JOURNAL OF BACTERIOLOGY, Nov. 1983, p. 498–506
0021-9193/83/110498-09$02.00/0

Vol. 156, No. 2

Conservation of Genetic Linkage in Nonisogenic Isolates of *Candida albicans*

RUSSELL POULTER* AND VICKIE HANRAHAN

Department of Biochemistry, University of Otago, Dunedin, New Zealand

Received 15 February 1983/Accepted 9 August 1983

A number of laboratories are now engaged in the genetic analysis of *Candida albicans*. This diploid yeast, the major fungal pathogen of humans, is imperfect. Parasexual techniques have been devised for complementation and recombination analysis in this organism. This paper attempts to address the question of the extent to which nonisogenic strains of *C. albicans* have conserved a common genetic map. This analysis is a prerequisite for the integration of work done in different laboratories and may also provide useful information on the taxonomy of the genus *Candida*. The paper also reports the analysis of an interspecific hybrid between *C. albicans* and *Candida stellatoidea*. The method employed in these studies was the analysis of the mitotic recombination relationships of a group of linked genes and their centromere. Strains carrying linked auxotrophic mutations were fused with isogenic and nonisogenic complementary strains to form tetraploids. The mitotic recombination analyses of these tetraploids suggest that in the isolates studied the genetic map is conserved. A comparison of tetraploid and diploid mitotic recombination analyses is also presented.

Candida albicans, a common causative agent of mycotic infection, is generally considered to exist naturally only in the asexual state (2). Recently, parasexual protocols have been reported for this organism (5, 7, 8, 10). The protocols for parasexual analysis all employ enzymic digestion to produce protoplasts, followed by the induction of protoplast fusion by polyethylene glycol. Fusion of complementing auxotrophs generates prototrophic fusion products which can be selected on appropriate media. Such fusion products are either multinucleate heterokaryons or, more rarely, uninucleate cells which have undergone karyogamy. Uninucleate prototrophic fusion products are spontaneously unstable, giving rise with a low frequency to auxotrophic derivatives (8). The mitotic instability of fusion products can be increased by exposure to UV irradiation (8). It has been suggested that the predominant form of mitotic instability is mitotic crossing-over (7, 10). The ploidy of *C. albicans* remains a matter of debate, some reports supporting haploidy (10) and others diploidy (1, 4, 7, 9, 11, 12). It has been suggested that protoplast fusion of naturally diploid cells gives rise to euploid tetraploids (8), but it may also give rise to aneuploid products (10).

The first report of genetic linkage in *C. albicans* was provided by Sarachek et al. (10) with derivatives of *C. albicans* WC. The report indicated that the *arg* allele carried by WC-5-4 and the *ade* allele carried by WCR-1-74 show *trans* linkage in the isogenic prototrophic fusion product of these two strains. Poulter et al. (7) reported the first *C. albicans* strain carrying linked auxotrophic mutations. The analysis employed derivatives of ATCC 10261 and provided evidence for linkage of *ade* and *met* alleles in a multiply auxotrophic strain, ATCC 10261 (here termed 10261) hOG24 (Ade^- Pro^- Met^-), and its derivative, 10261 hOG45 (Ade^- Pro^- Met^- His^- Lys^-). This interpretation was based on two forms of mitotic recombination analysis. First, fusion was performed between 10261 hOG24 (Ade^- Pro^- Met^-) and an isogenic strain, 10261 hOG282 (His^-); from this fusion a tetraploid product was isolated. After exposure to UV irradiation this prototrophic tetraploid heterozygote gave rise to Ade^- and Ade^- Met^- derivatives. Second, reversion of the diploid strain hOG45 to prototrophy was performed sequentially. The diploid heterozygotes produced by reversion were found to be unstable, some giving rise to Ade^- derivatives and some to both Ade^- and Ade^- Met^- derivatives. It was suggested that in the former revertant class the *ade* and *met* alleles were *trans* linked and that in the latter revertant class they were *cis* linked (7). In summary, both the tetraploid (fusion) mitotic crossing-over analysis and diploid (reversion) mitotic crossing-over analysis suggest linkage of the *ade* and *met* alleles carried by 10261 hOG24 (Ade^- Pro^- Met^-). These recombination analy-

ses further suggest that the linkage sequence is centromere-*met-ade*.

C. albicans is an imperfect yeast, and it is of interest to determine whether the arrangement of the genetic material has been conserved in different isolates or whether rearrangements have occurred. In a sexually reproducing organism there is considerable selective pressure against genetic rearrangement. In organisms like *C. albicans* which do not display a sexual cycle there might be little selective pressure for the conservation of genetic arrangement. This analysis is of interest for a number of reasons: it might help elucidate the taxonomic division of the genus *Candida*; it might facilitate strain discrimination within the species *C. albicans*; and it is necessary for the cooperative study of the genetics of this organism. The present methods of parasexual analysis permit study of the patterns of genetic linkage found in a variety of nonisogenic isolates.

The present paper describes the isolation from a prototroph (ATCC 22114, here termed 22114) of two triple auxotrophs (hOG311 and hOG357) requiring adenine, arginine, and methionine. The analysis of complementation by protoplast fusion supports the strain designations: 10261 hOG24 *ade1 pro met1*, 22114 hOG311 *ade1 arg1 met1*, 22114 hOG357 *ade1 arg1 met1*, WCR-1-74 *ade1 thr*, and WC-5-4 *his arg1*. This paper describes the formation of tetraploid strains by fusion of 10261 hOG24, 22114 hOG311, or 22114 hOG357 with complementing auxotrophs derived from seven nonisogenic strains. The analysis of the UV irradiation-induced auxotrophic derivatives of these prototrophic tetraploids derived from isogenic and nonisogenic fusions is described. In addition, the analysis of the UV-induced auxotrophic derivatives of diploid prototrophic revertants of 10261 hOG24 and 22114 hOG311 is presented. These analyses indicate that the arrangement of these genes in the seven *C. albicans* strains has been conserved. The genetic linkage suggested is centromere-*met1-arg1-ade1*. In addition, the analysis of a fusion between 10261 hOG24 and an auxotrophic derivative of *Candida stellatoidea* is presented. *C. stellatoidea* is generally considered to be closely related to *C. albicans* (3). The analysis suggests the conservation of the sequence centromere-*met1-ade1* in *C. stellatoidea*.

MATERIALS AND METHODS

Strains. The *C. albicans* mutants are derived from a number of prototrophic isolates, ATCC 10261, ATCC 22114, WC (10), A72 (a strain provided by A. Cassone), FC18 (11), and two Otago, New Zealand isolates, A4843 and U10279. The auxotrophic derivative of *C. stellatoidea* was produced from strain CDC B2213 (provided by K. A. Hobbis, Mycology Laboratory, National Health Institute, Wellington, New Zealand). Strains WCR-1-74 (ATCC 44990) and WC-5-4 (ATCC 44989) were obtained from the American Type Culture Collection. Strain A515 was obtained from P. T. Magee. The origin of some mutant auxotrophic strains isolated in this laboratory from 10261 has been described previously (7, 8). The auxotrophic strains employed in this analysis are described in Table 1, together with their origins. *C. stellatoidea* CDC B2213 grew on yeast extract-peptone (YEP) agar with the expected stellate morphology and failed to grow on medium with sucrose as the sole carbon source. The growth rate was, as expected, significantly slower than that of *C. albicans* (3).

Media and culture conditions. Long-term maintenance of all strains was effected in sterile 50% glycerol at −20°C. Strains were maintained for short periods on YEP agar slopes or plates at 4°C. For analysis of auxotrophs the organism was grown on a defined glucose-salts-biotin (GSB) medium supplemented as appropriate with 3×10^{-4} M adenine and 3×10^{-3} M of specific amino acids.

Isolation of auxotrophic strains. Strains were mutagenized by standard protocols (7) using either UV irradiation or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. All adenine-requiring auxotrophs described in this work grew as red colonies in YEP medium.

Reversion of auxotrophic alleles to prototrophy. The auxotrophic mutations present in hOG24, hOG311, and WCR-1-74 were reverted by spreading 10^4 cells per plate on selective medium lacking one essential nutrient, exposing to UV (60 s at 13 ergs mm^{-2} s^{-1}), and isolating revertant colonies prototrophic for the omitted nutrient. This procedure was repeated serially until the desired number of auxotrophic alleles had been reverted to prototrophy.

Induction of mitotic instability in the diploid revertants and tetraploid fusion products. Colonies of both the diploid and tetraploid prototrophic adenine heterozygotes were white on GSB. The spontaneous mitotic instability of the prototrophic diploid revertants, as measured by the appearance of red sectors on YEP, was low (1 sector per 10^4 colonies). The spontaneous mitotic instability of prototrophic tetraploid fusion products was so low that it could not be determined with accuracy. The frequency of mitotic instability of both diploid and tetraploid strains was increased by exposure to UV light immediately after plating cells onto YEP (60 s at 13 ergs mm^{-2} s^{-1}). UV-induced red adenine auxotrophs either occurred as pure colonies or as sectors on YEP agar. For some purposes both the red and white components of sectored colonies were purified and analyzed.

Protoplast formation, fusion, and regeneration. Procedures for protoplast formation, fusion, and regeneration were undertaken with published methods (7). *C. stellatoidea* formed osmotically fragile protoplasts when treated with standard protoplasting procedures.

Characterization of auxotrophic mutants. Mutants were characterized by adding amino acids (3×10^{-3} M) or other metabolites to GSB medium or supplemented GSB medium. The requirements of various strains are listed below.

10261 hOG6 (Ade^- Ura^- Lys^-) required adenine, uracil (3×10^{-2} M), and lysine. Cytosine could not be substituted for uracil, a result attributed to competitive uptake between adenine and cytosine (6). It is believed

TABLE 1. Strain designation, origin, phenotype, and genotype of strains used in this analysis

Strain[a]	Origin	Phenotype	Genotype
C. albicans			
10261			
hOG1	ATCC 10261	Ade$^-$	*ade1*
hOG2	hOG1	Ade$^-$ Pro$^-$	*ade1 pro*
hOG4	hOG1	Ade$^-$ Ura$^-$	*ade1 ura*
hOG6	hOG4	Ade$^-$ Ura$^-$ Lys$^-$	*ade1 ura lys*
hOG24	hOG2	Ade$^-$ Pro$^-$ Met$^-$	*ade1 pro met1*
hOG25	hOG24	Ade$^-$ Pro$^-$ Met$^-$ His$^-$	*ade1 pro met1 his*
hOG45	hOG25	Ade$^-$ Pro$^-$ Met$^-$ His$^-$ Lys$^-$	*ade1 pro met1 his lys*
hOG282	ATCC 10261	His$^-$	*his*
22114			
hOG310	ATCC 22114	Met$^-$	*met1*
hOG311	hOG310	Ade$^-$ Arg$^-$ Met$^-$	*ade1 met1 arg1*
hOG312	ATCC 22114	Met$^-$ Thr$^-$	*hom*
hOG318	hOG310	Arg$^-$ Met$^-$	*arg1 met1*
hOG357	hOG318	Ade$^-$ Arg$^-$ Met$^-$	*ade1 arg1 met1*
A72 hOG313	A72	Arg$^-$	*arg2*
A4843 hOG463	A4843	Lys$^-$	*lys*
U10279 hOG319	U10279	Ino$^-$	*ino*
FC18			
A50	FC18	Trp$^-$	*trp*
A515	A50	His$^-$ Trp$^-$	*his trp*
WC			
WCR-1	WC	Ade$^-$	*ade1*
WC-5	WC	His$^-$	*his*
WCR-1-74	WCR-1	Ade$^-$ Thr$^-$	*ade1 thr*
WC-5-4	WC-5	Arg$^-$ His$^-$	*arg1 his*
C. stellatoidea			
hOG430	CDC B 2213	Pro$^-$	*pro*
hOG465	hOG430	Pro$^-$ His$^-$	*pro his*
Tetraploid prototrophs			
dOG308	hOG282 × hOG24		
dOG321	hOG282 × hOG311		
dOG322	hOG312 × hOG24		
dOG323	hOG312 × hOG311		
dOG324	hOG313 × hOG24		
dOG325	hOG313 × hOG311		
dOG506	hOG465 × hOG24		
dOG533	hOG319 × hOG357		
dOG534	A515 × hOG357		
dOG535	hOG463 × hOG357		
dOG536	WC-5-4 × hOG24		

[a] The strain prefix ATCC and the clinical isolate prefix (e.g., 10261) are commonly omitted.

that uracil uptake is due to passive diffusion, and therefore there is no competition between adenine and uracil uptake. Uridine (3×10^{-3} M) could be substituted for uracil.

10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$) required adenine, proline, and methionine. Homocysteine, an intermediate in methionine biosynthesis, could not be used in place of methionine.

22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$) required adenine, arginine, and methionine. Homocysteine could not be used in place of methionine. Ornithine, an intermediate in arginine biosynthesis, could not be used in place of arginine. The Arg$^-$ phenotype was slightly leaky after prolonged incubation. The phenotype was expressed with greater clarity if 3×10^{-3} M canavanine was added to the medium. Canavanine is an analog of arginine and an inhibitor of arginine biosynthesis. Prototrophic strains were unaffected by this concentration of canavanine.

22114 hOG357 (Ade$^-$ Arg$^-$ Met$^-$) resembled hOG311 except that the independently isolated *arg* mutation was nonleaky.

WC-5-4 (Arg$^-$ His$^-$) required arginine and histidine; ornithine could not be used in place of arginine.

A72 hOG313 (Arg$^-$) required arginine. Ornithine could be substituted for arginine.

WCR-1-74 (Ade$^-$ Thr$^-$) required adenine and threonine; homoserine, an intermediate in both threonine and methionine biosynthesis, could not be used in place of threonine.

22114 hOG312 (Met$^-$ Thr$^-$) required methionine and threonine. Homoserine could be used in place of both of these supplements. Homocysteine could be used in place of methionine.

A4843 hOG463 (Lys$^-$) required lysine.
U10279 hOG319 (Ino$^-$) required inositol.
FC18 A515 (His$^-$ Trp$^-$) required histidine and tryptophan.
10261 hOG282 (His$^-$) required histidine.
C. stellatoidea CDC B2213 hOG465 (Pro$^-$ His$^-$) required proline and histidine.

RESULTS

Complementation analysis of auxotrophs. The results of the various fusions performed are described in Table 2. Tetraploid prototrophic products of 11 of the fusions, designated dOG308, 321, 322, 323, 324, 325, 506, 533, 534, 535, and 536, were used in subsequent analysis. The colonial morphology and sucrose utilization of dOG506 (the hybrid "*Candida albastella*") resembled that of *C. albicans*. The complementation analysis of the phenotypic characters is described below.

(i) Arginine. The data presented in Table 2 suggest that the arginine mutations carried by 22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$), 22114 hOG357 (Ade$^-$ Arg$^-$ Met$^-$), and WC-5-4 (Arg$^-$ His$^-$) are allelic since they failed to complement. In addition, the strains cannot utilize ornithine in place of arginine (see above). The gene corresponding to this mutant allele is designated *arg1* in Table 1. In contrast, the arginine mutation carried by A72 hOG313 (Arg$^-$) complements with 22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$). A72 hOG313 (Arg$^-$) can grow on ornithine-supplemented medium, and it is therefore described in Table 1 as carrying a defective *arg2* allele.

(ii) Adenine. It is apparent from the lack of complementation that the adenine mutations carried by 22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$), 22114 hOG357 (Ade$^-$ Arg$^-$ Met$^-$), 10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$), and WCR-1-74 (Ade$^-$ Thr$^-$) are allelic. These strains are described as carrying *ade1* mutant alleles in Table 1.

(iii) Methionine. The methionine mutation carried by 22114 hOG310 (Met$^-$), and therefore its derivatives 22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$) and 22114 hOG357 (Ade$^-$ Arg$^-$ Met$^-$), is allelic to the 10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$) methionine mutation since hOG24 fails to complement with hOG310. These strains cannot use homocysteine in place of methionine; they are described as carrying *met1* alleles. In contrast, 22114 hOG312 (Met$^-$ Thr$^-$) shows complementation with both 10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$) and 22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$). The 22114 hOG312 (Met$^-$ Thr$^-$) strain can utilize homocysteine in place of methionine. The auxotrophic requirements of 22114 hOG312 (Met$^-$ Thr$^-$), which appeared as a result of a single mutagenic step, are satisfied by the single supplement homoserine. Strain hOG312 is described in Table 1 as carrying a *hom* mutant allele.

Induction of mitotic instability in prototrophic fusion products and the analysis of auxotrophic requirements. The 11 prototrophic tetraploid strains designated dOG308, 321, 322, 323, 324, 325, 506, 533, 534, 535, and 536 reflect various isogenic and nonisogenic fusions. These strains were exposed to UV irradiation immediately after plating on YEP. After incubation of these plates, red derivatives were isolated and analyzed for their auxotrophic requirements. The results of this analysis are given in Tables 3 and 4.

The results described in Tables 3 and 4 are interpreted in the following manner. The red (Ade$^-$) character appears in the derivatives as the result of the loss of the two functional *ade1*$^+$ alleles present in the tetraploid heterozygote. In all of these tetraploids the loss of the *ade1*$^+$ alleles is frequently associated with the loss of

TABLE 2. Complementation analysis based on fusion of strains on minimal medium (GSB)

Strain	Complementation[a]		
	10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$)	22114 hOG311 (Ade$^-$ Arg$^-$ Met$^-$)	22114 hOG357 (Ade$^-$ Arg$^-$ Met$^-$)
10261 hOG24 (Ade$^-$ Pro$^-$ Met$^-$)	–	–	–
10261 hOG6 (Ade$^-$ Ura$^-$ Lys$^-$)	–	–	–
22114 hOG310 (Met$^-$)	–	–	–
WC-5-4 (Arg$^-$ His$^-$)	+ (dOG536)	–	–
WCR-1-74 (Ade$^-$ Thr$^-$)	–	–	–
10261 hOG282 (His$^-$)	+ (dOG308)	+ (dOG321)	
22114 hOG312 (Met$^-$ Thr$^-$)	+ (dOG322)	+ (dOG323)	
A72 hOG313 (Arg$^-$)	+ (dOG324)	+ (dOG325)	
U10279 hOG319 (Ino$^-$)			+ (dOG533)
A4843 hOG463 (Lys$^-$)			+ (dOG535)
B2213 hOG465 (Pro$^-$ His$^-$)	+ (dOG506)		+
FC18 A515 (His$^-$ Trp$^-$)			+ (dOG534)

[a] +, Complementation; – denotes its absence.

TABLE 3. Analysis of red (adenine) auxotrophs derived from tetraploid prototrophs having hOG24 as one parent

Strain	Origin	No. of reds analyzed	No. of auxotrophs[a]		Frequency, $Ade^-/Ade^- + Ade^- Met^-$
			Ade^-	$Ade^- Met^-$	
dOG308	10261 hOG24 ($Ade^- Pro^- Met^-$) × 10261 hOG282 (His^-)	84	11	73	0.13
dOG322	10261 hOG24 ($Ade^- Pro^- Met^-$) × 22114 hOG312 ($Met^- Thr^-$)	64	12	52	0.19
dOG324	10261 hOG24 ($Ade^- Pro^- Met^-$) × A72 hOG313 (Arg^-)	96	15	81	0.16
dOG506	10261 hOG24 ($Ade^- Pro^- Met^-$) × B2213 hOG465 ($Pro^- His^-$)	50	7	43	0.14
dOG536	10261 hOG24 ($Ade^- Pro^- Met^-$) × WC-5-4 ($Arg^- His^-$)	79	22	57	0.28

[a] Only the Ade^- and Met^- phenotypes are tabulated. The following additional auxotrophies occurred: dOG308, none; dOG322, one $Ade^- Met^- Thr^-$; dOG324, six $Ade^- Arg^- Met^-$ and one $Ade^- Arg^- Met^- Pro^-$; dOG506, none; dOG536, none.

the *met1*$^+$ alleles; this suggests that the *ade1* and *met1* loci are linked in all seven *C. albicans* isolates, and also in the *C. stellatoidea* isolate. The similarity of the relative Ade^- to $Ade^- Met^-$ frequency in the red derivatives of the 11 tetraploid strains suggests that the relative position of these genes with respect to each other and the centromere has also been conserved in the eight isolates. The conserved sequence is centromere-*met1-ade1*. Two mitotic crossing-over events between the centromere and the proximal gene (*met1*) would generate $Ade^- Met^-$ derivatives. Mitotic crossing-over in the segment between *met1* and *ade1* would generate simple Ade^- derivatives, with the *met1* gene remaining heterozygous.

Fusions involving 22114 hOG311 ($Ade^- Arg^- Met^-$) or 22114 hOG357 ($Ade^- Arg^- Met^-$) as one parent (Table 4) revealed information concerning the linkage of the *arg1* gene. These tetraploids gave rise, after UV irradiation, to similar frequencies of Ade^-, $Ade^- Arg^-$, and $Ade^- Arg^- Met^-$ derivatives. This result suggests that the relative sequence is centromere-*met1-arg1-ade1*. If the data of Table 3 and Table 4 are aggregated, then the frequency ratio of Ade^- to $Ade^- + Ade^- Met^-$ is 161/803 (0.2). The aggregated Ade^- to $Ade^- + Ade^- Arg^-$ frequency ratio from Table 4 is 34/431 (0.08). This suggests a tetraploid mitotic recombination map with the following relative positions: centromere to *met1*, 0.80; *met1* to *arg1*, 0.12; *arg1* to *ade1*, 0.08. The tetraploid strain dOG536 carries the *arg1* allele in *trans* to the *ade1* and *met1* alleles. This strain was exposed to UV irradiation on YEP medium, and six sectored colonies (approximately half red, half white) were analyzed. Of the six sectored colonies, three were found to consist of an Ade^- (red) sector and a (white) prototrophic sector; one was found to consist of an $Ade^- Met^-$ (red) sector and a (white) prototrophic sector; two were found to consist of an $Ade^- Met^-$ (red) sector and an Arg^- (white) sector. This result supports that of Sarachek et al. (10) and confirms the conservation of the sequence centromere-*met1-arg1-ade1* in isolate WC. The result also supports the belief that mitotic crossing-over is the predominant type of mitotic instability induced by UV irradiation, since only this mechanism would give rise to the observed reciprocal products.

Reversion of auxotrophic alleles in diploid strains. The following reversions were performed by the protocol described above. Strain 10261 hOG24 ($Ade^- Pro^- Met^-$) was reverted to give six strains that had lost the requirement for adenine. These strains were designated hOG24-1, -2, -3, -4, -5, and -6. Each of these was then reverted for methionine; several revertants were isolated from some of the strains. These Pro^- revertants were given an additional suffix; for example, hOG24-1.1 and hOG24-1.2 are two independent Pro^- revertants of hOG24-1 ($Pro^- Met^-$). A similar reversion sequence was employed with hOG311 ($Ade^- Arg^- Met^-$) to give

TABLE 4. Analysis of red (adenine) auxotrophs derived from tetraploid prototrophs having hOG311 or hOG357 as one parent

Strain	Origin	No. of reds analyzed	No. of red auxotrophs[a]				Frequency,[b] $Ade^-/Ade^- + Ade^- Arg^-$	Frequency,[c] $Ade^-/Ade^- + Ade^- Met^-$
			Ade^-	$Ade^- Met^-$	$Ade^- Arg^-$	$Ade^- Arg^- Met^-$		
dOG321	22114 hOG311 ($Ade^- Arg^- Met^-$) × 10261 hOG282 (His)	73	6	0	8	59	0.08	0.19
dOG323	22114 hOG311 ($Ade^- Arg^- Met^-$) × 22114 hOG312 ($Met^- Thr^-$)	111	8	0	6	97	0.07	0.13
dOG325	22114 hOG311 ($Ade^- Arg^- Met^-$) × A72 hOG313 (Arg^-)	81	7	1	12	61	0.10	0.24
dOG533	22114 hOG357 ($Ade^- Arg^- Met^-$) × U10279 hOG319 (Ino^-)	59	5	0	21	33	0.09	0.44
dOG534	22114 hOG357 ($Ade^- Arg^- Met^-$) × FC18 A515 ($His^- Trp^-$)	52	4	1	9	38	0.10	0.25
dOG535	22114 hOG357 ($Ade^- Arg^- Met^-$) × A4843 hOG463 (Lys^-)	54	2	0	6	46	0.04	0.15

[a] Only the Ade^-, Arg^-, and Met^- phenotypes are tabulated. The following additional auxotrophies occurred: dOG321, three $Ade^- Arg^- Met^- His^-$; dOG323, three $Ade^- Arg^- Met^- Thr^-$; dOG325, none; dOG533, one $Ade^- Arg^- Ino^-$ and five $Ade^- Arg^- Met^- Ino^-$; dOG534, three $Ade^- Arg^- Met^- His^-$, one $Ade^- Arg^- Met^- His^- Trp^-$, and two $Ade^- Arg^- Met^- Lys^-$; dOG535, one $Ade^- Arg^- Lys^-$ and five $Ade^- Arg^- Met^- Lys^-$.

[b] To calculate this frequency the Ade^- and $Ade^- Met^-$ are aggregated, as are the $Ade^- Arg^-$ and $Ade^- Arg^- Met^-$ classes. Effectively, the Met^- status of the strains is therefore ignored.

[c] To calculate this frequency the Arg^- status of the strains is ignored.

four Arg^- revertants and two fully prototrophic revertants.

Recovery of red (adenine auxotroph) derivatives from revertant diploids and analysis of auxotrophic requirements. The Pro^- diploid revertant strains derived from hOG24 grew as white colonies on YEP medium. They gave rise spontaneously to red (adenine auxotroph) sectors on YEP medium, but only at a frequency of approximately 1 per 10^4 colonies. After plating on YEP and exposure to UV, all strains gave rise to red, adenine-requiring sectors at a high frequency. A number of red derivatives of each of the revertant diploid strains were isolated and analyzed for their nutritional requirements. Each isolate, as anticipated, required adenine; some isolates were found to have additional requirements (Table 5).

The data of Table 5 suggest the following points. Some of the revertant strains are interpreted to be carrying the *ade1* and *met1* mutant alleles in *cis* linkage since they displayed frequent coincident reappearance of the Ade^- and Met^- characters. The other revertants do not show such coincident reappearance and are considered to carry the *ade1* and *met1* alleles in *trans* linkage. Second, the data of the *cis*-linked strains support the gene order suggested by the tetraploid analysis, centromere-*met1*-*ade1*. Third, the data are statistically non-homogeneous with respect to the Ade^- and $Ade^- Met^-$ relative frequency in the *cis*-linked strains. This

TABLE 5. Analysis of red (adenine) auxotrophs derived from diploid (Pro^-) revertants of hOG24 (Ade^- Met^- Pro^-) and diploid (Arg^-) revertants of hOG311 (Ade^- Arg^- Met^-)

Revertant strain	Origin[a]	No. of red derivatives analyzed	Auxotrophs		Frequency, Ade^-/Ade^- + Ade^- Met^-
			Ade^-	Ade^- Met^-	
hOG24-1.1	hOG24-1	64	17	47	0.27
hOG24-1.2	hOG24-1	89	26	63	0.29
hOG24-1.3	hOG24-1	65	18	47	0.28
hOG24-1.4	hOG24-1	66	65	1	*trans*
hOG24-1.5	hOG24-1	34	10	24	0.29
hOG24-1.6	hOG24-1	115	28	87	0.24
hOG24-2.1	hOG24-2	105	18	87	0.17
hOG24-3.2	hOG24-3	103	14	89	0.14
hOG24-4.0	hOG24-4	102	18	84	0.18
hOG24-5.1	hOG24-5	100	40	60	0.40
hOG24-5.2	hOG24-5	97	47	50	0.49
hOG24-6.1	hOG24-6	50	16	34	0.32
hOG24-6.2	hOG24-6	50	32	18	0.64
hOG24-6.3	hOG24-6	51	19	32	0.37
hOG24-6.4	hOG24-6	60	59	1	*trans*
hOG24-6.5	hOG24-6	50	50	0	*trans*
hOG311-1.1	hOG311-1	106	32	74	0.30
hOG311-2.1	hOG311-2	95	45	50	0.47
hOG311-3.1	hOG311-3	102	46	56	0.45
hOG311-3.2	hOG311-3	48	48	0	*trans*

[a] hOG24-1, -2, -3, -4, -5, and -6 were independent (Pro^- Met^-) revertants of hOG24 (Ade^- Met^- Pro^-). All hOG24 derivatives described in this Table were Pro^-. hOG311-1, -2, and -3 were independent (Arg^- Met^-) revertants of hOG311 (Ade^- Arg^- Met^-). All hOG311 derivatives described in this Table were Arg^-.

last point, and a quantitative consideration of the data, will be returned to in the discussion.

Two fully prototrophic derivatives of 22114 hOG311 (Ade^- Arg^- Met^-), designated hOG427 and hOG428, were plated on YEP and exposed to UV irradiation. UV-induced red derivatives were analyzed and were found to be as follows: from hOG427, 39 Ade^-, 67 Ade^- Arg^-, and 41 Ade^- Arg^- Met^-; from hOG428, 21 Ade^-, 38 Ade^- Arg^-, and 41 Ade^- Arg^- Met^-. These data support the conclusion of the tetraploid data (Table 4), suggesting a relative gene order of centromere-*met1-arg1-ade1*.

WCR-1-74 (Ade^- Thr^-) was reverted on GSB-threonine medium, and five strains which had lost the requirement for adenine were isolated and purified. All five strains grew white on YEP. After exposure to UV irradiation they gave rise to a high frequency of red (Ade^-) derivatives. This behavior suggests that the revertants carry the *ade* allele heterozygously and indicates that WCR-1-74 is diploid.

DISCUSSION

The degree of conservation of genetic similarity between isolates of *C. albicans* can be studied by using strains carrying genetically linked auxotrophic mutations. The methods described in this paper have provided three such strains, 10261 hOG24 (Ade^- Pro^- Met^-) with linked *ade1* and *met1* alleles and the two strains 22114 hOG311 (Ade^- Arg^- Met^-) and hOG357 (Ade^- Arg^- Met^-) with linked *ade1*, *arg1*, and *met1* alleles. These strains carry an easily selected red adenine (*ade1*) auxotrophic allele at the distal end of the series of mutant loci; this facilitates visual selection of recombinants and permits the quantitative study of recombination frequencies.

Before this report only two examples of linkage have been described in *C. albicans*. Poulter et al. (7) reported *cis* linkage of the *ade1* and *met1* alleles in 10261 hOG24 and in hOG45, a derivative of hOG24. Sarachek et al. (10) reported *trans* linkage of the *ade* allele carried by WCR-1-74 and the *arg* allele carried by WC-5-4. The present study integrates the two previous analyses by selecting from a third distinct isolate a strain carrying mutant alleles corresponding to the 10261 *ade1* and *met1* loci and the WC *ade* and *arg* loci. This was achieved by the isolation of strains hOG311 (Ade^- Arg^- Met^-) and hOG357 (Ade^- Arg^- Met^-) from strain 22114. Complementation tests indicate that the *ade* mutations carried by 10261 hOG24, WCR-1-74, 22114 hOG311, and 22114 hOG357 are noncomplementing allelic series of a gene designated *ade1*. Complementation tests further indicate that the *arg* mutations carried by WC-5-4, 22114 hOG311, and 22114 hOG357 are noncomplementing alleles at a locus designated *arg1*. Similarly, complementation tests indicated that the *met* mutation carried by 10261 hOG24 is allelic

to the *met* mutation carried by 22114 hOG310 and its derivatives hOG311 and hOG357. These *met* alleles are assigned to the locus *met1*.

Isogenic fusions, for example, 10261 hOG24 (Ade^- Pro^- Met^-) with 10261 hOG282 (His^-), provide the simplest evidence of linkage between these loci. The prototrophic tetraploid products of such fusions are unstable. UV irradiation-induced mitotic crossing-over gives rise to red auxotrophic derivatives. The analysis of such red derivatives indicates the presence or absence of linkage between loci. Mitotic crossing-over results in the loss of the $ade1^+$ alleles and may involve coincident loss of prototrophic alleles carried heterozygously in *cis* with the $ade1^+$ alleles. The red derivatives may as a consequence show coincident reappearance of homozygosity for the *ade1* allele and for other linked mutant alleles carried heterozygously in *cis* with the *ade1* allele. Isogenic fusion analysis established that the sequence of genes is centromere-*met1-ade1* in 10261 hOG24 and centromere-*met1-arg1-ade1* in 22114 hOG311.

Nonisogenic fusions have also been performed between complementing auxotrophs. Nonisogenic tetraploids were subjected to UV irradiation, and red auxotrophic derivatives were isolated and analyzed. The results of the isogenic analyses resemble qualitatively and quantitatively the results of the analogous nonisogenic analyses. Thus the analysis of the isogenic tetraploid dOG308 (hOG24 × hOG282) resembles that of the analogous nonisogenic tetraploids, for example, dOG322 (hOG24 × hOG312) and dOG314 (hOG24 × hOG313). Mitotic crossing-over gives rise to coincident loss of the ade^+ and other *cis*-linked prototrophic alleles and provides evidence that the loci are linked in the diploid strain carrying these *cis*-linked functional alleles. The study of sectored colonies of dOG536 permits analysis of the *trans*-linked *ade1* and *arg1* alleles and confirms the linkage data provided by Sarachek et al. (10), that is, that *arg1* is linked to *ade1* and placed proximally. By using nonisogenic fusions employing as one parent hOG24, hOG311, or hOG357 and employing both *cis* and *trans* analysis, we have confirmed the sequence centromere-*met1-arg1-ade1* in the *C. albicans* strains analyzed, that is, 10261, 22114, A72, WC, FC18, A4843, and U10279.

The genetic map of the *C. albicans* chromosome analyzed is conserved in all of the isolates of disparate origin. If this conclusion applies to other linkage groups, recombination mapping will be of doubtful use in biotyping isolates of *C. albicans*, but the technique is of potential use in resolving the relationship between species of the genus *Candida*. The presence of a conserved genomic pattern and the ability to perform mitotic crossing-over would suggest close affinity between two species, and the absence of these properties would suggest the converse. The conservation of the sequence centromere-*met1-ade1* in both *C. albicans* and *C. stellatoidea* supports the belief that these two imperfect species are closely related.

The conservation of linkage in various isolates of *C. albicans* suggests that it will be possible to employ auxotrophs of disparate origin in the mapping of this organism and that it will therefore be possible to integrate research done in various laboratories. Both complementation and recombination are likely to prove useful in the analysis of *C. albicans*; the present paper is the first report of the definition of genes in *C. albicans* by phenotypic complementation and recombination analyses. The loci *ade1* (red adenine), *met1* (growth on methionine but not homocysteine), and *arg1* (growth on arginine but not ornithine) are represented by noncomplementing alleles derived from several strains, and these genes are linked in the sequence centromere-*met1-arg1-ade1* in the seven isolates analyzed.

Recombination analysis in revertant diploid heterozygotes was undertaken to study the nature of the process of recombination and to further clarify the question of ploidy. The analysis of recombination in protoplast fusion products can be interpreted in terms of the natural isolates being haploid (10) or diploid (7). The genetic evidence supporting the diploid hypothesis is derived largely from the analysis of recombination in revertant auxotrophs (7, 11, 12). It is desirable, therefore, that a quantitative study of recombination be made in revertants. For this purpose multiple auxotrophs, 10261 hOG24 and 22114 hOG311, were used. These strains carry allelic (red) *ade1* mutations which can be easily selected and have in addition proximally placed allelic *met1* mutations and, in the case of hOG311, a proximally placed *arg1* mutation. These strains permit a quantitative analysis of recombination in revertants. The observations confirm that *C. albicans* is naturally diploid and that the genetic map is centromere-*met1-arg1-ade1*.

The previous analysis (7) of 10261 revertants employing derivatives of hOG45 has now been reproduced in Pro^- revertants of 10261 hOG24. Revertant hOG24 strains displaying both *cis* and *trans* linkage of the *ade1* and *met1* alleles have been detected. The *cis* strains gave rise to both Ade^- and Ade^- Met^- derivatives, the Ade^- frequency being in the range of 0.14 to 0.64 (this variation will be considered below). A similar result has been found in the prototrophic revertants of 22114 hOG311. Two hOG311 prototrophic revertants were analyzed and were shown

to give rise to red auxotrophic derivatives which were phenotypically Ade$^-$, Ade$^-$ Arg$^-$, or Ade$^-$ Arg$^-$ Met$^-$. These prototrophic revertants were therefore *cis* linked for the *ade1*, *met1*, and *arg1* alleles and gave occasional coincident reappearance of all three auxotrophic characters. In addition, analysis was performed on a number of hOG311 derivatives revertant for Ade$^-$ and Met$^-$ but still requiring arginine. Revertants displaying both *cis* and *trans* linkage of the *ade1* and *met1* alleles were detected in these hOG311 Arg$^-$ revertants. The Ade$^-$ to Ade$^-$ + Ade$^-$ Met$^-$ frequency ratios in the *cis* revertants of hOG311 were in the range of 0.30 to 0.47.

One of the reasons for analyzing a large number of revertant hOG24 *ade1/ade1*$^+$ *met1/met1*$^+$ heterozygotes was to provide a quantitative comparison of the Ade$^-$ and Ade$^-$ Met$^-$ frequencies in *cis ade1 met1* heterozygous diploids and tetraploids. The tetraploid mitotic instability reflects a double mitotic crossing-over; the diploid mitotic instability reflects a single mitotic crossing-over. If the Ade$^-$ to Ade$^-$ + Ade$^-$ Met$^-$ frequency ratio in the tetraploid system is y, then this ratio in the diploid system should be $1 - \sqrt{1 - y}$.

In the isogenic hOG24 × hOG282 tetraploid the Ade$^-$ to Ade$^-$ + Ade$^-$ Met$^-$ frequency ratio in red adenine derivatives was found to be 0.13. This frequency in hOG24 diploid revertants was therefore expected to be $1 - \sqrt{1 - 0.13}$ or 0.07. Consideration of Table 5 indicates that this expectation was not fulfilled. The data are statistically non-homogeneous but indicate a much higher Ade$^-$ frequency. A similar discrepancy between expectation and observation was encountered in the comparison of hOG311 revertants and tetraploids. It is unclear how this should be interpreted. One possible interpretation is that recombination frequencies in the diploids are being perturbed by the presence of recessive lethal alleles located between the centromere and *met1*. Recessive lethal alleles have been detected in another strain of *C. albicans* (13). It is possible that such alleles could arise either during the isolation of the original mutants or during the reversion protocol. Alternatively, they might be present in the original prototrophic strain. The presence of such heterozygous recessive lethal alleles would distort the diploid recombination frequencies because they would give rise to homozygous lethal alleles after UV irradiation. In the strains analyzed a recessive lethal allele positioned between the centromere and *met1* would result in an increased *Ade*$^-$ to Ade$^-$ Met$^-$ frequency ratio. This would occur because UV-induced mitotic crossing-over would result in some of the Ade$^-$ Met$^-$ derivatives being lethal homozygotes. Heterozygous lethal alleles would be undetected in the tetraploid recombination analysis. A consideration of the various possible events that might occur during a double mitotic crossing-over in an isogenic tetraploid will confirm that a recessive lethal allele cannot be brought to homozygosity by any plausible sequence of events which brings the *ade* allele to homozygosity. The apparent quantitative discordance between the tetraploid and diploid recombination analyses requires further study in other strains.

LITERATURE CITED

1. **Kakar, S. N., and P. T. Magee.** 1982. Genetic analysis of isoleucine-valine, methionine, and arginine alleles by complementation. J. Bacteriol. **151**:1247–1252.
2. **Kwon-Chung, K. J.** 1974. Genetics of fungi pathogenic for man. Crit. Rev. Microbiol. **3**:115–133.
3. **Odds, F. C.** 1979. *Candida* and candidosis. Leicester University Press, Leicester, England.
4. **Olaiya, A. F., and S. J. Sogin.** 1979. Ploidy determination of *Candida albicans*. J. Bacteriol. **140**:1043–1049.
5. **Pesti, M., and L. Ferenczy.** 1982. Protoplast fusion hybrids of *Candida albicans* sterol mutants differing in nystatin resistance. J. Gen. Microbiol. **128**:123–128.
6. **Polak, A., and M. Grenson.** 1973. Evidence for a common transport system for cytosine, adenine and hypoxanthine in *Saccharomyces cerevisiae* and *Candida albicans*. Eur. J. Biochem. **32**:276–282.
7. **Poulter, R., V. Hanrahan, K. Jeffery, D. Markie, M. G. Shepherd, and P. A. Sullivan.** 1982. Recombination analysis of naturally diploid *Candida albicans*. J. Bacteriol. **152**:969–975.
8. **Poulter, R., K. Jeffery, M. H. Hubbard, M. G. Shepherd, and P. A. Sullivan.** 1981. Parasexual genetic analysis of *Candida albicans* by spheroplast fusion. J. Bacteriol. **146**:833–840.
9. **Riggsby, W. S., L. J. Torres-Bauza, J. W. Wills, and T. M. Townes.** 1982. DNA content, kinetic complexity, and the ploidy question in *Candida albicans*. Mol. Cell. Biol. **2**:853–862.
10. **Sarachek, A., D. D. Rhoads, and R. H. Schwarzhoff.** 1981. Hybridisation of *Candida albicans* through fusion of protoplasts. Arch. Microbiol. **129**:1–8.
11. **Whelan, W. L., and P. T. Magee.** 1981. Natural heterozygosity in *Candida albicans*. J. Bacteriol. **145**:896–903.
12. **Whelan, W. L., R. M. Partridge, and P. T. Magee.** 1980. Heterozygosity and segregation in *Candida albicans*. Mol. Gen. Genet. **180**:107–113.
13. **Whelan, W. L., and D. R. Soll.** 1982. Mitotic recombination in *Candida albicans*: recessive lethal alleles linked to a gene required for methionine biosynthesis. Mol. Gen. Genet. **187**:477–485.