
II	*his7, lys2*	28	0	0	0
III	*MAT*	98	0	0	0
IV	*trp1*	10	0	0	0
V	*his1*	7	0	0	0
VI	*cdc14*	10	0	0	0
VII	*ade5*	19	0	0	0
VIII	*arg4*	10	0	0	0
IX	*lys1*	7	0	0	0
X	*ilv3*	2	5	1	0
	met3	2	3	0	0
	ura2	3	6	9	0
XI	*ura1*	13	0	0	0
XII	*asp5*	15	0	2	0
XIII	*rna1*	12	0	0	0
XIV	*pet8, lys9*	11	0	0	0
XV	*ade2, arg1*	21	0	0	0
XVI	*aro7*	15	0	0	1
XVII	*met2*	12	0	0	1

strains will not grow on INO⁻ medium; whereas, they will grow at 25° and 30°. Wild-type revertants grow on INO⁻ medium at all three temperatures. Of course, the Ino⁻ haploid segregants will not grow on INO⁻ medium at any temperature. Thus, the disomic clones can always be distinguished and selected from nondisjunctant or revertant clones. Furthermore, the chromosome-*X* disomic strain reported here is stable with respect to all other chromosomes. In all of the crosses performed, no instance of aneuploidy for any other chromosome was detected. In addition, spore viability in general was 90% or more in the crosses from which the data in Table 5 is derived.

In order to place the *ino1* locus on the chromosome-*X* map, a series of crosses was made between *ino1* strains and strains carrying other chromosome-*X* markers. Tetrad analysis revealed no linkage between *ino1* and *ilv3*, *met3*, *cdc8* or *cdc11*; linkage of *ino1* to *ura2* was detected. We were informed of linkage of *cdc6* to *ura2* by G. KAWASAKI (personal communication; KAWASAKI 1980), and of *arg3* to *ura2* by F. MESSENGUY and F. HILGER (personal communication). These investigators kindly supplied the appropriate strains, and crosses were made between the various markers. These data are displayed in Table 6. The *ino1* locus is 11.1 cM from *ura2* and 21.8 cM and *cdc6*, using the mapping formulas of PERKINS (1949). The relative distance between *cdc6* and *ura2* is quite similar to the results of KAWASAKI (1980), who reported a map distance of 25 cM between *ura2* and *cdc6*. F. MESSENGUY and F. HILGER (personal communication) report that they have detected 25% recombination between *ura2* and *arg3*. This distance is consistent with the order of markers given in Figure 1 and is similar in magnitude to the map distance obtained in our crosses. The data allow an ordering of the four loci on chromosome *X* as shown in Figure 1, the new map for chromosome *X*. None of the markers displays centromere linkage; thus, it is not possible on the basis of these data to determine the order of this group of genes with respect to the centromere. Crosses were not performed with *SUP7* or *mak17*.

TABLE 6

Tetrad analysis of crosses involving markers on chromosome X

Cross	Tetrads given as total PD:NPD:TT for marker pairs					
	ino1:*ura2*	*ino1*:*cdc6*	*ino1*:*arg3*	*cdc6*:*ura2*	*cdc6*:*arg3*	*ura2*:*arg3*
ino1 cdc6 lys2 **a** × *arg3*	——	24:0:22	14:1:31	——	9:6:31	——
arg3 α × *ura2 lys2* **a**	——	——	——	——	——	17:1:28
inol cdc6 lys2 **a** × *ura2*	57:0:16	45:0:28	——	31:0:42	——	——
ino1 lys2 α × *ura2* **a**	45:0:13	——	——	——	——	——
ino1 lys2 α × *cdc6* **a**	——	12:0:13	——	——	——	——
Totals	120:0:29	81:0:63	14:1:31	31:0:42	9:6:31	17:1:28
$\frac{6NPD+TT}{2 \text{ Total}}$	11.1	21.8	40.2	28.8	72.8	32.6

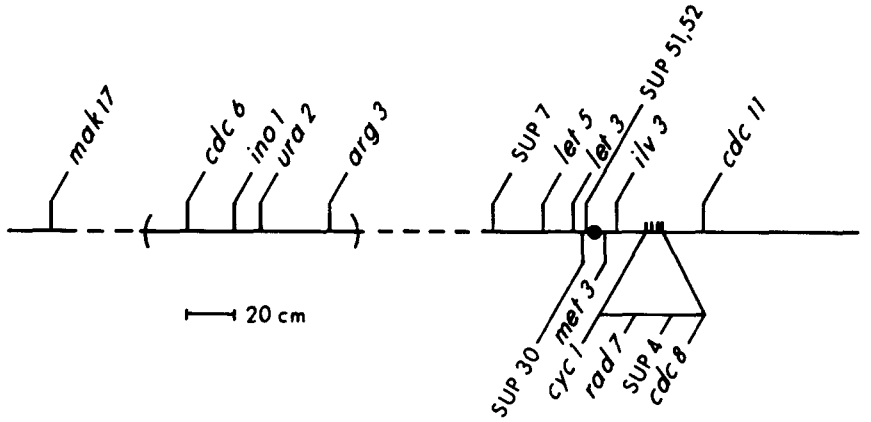

FIGURE 1.—Map of chromosome *X* of *S. cerevisiae*. Relative positions of *ino1*, *ura2*, *arg3* and *cdc6*, based upon recombination frequencies given in Table 6. The remainder of the map is based on data of HAWTHORNE and MORTIMER (1968); MORTIMER and HAWTHORNE (1973); LAWRENCE *et al.* (1975); LIEBMAN *et al.* (1977) and WICKNER (1979). Parentheses ( ) indicate that the order relative to the centromere is not yet established. Dashed line indicates region where precise map distance is not established.

DISCUSSION

The *ino1* locus has been shown by trisomic analysis to be located on chromosome *X*. In performing the analysis, we produced moderately stable $n + 1$ strains disomic for chromosome *X*. We have shown that these disomic strains contain no aneuploidy other than that for chromosome *X*. These disomic strains are not completely stable with respect to the chromosome *X* disomy. However, we have a selective method for maintaining and testing for the presence of the complementing *ino1* alleles. Thus, these strains could be successfully employed in studies of gene dosage or nondisjunction of chromsome *X*. Pairwise crosses with markers on chromosome *X* show that *ino1* is located between *ura2* and *cdc6*, and the new map of that arm of chromosome *X* is presented in Figure 1.

Biochemical and immunological data in this laboratory have shown that the *ino1* locus is the structural gene for inositol-1-phosphate synthase, the major enzyme involved in isositol biosynthesis (DONAHUE and HENRY 1981). The enzyme is a tetramer of 240,000 daltons comprised of identical subunits. The gene product of the *ino1* locus is the 62,000 dalton subunit of this enzyme. The *ino* loci, other than *ino1*, have yet to be explained biochemically. Some may be defective in the complex regulatory machinery controlling the level of inositol-1-phosphate synthase activity (CULBERTSON, DONAHUE and HENRY 1976 a & b). In addition, evidence accumulated in this laboratory shows that *ino2* and *ino4* mutants have complex pleiotrophic defects in phospholipid metabolism (HENRY *et al.* 1981).

The failure to detect nonsense mutants at the *ino1* locus is an unexplained phenomenon. It is hard to believe that a failure to obtain nonsense mutants is due to the lack of appropriate mutable sites in a gene encoding a product of 62,000

daltons. Normally, among mutants at a variety of loci in yeast, the frequency of suppressible alleles is 15% to 50%. The proportionate occurrence of such mutants has been extensively discussed by CHATTOO *et al.* (1979). In the present report, more than 65 allelic representatives of *ino1* were screened by one of two different methods for suppressibility. Since no suppressible alleles were detected, the proportion of suppressible mutants at this locus must be less than 2%. The failure to find suppressible mutants cannot be attributed to the screening methods used since, in a much smaller sample of *ino2* alleles, suppressible mutants of both the ochre and amber variety were detected.

Two other loci in yeast are characterized by a lack of suppressible alleles: *can1* and *his1*. Only a few nonsense suppressible alleles have been identified for the *can1* locus; whereas, not one nonsense mutation has ever been identified for the *his1* locus (SNOW 1978). The *can1* locus encodes the genetic information for the synthesis of arginine permease, a membrane-associated protein that transports the amino acid arginine. It is assumed that the relative lack of nonsense mutant representation at this locus might indicate a structural function of the protein for maintenance of membrane integrity. The *his1* locus encodes the genetic information for the first enzymatic function of histidine biosynthesis, phosphoribosyl transferase (FINK 1964). The biosynthetic pathways of histidine, arginine, tryptophan and lysine have been shown to be coordinately regulated by mechanisms of represssion and derepression. Starvation of cells for either histidine, tryptophan, lysine or arginine results in the coordinate derepression of the four biosynthetic pathways (WOLFNER *et al.* 1975). The *his1* gene product is sensitive to feedback inhibition by histidine (RASSE-MESSENGUY and FINK 1974). Mutants (*tra*) of *S. cerevisiae* resistant to the histidine analog triazolealanine have been shown to be resistant to feedback control (RASSE-MESSENGUY and FINK 1974) and are fully derepressed for the enzymes of the four amino acid biosynthetic pathways (WOLFNER *et al.* 1975). Additionally, the mutants are temperature sensitive for growth, being defective in the cell cycle early in the G_1 phase. This class of mutants has been shown to be tightly linked to the *his1* locus (FOGEL, LAX and HURST 1978; LAX and FOGEL 1978; RASSE-MESSENGUY and FINK 1974; WOLFNER *et al.* 1975). The lack of nonsense mutants at this locus suggests that the *his1* gene product has an additional function aside from its catalytic function in histidine biosynthesis. The additional function would appear to be essential for coordinating amino acid biosynthesis with other cellular processes in order to maintain a properly balanced cell division cycle.

The absence of suppressible alleles of the *ino1* locus may be explained in a similar fashion, *i.e.*, the *ino1* gene product may play some role in addition to its biosynthetic function. It might, for example, be involved in some manner in coordinating the biosynthesis of phospholipids derived from inositol or in coordinating phospholipid biosynthesis with general growth and division. Whatever the function, to explain the absence of suppressible alleles, the gene product must be essential to some aspect of growth or metabolism, and the total absence of the gene product must not be correctable by the presence of inositol in the growth medium. Such a postulated essential function can be identified only by the isolation of

appropriate conditional lethal mutants linked to *ino1*. Efforts to obtain such mutants are in progress.

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