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Chromosomal Rearrangement in *Candida stellatoidea* Results in a Positive Effect on Phenotype

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When type I *Candida stellatoidea* is plated onto sucrose agar at levels in excess of 10^8 cells, some isolates spontaneously form sucrose-positive colonies. These isolates do not display typical type I phenotypes but instead exhibit phenotypes intermediate between type I *C. stellatoidea* and *C. albicans*. Also, this phenotypic change only occurs in conjunction with a chromosomal rearrangement. These rearrangements have been studied in a strain naturally marked for methionine auxotrophy. Chromosome-size DNA bands separated by pulsed-field gel electrophoresis were probed with genes cloned from *C. albicans*. The hybridization pattern indicated that the genes on several chromosomes underwent extensive rearrangement.

Pulsed-field gel electrophoresis was first developed in 1984 by Schwartz and Cantor (28). Subsequent modifications (2, 3) have resulted in a technique that allows separations of chromosomes up to 12 Mb in size (21). It has been used extensively to study the karyotypes of numerous eukaryotic organisms (17, 20, 22, 28, 29) and is becoming an increasingly popular and standard tool in molecular biology. Among the many uses of this technique is the determination of chromosome size, number, and gene linkage.

Candida albicans is an opportunistic, yeastlike pathogen known to cause serious infection in immunosuppressed patients. An important characteristic of this organism is its ability to undergo rapid changes in colonial morphology, called switching (30, 31). In addition to morphological variation, chromosomal sizes may also vary in conjunction with switching (24). We have investigated a related phenomenon in type I *Candida stellatoidea*, a subspecies of *C. albicans*. However, instead of karyotypic variation arising at random in conjunction with a switch in colonial morphology, chromosomal rearrangements in this organism occur in response to plating on sucrose medium, which the organism normally cannot assimilate. Rearrangements result in the emergence of new phenotypes, one of which is the ability to assimilate sucrose. The rearrangement is specific for those isolates which acquire the ability to utilize sucrose, and it confers a selective advantage on the organism by virtue of the number of phenotypes affected. Among them are changes in growth rate, carbon assimilation pattern, and virulence in mice.

In this study we followed chromosomal rearrangements in an isolate of type I *C. stellatoidea* with a genetically marked strain to facilitate proof of isogenicity and identified a number of phenotypic effects associated with these rearrangements. A genetic mechanism is proposed, based on data accumulated from hybridizations of cloned genes to blots of pulsed-field gels.

MATERIALS AND METHODS

Strains and genes. All isolates and cloned sequences are listed in Table 1. Cultures were stored in 15% glycerol at −70°C or maintained on Sabouraud agar slants at room temperature. Prior to each experiment, cultures were transferred onto YEPD agar (1.0% yeast extract, 2.0% peptone, 2.0% dextrose, 2.0% agar) or Sabouraud agar, and incubated at 30°C for 24 to 72 h unless otherwise indicated.

Phenotypic analysis. To rule out contaminating *C. albicans*, which is indistinguishable from sucrose-positive (Suc^+) *C. stellatoidea*, only the genetically marked strain B-4462 was used for phenotypic analysis. This strain was found to be naturally auxotrophic for methionine biosynthesis, a phenotype usually not found in wild-type strains. Several other isolates containing auxotrophic markers induced by mutagenesis failed to yield Suc^+ colonies.

Suc^+ clones were recovered from B-4462 by the following procedure. First, 10^6 cells derived from a single large colony were used to inoculate a YEPD agar slant. As a control, 10^8 cells were plated onto sucrose agar to ensure that Suc^+ revertants were not already present in the inoculum. After 72 h, 5×10^8 cells were scraped from the YEPD agar slant, washed twice with distilled water, and plated onto MIN-SUC agar (yeast nitrogen base without amino acids, 6.7 g/liter; 2.0% sucrose; 2.0% agar) supplemented with 20 μg of methionine per ml. Plates were incubated at 30°C for 2 weeks. Spontaneous Suc^+ colonies were isolated and purified on MIN-SUC agar. One of the Suc^+ isolates (B-4462SU1A) was used for all analyses unless otherwise indicated.

Growth rates were determined spectrophotometrically by taking hourly readings of the absorbance (optical density at 600 nm) of cells grown in 50 ml of YEPD broth in 125-ml flasks, at 30°C, while shaking at 250 rpm.

Determination of glycerol utilization at 37°C was performed as described previously (26), with the exception that the glycerol agar was supplemented with yeast nitrogen base with amino acids (Difco Laboratories, Detroit, Mich.) to complement the methionine auxotrophy of B-4462.

Virulence of the isolates was tested by using the mouse model as described previously (14). Ten National Institutes of Health BALB/c female mice per isolate were injected in the lateral tail vein with 0.2 ml of a cell suspension made up in 0.8% saline. Survival of the mice was monitored for 45 days.

Pulsed-field gel electrophoresis. Agarose plugs were pre-

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TABLE 1. Yeast strains and genes

Strain or gene	Source identification	Source
Type I *C. stellatoidea*		
B-4460	Y-2361	C. P. Kurtzman
B-4461	Y-2442	C. P. Kurtzman
B-4462	Y-2443	C. P. Kurtzman
B-4463	Y-2444	C. P. Kurtzman
B-4464	Y-2445	C. P. Kurtzman
B-4465	Y-2446	C. P. Kurtzman
B-4466	Y-2447	C. P. Kurtzman
B-4404	SR1145	W. S. Riggsby
B-4406	SR1146	W. S. Riggsby
B-4252	11006	ATCC[a]
B-4257	36232	ATCC
Type II *C. stellatoidea*		
B-4365	20408	ATCC
C. albicans		
B-4509	1012A	B. B. Magee
B-4497	18804	ATCC
B-4201	C9	P. T. Magee
B-311	B-311	K. J. Kwon-Chung
JB71	JB71	J. Bennett
JB93	JB93	J. Bennett
JB172	JB172	J. Bennett
280	280	J. Bennett
863	863	J. Bennett
856	856	J. Bennett
1127	1127	J. Bennett
Gene		
DHFR	pML20	R. Kelly
LYS2	pTK2-9-1	B. B. Magee
LEU2	pLE219	R. Kelly
BEN^r	clone 4	Y. Koltin
SOR2	pYSK230	B. B. Magee
ADE2	pSM7	R. Kelly
URA3	pET39	R. Kelly
SUC	CASUC1	R. Kelly
ILV2	10A	M. Maceachern
MGL1	pYSK210	B. B. Magee
RDN1	CA4	J. Hicks
ACT1	pCACT	R. Kelly
TUB2	pHA5100	R. Kelly
CDC21	pBS+	B. B. Magee
TRP1	pJG-T1	B. B. Magee
ADE1	pADE1-1	R. Kelly
CAG1	CAG1	J. Hicks
GAL1	pGALS15	Y. Koltin
HIS3	pAR84.3	R. Kelly
C. albicans repeated sequence	27A	S. Scherer

[a] ATCC, American Type Culture Collection.

pared by using a modification of the procedure of Merz et al. (20). Some 3×10^8 cells from a 20-h Sabouraud agar plate grown at 30°C were washed twice with 1.0 ml of washing buffer (0.5 M EDTA, 0.1 M Tris, pH 7.5) in a 2.0-ml tube (Sarstedt, Numbrecht, Federal Republic of Germany). Cells were resuspended in TEME (100 mM Tris [pH 8.0], 5 mM EDTA [pH 8.00]) containing 5.0% 2-mercaptoethanol and incubated in a 37°C water bath for 30 min. Suspensions were pelleted, washed once in MES-sorbitol [20 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.0), 1 M sorbitol], and resuspended in SCE (1 M sorbitol, 0.1 M sodium citrate [pH 5.8], 0.01 M EDTA [pH 8.0]). A 50-μl portion of a 10-mg/ml stock spheroplasting solution (Zymolyase 20T; ICN Immunobiologicals, Lisle, Ill.) made up in SCE was added to the suspension, which was vortexed briefly. A 300-μl amount of 1.0% low-melting agarose (Incert agarose; FMC, Rockland, Maine) made up in 0.125 M EDTA, pH 7.5, held at 37°C, was immediately added to the tubes, which were mixed by inversion. Molten plugs were poured into a 96-well microtiter plate on ice. Plugs were removed to 2.0 ml of LET (0.45 M EDTA [pH 9.0], 10 mM Tris [pH 8.0]) containing 1.0% 2-mercaptoethanol in a 10.0-ml tube and incubated for 24 h at 37°C. LET was removed and NDS (LET, 1.0% sarcosine [pH 9.0], 2 mg of proteinase K per ml) was added followed by incubation at 50°C for 20 h. Prior to loading, plugs were rinsed twice in 10 volumes of running buffer (0.5× TBE) at 5°C for 1 h each. Rinsed plugs were removed to Eppendorf tubes and melted at 67°C. Samples were gently loaded into the wells of a 0.6% agarose gel (chromosomal grade agarose; Bio-Rad Laboratories, Richmond, Calif.) made up in 0.5× TBE, using a 1.0-ml syringe and 19-gauge needle.

Electrophoresis was performed in a Bio-Rad CHEF DRII unit at 70 V, using a dual ramp switch time of 180 to 300 s for 30 h followed by 420 to 900 s for 66 h. Gels were stained for 15 min in ethidium bromide, destained for 3 h in distilled water, and then photographed.

DNA isolation and restriction analysis. DNA was extracted from yeast cells, using a modification of previously described methods (1, 8). Cells were grown overnight at 30°C on Sabouraud agar plates. Cells (10^9) were harvested and washed once in distilled water. Cells were suspended in TEME with 5.0% 2-mercaptoethanol, incubated in a water bath at 37°C for 30 min, and then washed in MES-sorbitol. Pellets were resuspended in 1.5 ml of SCE followed by addition of 100 μl of spheroplasting solution (45 mg of Zymolyase 20 T per ml in SCE). The suspension was incubated in a water bath at 37°C for 30 min and then centrifuged at $1,200 \times g$ for 5 min. Pellets were resuspended in 600 μl of lysing solution (4.5 M guanidinum-HCl, 0.1 M EDTA [pH 8.00], 0.15 M NaCl, 0.05% sarcosyl [pH 8.0]) and incubated at 65°C for 10 min with frequent shaking. Suspensions were removed, transferred to Eppendorf tubes, and allowed to cool for 30 min prior to pelleting at high speed. Supernatants were carefully removed in 200-μl aliquots and pipetted under the surface of 2.0 ml of ethanol at room temperature. DNA was removed by spooling, rinsed in 70% ethanol, allowed to dry, and resuspended in 500 μl of distilled water. Samples were treated with RNase, chloroform-isoamyl alcohol extracted, and ethanol precipitated. Pellets were redissolved in 200 μl of TE. Yields were approximately 10 to 20 μg depending on the strain.

Restriction digests were performed by the manufacturer's specifications (GIBCO-Bethesda Research Laboratories, Gaithersburg, Md.), using 2 μg of DNA and 10 U of enzyme and with digestion times of 4 h. Samples were electrophoresed in a 0.8% agarose gel at 1.5 V/cm for 20 h.

Blotting and hybridizations. Gels were blotted onto nylon filters (Schleicher & Schuell, Keene, N.H.) and probed by standard methods (18) at high stringency. Damp filters were autoradiographed and stripped according to manufacturer's specifications.

Ploidy determination. Genetic determination of ploidy was performed with an arginine auxotroph (B-4252 *arg/arg*) and a heterozygous revertant (B-4252 *ARG/arg*) derived from the type culture of *C. stellatoidea* (B-4252) by the method used for analysis of type II *C. stellatoidea* (16).

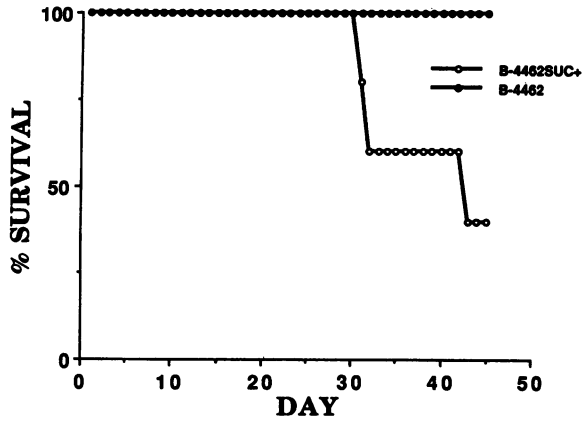

FIG. 1. Suc$^-$ versus Suc$^+$ type I *C. stellatoidea* virulence in mice. Dosages were 5×10^6 intravenously for Suc$^+$ and Suc$^-$. Ten mice per isolate were injected and checked daily for mortality for a period of 45 days.

RESULTS

Phenotypes associated with Suc$^+$ conversion. The recovery of Suc$^+$ clones was found to be dependent on a number of factors. Among them were strain, age of culture, and length of incubation. Nutritionally marked Suc$^+$ clones were recovered from B-4462 as well as other isolates. The isolates recovered from this strain retained the methionine marker, showing that the Suc$^+$ clones and corresponding phenotypes were not the result of contamination by *C. albicans*. Suc$^+$ revertants were recovered at frequencies of 1×10^{-7} to 3×10^{-7} for B-4462 and B-4460, while other unmarked isolates (B-4252 and B-4406) yielded revertants at much lower frequencies. In other strains (B-4404, B-4459, and B-4465), no Suc$^+$ colonies were recovered. In addition to the ability to utilize sucrose, Suc$^+$ isolates were found to exhibit three new phenotypes. These were faster growth rate, enhanced virulence, and the ability to utilize glycerol at 37°C.

The increase in growth rate following reversion to Suc$^+$ was found to be significant. The doubling time of B-4462 Suc$^-$ was determined to be 115 min, consistent with other type I *C. stellatoidea* strains. However, doubling times of Suc$^+$ clones were observed to have decreased to 97 min. This growth rate was roughly intermediate between those of type I *C. stellatoidea* and the *C. albicans* reference isolate B-311, which had a doubling time of approximately 70 min, consistent with other *C. albicans* isolates (11).

Conversion to Suc$^+$ also had an effect on virulence (Fig. 1). At a dosage of 5×10^6, Suc$^+$ revertants were found to kill 60% ($P < 0.01$) of the infected mice by 45 days, while the Suc$^-$ parental culture killed none. This effect on virulence was also observed for Suc$^+$ clones derived from other strains of type I *C. stellatoidea* (data not shown).

Glycerol utilization at 37°C was determined to be a very accurate diagnostic criterion for type I *C. stellatoidea*. None of the isolates identified as type I *C. stellatoidea* could grow under these conditions. This was in contrast to type II *C. stellatoidea* and *C. albicans*, which could grow. When numerous Suc$^+$ clones isolated from type I *C. stellatoidea* were tested under these conditions, all were capable of growth.

Karyotypes and DNA fingerprints. Unlike *C. albicans*, type I *C. stellatoidea* tended to produce more hyphal cells than yeast cells in broth cultures, regardless of medium.

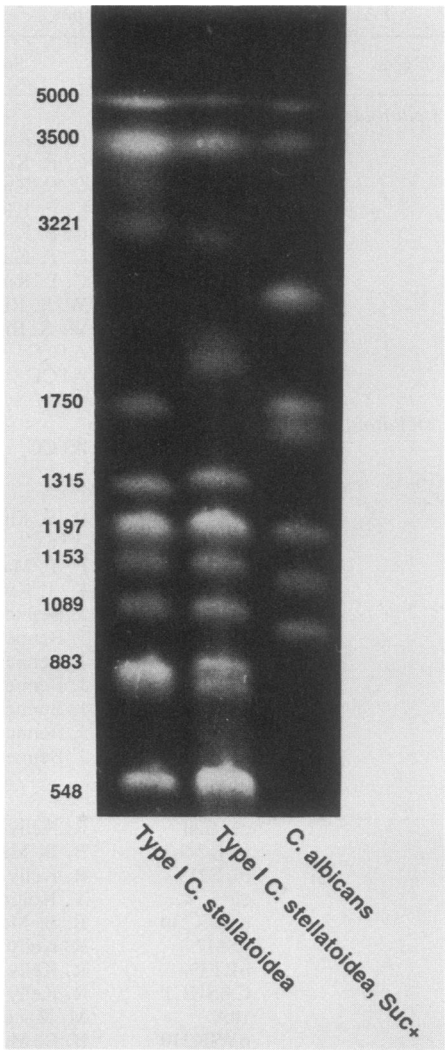

FIG. 2. Karyotypes of Suc$^-$ and Suc$^+$ *C. stellatoidea* showing 11 major bands comprising 13 chromosomes ranging in size from 560 kb to 5.0 Mb. *C. albicans* B-4509 is shown for comparison, with 8 bands comprising 8 chromosomes.

When suspensions containing mostly hyphal cells were used for plug formation, poor karyotypes were obtained. Compared with other conventional media, Sabouraud agar was found to be the best for growth of predominantly yeast cells. Hyphal formation by type I *C. stellatoidea* was delayed on this agar, and yeast cell concentrations were found to be sufficient for plug formation or DNA extraction after 20 h of growth.

Isolates of type I *C. stellatoidea* were found to contain 11 to 13 chromosomes ranging from approximately 560 to 5,000 kb in size (Fig. 2). In addition, the karyotypes of multiple isolates were found to exhibit the same heterogeneity as multiple *C. albicans* isolates (Fig. 3). As in *C. albicans*, conserved regions could be identified in type I *C. stellatoidea* (1,300 to 600 kb) which appear to be species specific. In addition to the conserved regions, type I *C. stellatoidea* contains regions in the 1,750- to 5,000-kb range which are quite variable, just as they are in *C. albicans*. These regions generally contain the larger chromosomes where the ribosomal genes are located.

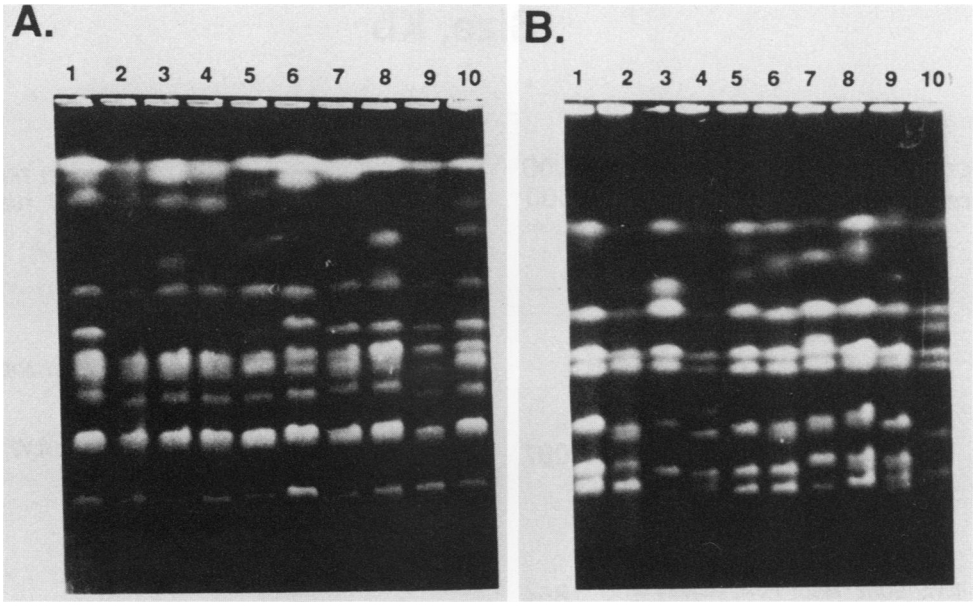

FIG. 3. CHEF electrophoresis of multiple isolates of type I *C. stellatoidea* and *C. albicans*. Run conditions were as described in Materials and Methods with the exception of the *C. albicans* isolates, which were run in a 0.4% gel at 70 V. (A) Type I *C. stellatoidea*, lanes 1 to 10: B-4461, B-4462, B-4463, B-4464, B-4465, B-4466, B-4252, B-4257, B-4404, and B-4406. (B) *C. albicans*, lanes 1 to 10: JB71, JB93, JB172, B-4497, B-4509, B-4201, 280, 863, 856, and 1127.

When ethidium bromide-stained gels of Suc^+ and Suc^- karyotypes were compared, there appeared to be only slight variation in chromosome sizes of the Suc^+ revertants, with all revertants having the same general rearrangement pattern. In this pattern four differences were observed. The 1,750-kb chromosome increased in size to approximately 1,850 kb, the chromosome located at 1,315 kb disappeared and was replaced by a slightly larger chromosome (1,393 kb in size), the chromosome located in the 883-kb size range increased to 953 kb, and the chromosome located at 3,350 kb also disappeared. This rearrangement pattern was observed for every clone derived from the same isolate.

Variation in karyotype of *C. albicans* is also reflected in variable DNA fingerprints. Only the most closely related *C. albicans* isolates have the same fingerprint when probed with 27A, a *C. albicans* species-specific, dispersed, repeated sequence useful for epidemiological purposes (27). To determine whether this was the case for type I *C. stellatoidea*, we probed *Eco*RI-digested DNA from 10 isolates with this sequence. Fingerprinting of this organism results in an extremely homogeneous pattern (Fig. 4). Furthermore, although the sequence hybridizes only to *C. albicans* and *C. stellatoidea* DNA, the fingerprint patterns of these two organisms are quite different both in hybridization pattern and number of bands.

Linkage mapping and identification of gene rearrangements. The chromosomal locations of 18 genes in Suc^- and Suc^+ isolates were determined by Southern hybridization to blots of contour-clamped homogeneous electric field (CHEF) electrophoresis gels and are shown in Fig. 5. Linkage mapping, using cloned genes to probe the karyotypes of Suc^- and Suc^+ isolates, yielded two general hybridization patterns. These patterns consisted of hybridization to one or two bands (Fig. 6) similar to a previous report for another Suc^- type I *C. stellatoidea* (23). This arrangement is not strain specific, but instead appears to be characteristic of all type I *C. stellatoidea* (data not shown). Furthermore,

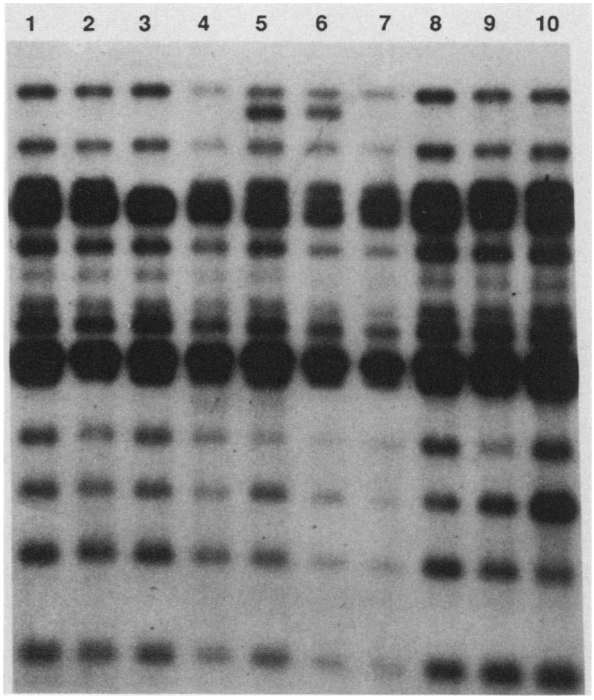

FIG. 4. Conserved hybridization pattern of *Eco*RI-digested type I *C. stellatoidea* genomic DNA probed with 27A. Only the most closely related *C. albicans* isolates show similar hybridization patterns. This is in contrast to type I *C. stellatoidea* which, regardless of source, displays a homogeneous pattern for all isolates. Lanes 1 to 10: B-4461, B-4462, B-4463, B-4464, B-4465, B-4466, B-4252, B-4257, B-4404, and B-4406.

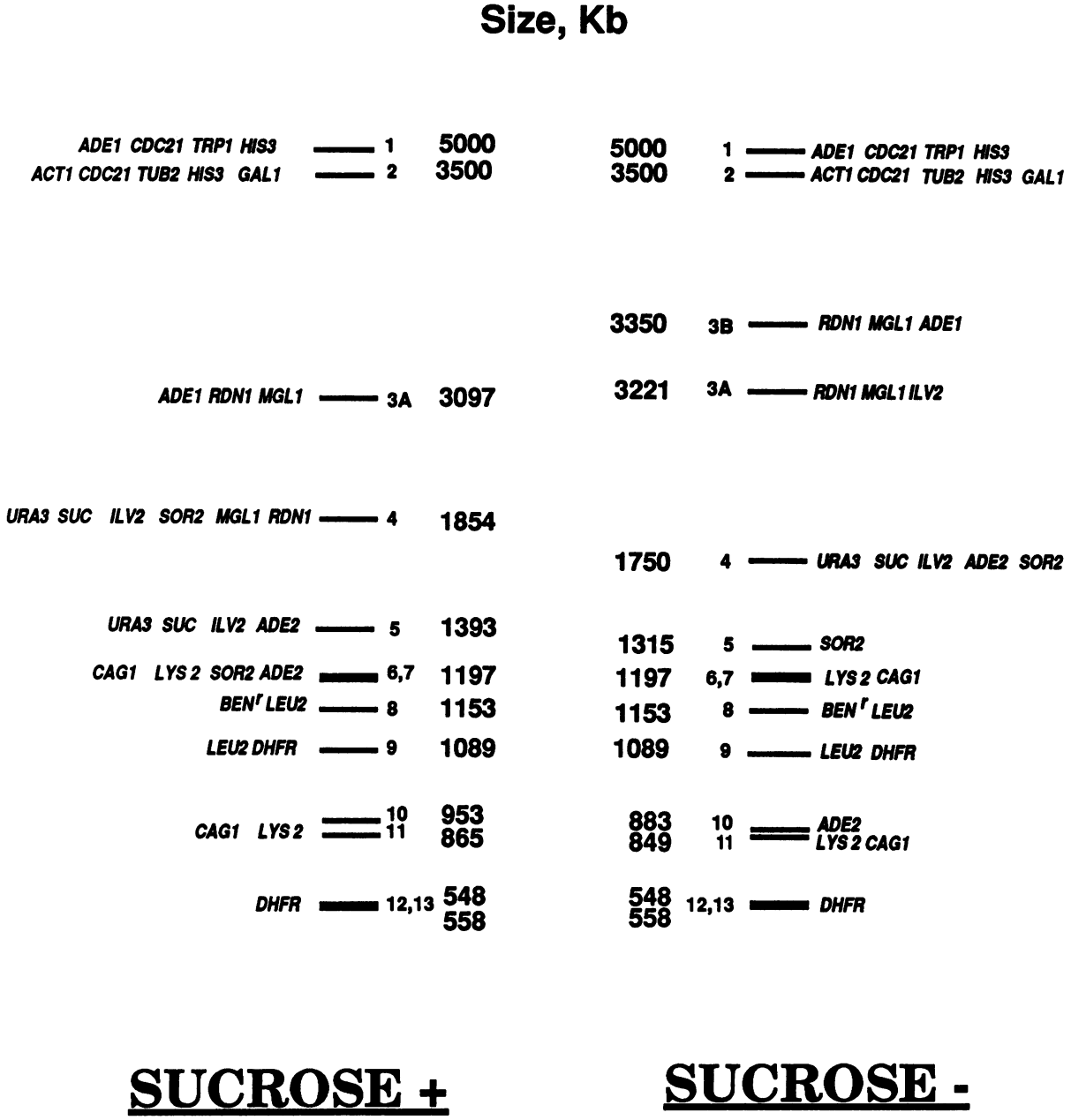

FIG. 5. Linkage map of type I *C. stellatoidea* Suc$^-$ and Suc$^+$ derivatives showing chromosome size, number, and linkage. Sizes were determined by statistical comparison with standard strains of *S. cerevisiae* and *S. pombe*. Heavy lines indicate the presence of more than two chromosomes (1,197, 883, and 548 kb).

while genes rarely hybridize to more than one chromosome in *C. albicans*, 12 genes were found to hybridize to two bands in type I *C. stellatoidea*. They are *DHFR*, *LYS2*, *ADE2*, *ADE1*, *SOR2*, *LEU2*, *ILV2*, *CDC21*, *CAG1*, *MGL1*, *HIS3*, and *RDN1*. In addition to these patterns, a third pattern indicative of extensive rearrangement was observed (Fig. 7). Rearranged isolates which were detected by reversion to Suc$^+$ resulted in a positional change of numerous genes which may be summarized as follows. In Suc$^-$ isolates, *MGL1* and *RDN1* hybridize to two bands designated chromosomes (Chr) 3A and 3B. *URA3*, *SUC*, and *ILV2* hybridize to a single band (Chr 4), while *ADE2* and *SOR2* hybridize to two bands (Chr 4 and 10 and Chr 4 and 5, respectively). In the Suc$^+$ revertants, *MGL1* and *RDN1* hybridize to two bands of different sizes (Chr 3 and 4) than in Suc$^-$ (Chr 3A and 3B). *ADE2* moves from chromosomes 4 and 10 to chromosomes 5 and 6, while *SOR2* moves from chromosomes 3 and 4 to chromosomes 4 and 6. Furthermore, loci linked to *SUC* (*ADE2*, *SOR2*, *URA3*, and *ILV2*) are now found on chromosome 5 (*SUC*, *URA3*, *ILV2*, *ADE2*, and *SOR2*) in addition to chromosome 4 (*SUC*, *URA3*, *ILV2*, and *SOR2*). The occasional background observed with some of the probes was most likely due to the use of entire plasmids as probes, resulting in nonspecific

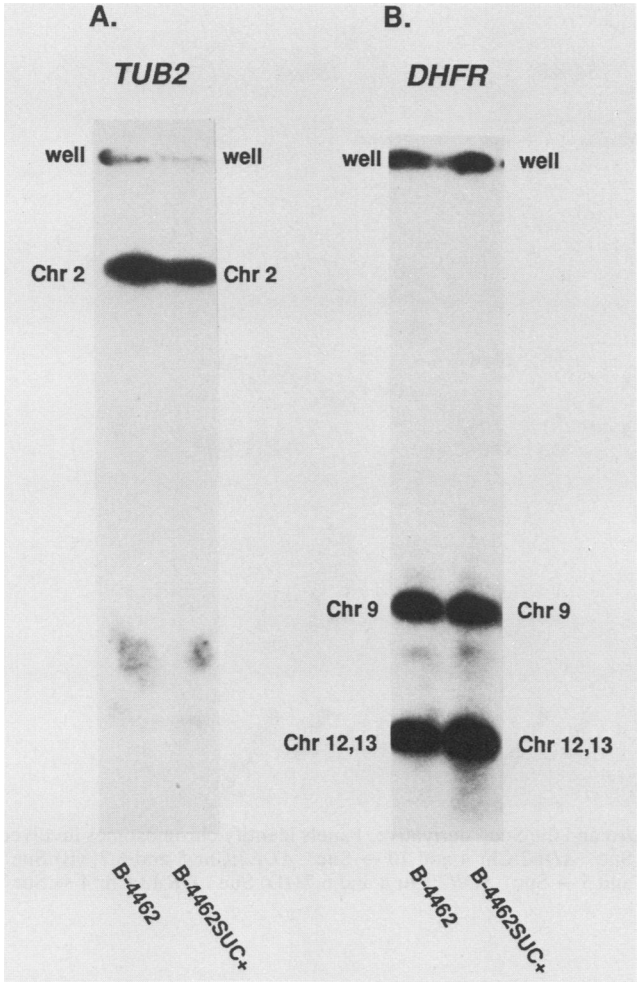

FIG. 6. Hybridization patterns of cloned genes to chromosomes separated by CHEF electrophoresis. B-4462 and B-4462 Suc$^+$ chromosomes separated by CHEF electrophoresis were Southern blotted onto nylon membranes. Membranes were repeatedly hybridized and stripped up to seven times with no detectable decrease in bound DNA. (A) Hybridization to *C. albicans TUB2* gene. Genes showing a single hybridization band were *TRP1*, *ACT1*, *GAL1*, *URA3*, *BEN*^{r}, and *SUC*. (B) Hybridization to *C. albicans DHFR* gene. Genes showing two hybridization signals were *DHFR*, *LYS2*, *ADE2*, *ADE1*, *SOR2*, *LEU2*, *ILV2*, *CDC21*, *HIS3*, *CAG1*, *MGL1*, and *RDN1*.

hybridization by vector or flanking sequences. When internal fragments were used, background was not detected.

The rearrangement appears to be specific since only slight changes are observed in ethidium bromide-stained gels. This is in contrast to the extensive changes observed in the hybridization pattern of numerous probes. That six independent Suc$^+$ isolates revealed identical rearrangement patterns is also indicative of the specificity. In all, seven genes residing on five chromosomes were involved.

Ploidy determination. Although the DNA content of type I *C. stellatoidea* cells was determined to be similar to that of *C. albicans* and type II *C. stellatoidea* (16), the ploidy of type I has not been determined. Genetic analysis of ploidy with an arginine auxotroph (*arg/arg*) of the type culture of type I *C. stellatoidea* suggests that it is diploid. Irradiation of a spontaneous revertant (*ARG/arg*) resulted in numerous sectored colonies appearing on minimal media (after replica plating). The majority of the sectors were found to be arginine auxotrophs (*arg/arg*), resulting in an overall frequency of 1.4%. The wild type (*ARG/ARG*), on the other hand, produced no auxotrophic sectors following UV irradiation with the same dosage. No auxotrophic sectors were produced when cells from the opposite side of a sectored colony were irradiated. These results would be expected if the revertant was originally heterozygous for a mutation in the arginine biosynthetic pathway and the induction of mitotic crossing over by UV irradiation resulted in a sectored colony. The colony would be homozygous in the auxotrophic sector for the original arginine mutation (*arg/arg*) and homozygous on the opposite side of the colony (prototrophic sector, *ARG/ARG*) for the wild-type gene. This is consistent with previously reported results on ploidy determinations for type II *C. stellatoidea* (16) and *C. albicans* (35).

DISCUSSION

In this study we have shown that some isolates of type I *C. stellatoidea* are capable of rearranging their chromosomes to yield an isolate with several characteristics distinct from the parent. Isolates possessing a rearranged karyotype are detected by the ability to assimilate sucrose. It is not clear whether reversion to Suc$^+$ is spontaneous (occurs during growth on YEPD agar) or induced (occurs during growth on sucrose agar). If it was spontaneous, all Suc$^+$ isolates obtained from each plating could have conceivably been siblings derived from a single Suc$^+$ cell. However, the following observations argue against this possibility: (i) Suc$^+$ colonies were never detected in the initial inoculum used to inoculate the YEPD agar; (ii) the event is always rare; (iii) purified Suc$^+$ isolates grow into visible colonies within 24 h when transferred onto sucrose agar (however, Suc$^+$ colonies never appear prior to 72 h, although they should have been visible in 24 h if they were already present in the initial inoculum); (iv) we observed that the karyotypes of Suc$^+$ isolates derived from two distinct strains, B-4460 and B-4462, were identical.

The ability to utilize sucrose may be due to activation of a cryptic gene, expression or derepression of a regulatory gene, or movement of a sequence to a location where it can be expressed. Even though it has not been possible to separate sucrose assimilation from the other phenotypes, it is unlikely that the ability to assimilate sucrose is responsible for their appearance. Instead, it is more probable that sucrose assimilation functions as a selectable marker, indicating that rearrangement has occurred and that the rearrangement itself is responsible for the new phenotypes. The number and types of phenotypic changes that occur are probably variable since karyotypic variation in both type I *C. stellatoidea* and *C. albicans* is extensive.

Once rearrangement has occurred, the most beneficial phenotypic change in type I *C. stellatoidea* is the change in growth rate, with the increase being 16%. The potential value of this effect on survival is seen in the change in virulence. Doses of 5×10^6 cells per mouse killed the majority of animals by 45 days for Suc$^+$ clones, in contrast to no killing for Suc$^-$ clones. However, it is unlikely that the ability to assimilate sucrose is a virulence factor (10). Just as in growth rate, virulence appears to be intermediate between *C. albicans* and type I *C. stellatoidea* as lowering the dose to 10^6 cells per mouse results in a reduction of mortality to 40% (data not shown). At this dosage, and dosages as low as $5 \times$

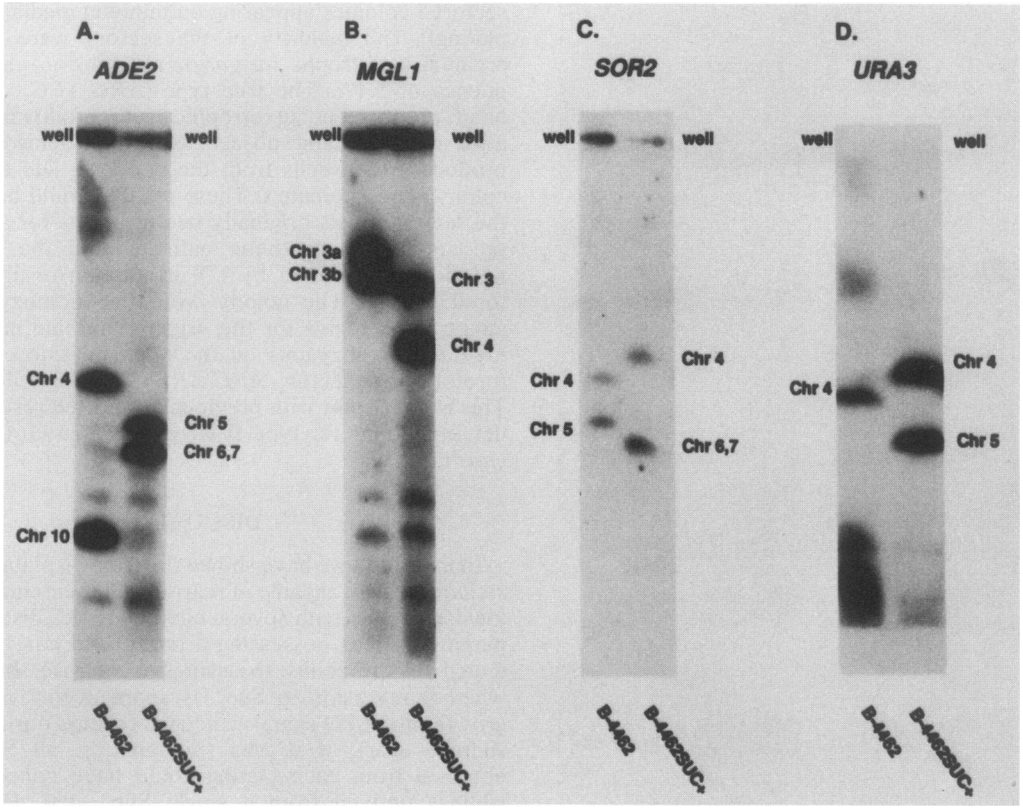

FIG. 7. Detection of chromosomal rearrangement in type I *C. stellatoidea* and the Suc$^+$ derivative. Panels identify chromosomes involved in the rearrangement by change in hybridization position of probes. (A) Suc$^-$ *ADE2*:Chr 4 and 10 → Suc$^+$ *ADE2*:Chr 5 and 6,7. (B) Suc$^-$ *MGL1*:Chr 3A and 3B → Suc$^+$ *MGL1*:Chr 3 and 4. (C) Suc$^-$ *SOR2*:Chr 4 and 5 → Suc$^-$ *SOR2*:Chr 4 and 6,7. (D) Suc$^-$ *URA3*:Chr 4 → Suc$^+$ *URA3*:Chr 4 and 5.

10^5 cells per mouse, a reference isolate of *C. albicans* kills 100% of the mice (12).

Apparently unrelated to growth rate is the ability of Suc$^+$ isolates to utilize glycerol at 37°C. Saracheck et al. have noticed that type I *C. stellatoidea* isolates normally cannot grow while *C. albicans* can grow well under these conditions. Possible explanations of this phenomenon may be uncoupling of electron transport or the induction of auxotrophies for certain amino acids (26).

A number of possible mechanisms can be proposed to account for the rearrangement that we observed. The complexity and low frequency of the event argue for a multistep process; however, construction of a specific, detailed model accounting for all participants and products is difficult since type I *C. stellatoidea* has no known sexual cycle. Instead, information must be derived from chromosomal mapping. This is complicated since two homologous chromosomes must be accounted for in spite of the fact that they do not always migrate together. In addition, linkage order and map distances are impossible to determine with precision due to the absence of meiotic products. Furthermore, determination of chromosome sizes is complicated by the need to calculate sizes based on statistical comparisons to mobilities of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* chromosomes. Most of these chromosomes lie outside the size range of type I *C. stellatoidea* chromosomes. In spite of these difficulties, at least two mechanisms appear to be plausible based on the available data. By using the *ADE2* gene as an example, one possible mechanism appears to be consistent with interchromosomal recombination between two nonhomologous chromosomes. In this case, two chromosomes (Chr 10 and 5) undergo similar alterations in their sizes. The observed products could have resulted from a reciprocal exchange which was detected by a change in position of the *ADE2* gene (size estimates may not be exact due to low resolution of pulsed-field gels for sizes smaller than 100 kb). A second possible mechanism is shown in Fig. 8 and is indicative of the complex nature of the event. It supposes that type I *C. stellatoidea* has direct repeat sequences capable of undergoing homologous, intrachromosomal, and interchromosomal recombination. As a result, the material between a repeat is capable of expulsion from one chromosome and reintegration into another without creating large, complex translocations. This type of mechanism has been widely documented in other yeasts as well as with bacterial transposable elements. For instance, in *S. cerevisiae*, Golin et al. (6) looked at the behavior of a pair of direct repeats flanking a *URA3* sequence which was present in each of two homologous chromosomes. They noted that loss of one pair from one homolog was often accompanied by loss of the second pair in the other homolog. This may explain why both Suc$^-$ homologs undergo a change in size instead of just one. Furthermore, they noted that reintegration took place at relatively high frequencies (ca. 3×10^{-5}). The second type of mechanism is also known to occur in concerted fashion and is flexible enough to account for the observed results. To determine whether the first mechanism (reciprocal recombination) could account for all rearrange-

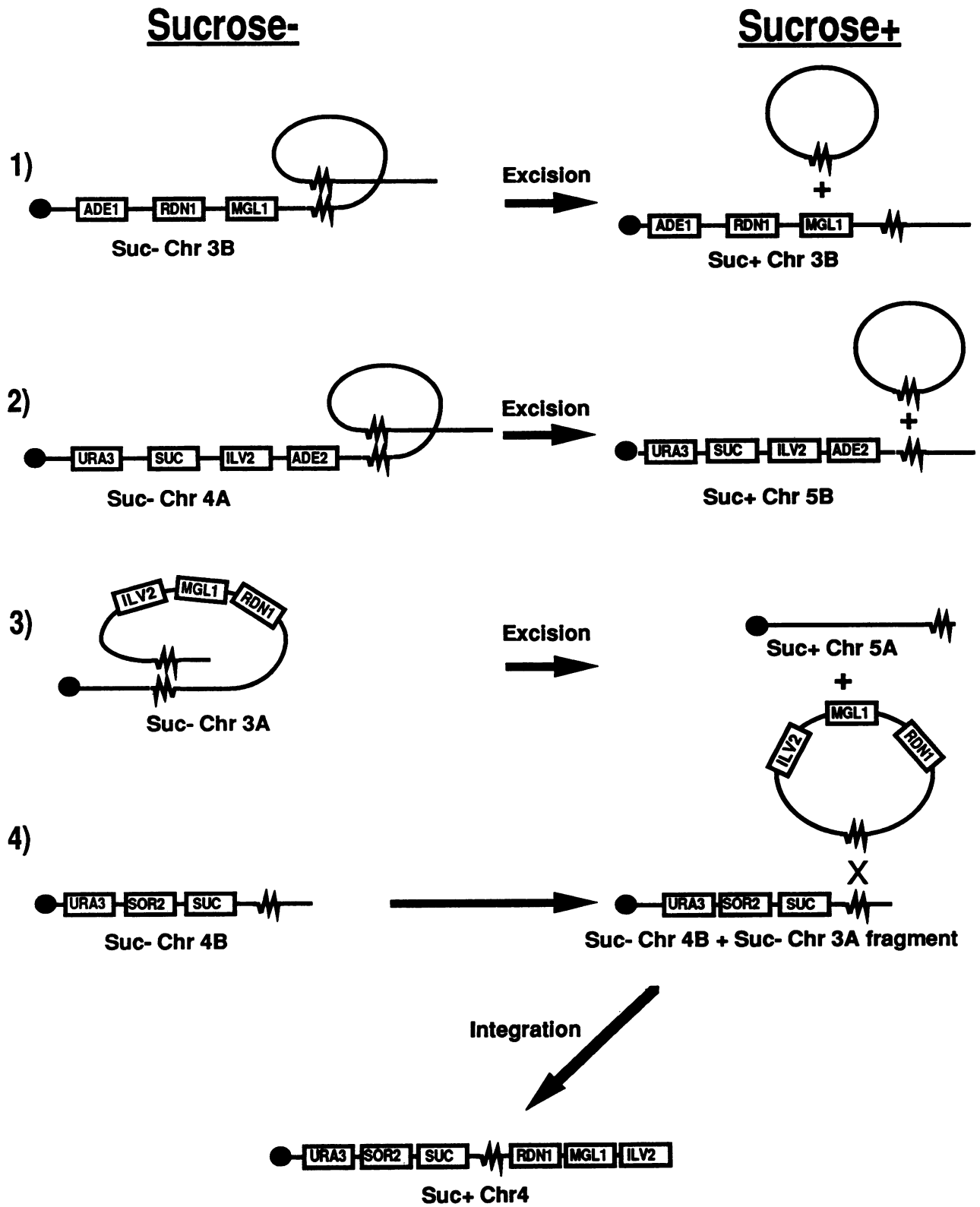

FIG. 8. One possible mechanism resulting in rearrangement depends on the presence of repeated sequences within the chromosome. Sequences can be expelled from one or both homologs followed by integration into another site. This mechanism assumes that the organism is diploid for all genes, excluding repeated sequences. 1) Excision of a fragment from Suc$^-$ chromosome 3B (one homolog) results in a deletion detected by change in position of Chr 3B to Suc$^+$ Chr 3B but not a change in linkage. 2) The same mechanism, which may be concerted, operates in Chr 4A. The result is an approximately 350-kb deletion. 3) and 4) Excision of a fragment containing *ILV2*, *MGL1*, and *RDN1* from Suc$^-$ Chr 3A. The fragment integrates into Suc$^-$ Chr 4B, resulting in Suc$^+$ Chr 4. The remainder of Suc$^-$ Chr 3A is similar in size to Suc$^+$ Chr 4 so it also migrates to this location.

ments, much more linkage data would be required since more than two chromosomes are involved, which creates the potential for many possible outcomes.

One potential source for karyotypic variation in type I *C. stellatoidea* and *C. albicans* can involve the chromosomes containing the rDNA genes (unpublished data). Because these genes are repeated, the homologs can undergo unequal recombination, leading to their separation in the pulsed-field gel. This is a fairly common occurrence and has been associated with switching (24). However, switching appears to be a random phenomenon, and a selective advantage has not been shown for a particular morphology. The rearrangements we have observed involve chromosomes other than the rDNA-containing ones. In addition, we have clearly demonstrated a selective advantage for this type of rearrangement, although at the present time it is not known what induces it to occur. Starvation-induced stress is one possibility presently under investigation. We observed that the recovery of sucrose-positive isolates increased with the age of the original culture and incubation time after plating onto sucrose agar. Cultures less than 48 h old tended not to yield Suc^+ colonies, while those approximately 4 weeks old yielded the most, although the viability begins to decline significantly at this age. Colonies on sucrose agar begin to appear at 72 h and continue to arise for 2 to 3 days afterward.

Analysis of karyotypes of numerous isolates of both type I *C. stellatoidea* and *C. albicans* reveals that these organisms are extremely heterogeneous in terms of chromosome sizes. However, in spite of the heterogeneity of karyotypes, DNA fingerprints of type I *C. stellatoidea* are extremely homogeneous, unlike those of *C. albicans*. One explanation is that all type I *C. stellatoidea* laboratory strains were originally derived from the same source. However, a recent clinical isolate displayed a fingerprint consistent with all other type I *C. stellatoidea* isolates (15). An alternative explanation may be that the sequence detected in fingerprinting is situated such that it is rarely affected by changes in chromosomal position.

Even though numerous strains were examined, not all yielded Suc^+ colonies. The observation of strain dependency may reflect the nature of the rearrangement. The presence of a specific sequence such as a transposable element or recombination hotspot is a possibility presently under investigation. Without the correct location of these sequences, rearrangement may not be able to occur or may be lethal to the organism if it does occur.

C. stellatoidea has been classified into two types, I and II (13), with type II being a sucrose-inhibitable, α-glucosidase mutant of *C. albicans* (10). The taxonomic relationship of type I *C. stellatoidea* to *C. albicans* has been the subject of much debate. Over the years this organism has gone from independent species, to subspecies, to variant. It is clear that it does not merit species status; however, it should not be considered a simple variant of *C. albicans*. Based on a previous study of the differences between the two organisms (9), one could conclude that type I *C. stellatoidea* behaves as a very debilitated *C. albicans*. That type I *C. stellatoidea* can even exist, given that it is in direct competition with *C. albicans* in the same ecological niche, is surprising. However, if wild-type type I *C. stellatoidea* is derived from *C. albicans* as a result of gross chromosomal rearrangement, coexistence with *C. albicans* would be explained in spite of the apparent competitive disadvantage. If this event is under genetic control, this gene(s) may represent a remnant of primitive life cycle genes, some of which were retained after colonization of warm-blooded animals. Induction of this gene(s) may occur in rare circumstances of severe stress with its function being to increase frequencies of recombination without reductional chromosomal segregation (i.e., haploidization). This "meiototic"-like division (5) could be what produces the great diversity in *C. albicans* in the absence of true meiosis. Type I *C. stellatoidea* may represent one possible outcome of this event, which in fact has been suggested previously (25). Previous observations of conserved restriction patterns (19), immunological relatedness (7), lack of a distinguishing DNA probe (4), common linkage groups (23), and the presence of small (<1,000 kb) chromosomes in some serotype B *C. albicans* isolates (data not shown) could be results supportive of this event. Finally, reports of sporulating cells (34), polyploidy (32), and delayed mitosis (33) in *C. albicans* may all be related genetic phenomena. If type I *C. stellatoidea* can be reproducibly isolated from *C. albicans*, it could become a useful genetic tool. Not only would this shed new light on *C. albicans* genetics, it could become a useful cloning vehicle for *C. albicans* sequences, using genes such as α-glucosidase as dominant markers.

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