UV and X-ray sensitivity of *Candida albicans* laboratory strains and mutants having chromosomal alterations

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Summary

Utilization of L-sorbose, D-arabinose or primary fluconazole resistance in *Candida albicans* are controlled by copy number of specific chromosomes. On the other hand, spontaneous morphological mutants have a wide range of chromosomal alterations. We have investigated the UV and X-ray sensitivity of these mutants, as well as *C. albicans* laboratory strains. While L-sorbose utilizing mutants had normal sensitivities, a large subclass of D-arabinose utilizing mutants was abnormally sensitive to UV. Spontaneous morphological mutants responded differently, an expected result because of the heterogeneous nature of their electrophoretic karyotypes. We suggest that the differences in UV and X-ray sensitivity are due to gene imbalance caused by some chromosomal alterations. In this respect, the radiation sensitivity is similar to other features impaired by changes in chromosomes, but is unlike the acquisition of the ability to utilize alternative nutrients or the acquisition of resistance to fluconazole. Our studies also revealed that strains of *C. albicans* heterozygous for the mating type loci exhibited the same X-ray sensitivity as homozygous or hemizygous strains, a finding which is in contrast to the properties of *Saccharomyces cerevisiae*, where heterozygous strains are more resistant. This feature of *C. albicans* strains may be indicative of an inefficient repair system that may be related to inefficiency of mating.

Key words

X-ray sensitivity, UV sensitivity, Electrophoretic karyotypes, Mating type locus, Ploidy, *Candida albicans*

Sensibilidad a la radiación UV y rayos X de cepas de laboratorio y mutantes de *Candida albicans* con alteraciones cromosómicas

Resumen

La utilización de L-sorbosa, D-arabinosa o la resistencia primaria al fluconazol en *Candida albicans* están controladas por un número de copias de cromosomas específicos. A su vez, mutantes morfológicos espontáneos presentan un amplio rango de alteraciones cromosómicas. Hemos investigado la sensibilidad a la radiación UV y a los rayos X de estos mutantes y de cepas de laboratorio de *C. albicans*. Mientras que los mutantes que utilizan L-sorbosa presentaron sensibilidades normales, una amplia subclase de mutantes que utilizan D-arabinosa fueron anormalmente sensibles a la radiación UV. Los mutantes morfológicos espontáneos respondieron de forma diferente, un resultado esperado por la naturaleza heterogénea de sus cariotipos elecroforéticos. Sugerimos que las diferencias en la sensibilidad a la radiación UV y a los rayos X se debe a un desequilibrio genético producido por algunas alteraciones cromosómicas. A este respecto, la sensibilidad a la radiación es similar a otras características dañadas por los cambios cromosómicos, pero no se parece a la adquisición de la capacidad de utilizar nutrientes alternativos o a la adquisión de la resistencia al fluconazol. Nuestros estudios revelan también que las cepas de *C. albicans* heterocigóticas para los loci de tipo de cruzamiento presentaron la misma sensibilidad a los rayos X que las cepas homocigóticas y hemicigóticas, un hallazgo que contrasta con las propiedades de *Saccharomyces cerevisiae*, donde las cepas heterocigóticas son más resistentes. Esta característica de las cepas de *C. albicans* puede ser indicativa de un sistema de reparación ineficaz que puede estar relacionado con la ineficacia del cruzamiento.

Palabras clave

Sensibilidad a rayos X-ray, Sensibilidad a rayos UV, Cariotipos electroforético, Locus de tipo de cruzamiento, Ploidía, *Candida albicans*

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It has long been known that *Candida albicans* is highly polymorphic, and spontaneously gives rise to high frequencies of mutants with altered colonial appearances, and with many other altered phenotypes [for reviews see 1-4]. In addition, colonies with various morphologies have been isolated from clinical patients [5]. After development of the DNA probes which defined common lineages [6], several studies revealed that differently-looking colonies from the same site of infection were mutants of a single strain [7,8]. Work by Rustchenko and colleagues demonstrated that various colonial morphologies were due to various spontaneous alterations in electrophoretic karyotypes [3,9,10], a condition which easily explained characteristic changes in many phenotypes. Most important, these mutants always had changes in utilization of different food supplies, as determined by assimilation profiles [for review see 12]. In contrast, many other phenotypes, such as, for example, the ability to form germ tube or chlamydospores etc., were sometimes but not always associated with chromosomal alterations. In addition, some phenotypes, as auxotrophy due to the loss of any of a number of biosynthetic functions, were never or perhaps seldom encountered among these spontaneous mutants [3].

Recently we elucidated how one particular type of chromosomal alteration, arising by nondisjunction, controls important functions in *C. albicans*. A causal relationship was established between monosomic-disomic condition of chromosome 5 and the expression of the *SOU1* gene responsible for L-sorbose utilization [13]. Also, utilization of another carbon source, D-arabinose, as well as first-occurring resistance to the drug fluconazole, were found to be controlled by similar mechanism, a specific chromosome copy number [14,11; E. Rustchenko, unpublished results]. We suggested that chromosome copy number is a general means to control a resource of potentially beneficial genes in *C. albicans* [13]. Because the chromosomal rearrangements in *C. albicans* are frequent [9,10,15], we speculated that repair systems may be affected. This hypothesis was tested by determining the UV- and X-ray sensitivity of laboratory strains and a variety of mutants having various chromosomal alterations.

MATERIALS AND METHODS

Nomenclature of C. albicans chromosomes. In this paper, the penultimate largest to smallest chromosomes of *C. albicans* are designated by Arabic numerals 1 to 7, whereas the largest chromosome, containing the ribosomal DNA cluster, is designated R [16]. The nomenclature used in our previous publications, consists of roman numerals I to VIII to designate the smallest to the largest chromosomes, respectively, and “a” and “b” to designate two homologues of different sizes. The following are equivalencies for the two types of chromosome assignments:

Chromosome	VIII	VII	VI	V	IV	III	II	I
	R	1	2	3	4	5	6	7

Strains. The strains used in this study are listed in Table 1. The *C. albicans* laboratory strain, 3153A [17] and its unstable derivative 300 [3], which is maintained in some laboratories instead of the original one, and which we consider to be just another spontaneous morphological mutant, were previously extensively characterized for their individual chromosomal patterns, assimilation profiles and a number of phenotypes [3,9-12]. We also examined a total of fourteen spontaneous mutants, m1 to m14, detected in population of 3153A due to their individually altered colonial forms, and previously characterized for their individually altered chromosomal patterns, assimilation profiles and various phenotypes [3,9-12]. In addition, we examined three of so-called non-germinative mutants, 301, 302 and 303, which were originally isolated from unstable variant 300 for their inability to form germ tube and later proved to be morphological mutants, and which possessed individually altered electro-karyotypes [3]. Finally, ten sorbose-positive, Sor1 to Sor10, and fifteen arabinose-positive, Ara1 to Ara15, mutants selected from strain 3153A on L-sorbose or D-arabinose containing media, respectively, were included in this study [14]. The karyotypic and phenotypic differences between detected morphological mutants and selected mutants of 3153A were addressed in our previous paper [12].

The survival of three *Saccharomyces cerevisiae* strains, two diploids, B-8722 and B-11961, heterozygous and homozygous by mating type, respectively, and haploid, B-6929, were used as for comparisons with *C. albicans* strains. The *S. cerevisiae* haploid strain no. 865 was used to obtain B-11961.

Media and maintenance. The standard rich medium, YPD [21], was used to grow liquid cultures for spreading cells on the plates. *C. albicans* and *S. cerevisiae* strains were incubated at 37°C and 30°C, respectively. Because *C. albicans* is particularly unstable, we used our

Table 1. *Candida albicans* and *Saccharomyces cerevisiae* strains.

Strain	Description or genotype, and karyotypic alterations	Reference or source
C. albicans laboratory strains		
3153A		[17]
WO-1		[18]
FC18		[19]
C9		[20]
Mutants derived spontaneously from 3153A		
m1-m14	Morphological mutants; single or multiple alterations of various chromosomes	[9,10]
Sor1-Sor10	L-sorbose utilizers; alteration of chromosome 5	[13,14]
Ara1, Ara2, Ara4, Ara5	D-arabinose utilizers; alteration of chromosome 6	[14]
Ara6-Ara15	D-arabinose utilizers; alteration of chromosome 2	[14]
Ara3	D-arabinose utilizer; alteration of chromosome 4	[14]
300	Derivative sometimes used instead of 3153A	[3]
301, 302, 303	Mutants of 300 unable to germinate	[3]
S. cerevisiae		
B-8722	*MAT***a**/*MAT*α *ura3/ura3 trp1/trp1 leu2/leu2 can1/can1*	F. Sherman
B-11961	*MAT***a**/*MAT***a** *ura3/ura3 trp1/trp1 leu2/leu2 can1/can1*	This study
B-6929	*MAT***a** *his3*-D*1 trp1-289 ura3-52*	F. Sherman
No. 865	*MAT*α *his3*-D*200 trp1-289 ura3-52*	F. Sherman

previously described maintenance procedures, which included keeping strains exclusively as stocks at -70°C on 15% glycerol and avoiding subcloning in order to preserve an original population [10,11].

UV irradiation. Strains were taken from -70°C stocks as a mass of cells, inoculated and grown to the end of stationary phase to avoid budding and resulting in a vast majority of single cells, and then gently sonicated to dissociate clumps. At least 90% of cells were viable after this procedure as estimated by platings. Using a cell counting chamber, appropriate dilutions were prepared and triplicates of YPD plates spread with approximately 200-300 cells per plate. Cells on the surface of open Petri plates were exposed to ultraviolet light (UV) in a custom-built irradiator containing germicidal lamps (General Electric G8T5), for 4, 8, 12, and 16 seconds. The plates with *C. albicans* and *S. cerevisiae* were incubated for 48 and 72 hours, respectively, in the dark to avoid photoreactivation before counting the grown colonies. The dose rate at the agar surface was 5 J/m^2/s, as determined by a germicidal photometer (International Light, Inc.). The triplicates of non-irradiated plates with the appropriate dilutions were included in each experiment as a control. Percent survival for each strain was calculated and presented by a survival curve. Strain 3153A, from which all mutants were derived, was assayed in three independent experiments and the range was calculated as a percentage for each dose and used to estimate the variability.

X-ray irradiation. Strains were grown, prepared and plated as described above for UV irradiation. Cells were X-irradiated on the surface of solid medium in open plastic Petri dishes with a Machlett OEG-60-7 X-ray tube, powered by a custom-made X-ray generator (Picker Corp.). The unit was operated at 50 KVP and 25 ma, with only inherent filtration. The dose rate at the surface of the plates was determined to be 28 kilorads/min, using a Model 555 Radcon II ratemeter with a 555-100 LA probe (Victoreen Instrument Division). Sets of triplicate plates were irradiated for 15, 30, 45 and 60 seconds.

Selection of S. cerevisiae strains homozygous for mating type. Approximately one thousand cells of *S. cerevisiae* B-8722 (*MAT***a**/*MAT*α) were plated on YPD plates and immediately irradiated with UV for 10 sec, resulting in approximately 90% of survival. The mutants homozygous for the mating type locus arise by mitotic crossing-over, which is enhanced by UV treatments. The screen for mutants homozygous for the mating type locus was performed by crossing irradiated diploid cells with either one of two haploid strains B-6929 (*MAT***a**) or no. 865 (*MAT*α). The homozygous *MAT***a**/*MAT***a** strain, B-11961, was isolated by this procedure and its survival curve was used for comparison to the survival curves of the *C. albicans* strains.

RESULTS AND DISCUSSION

The X-ray sensitivity of the laboratory strains of *C. albicans*, FC18, 3153A, C9 and WO-1, was compared to various strains of *S. cerevisiae*, including the *MAT***a** (B-6929) haploid strain, and the *MAT***a**/*MAT*α (B-8722) and *MAT***a**/*MAT***a** (B-11961) diploid strains, which are heterozygous and homozygous, respectively, at the mating type locus. As previously reported [22,23], and shown in figure 1, *MAT***a**/*MAT*α diploid strains are far more resistant to the lethal effect of ionizing radiation than haploid strains, and more resistant than diploid strains homozygous for the *MAT* locus. Thus, the X-ray sensitivity of the homozygous *MAT***a**/*MAT*a diploid strain is between that of the haploid and the heterozygous *MAT***a**/*MAT*α diploid

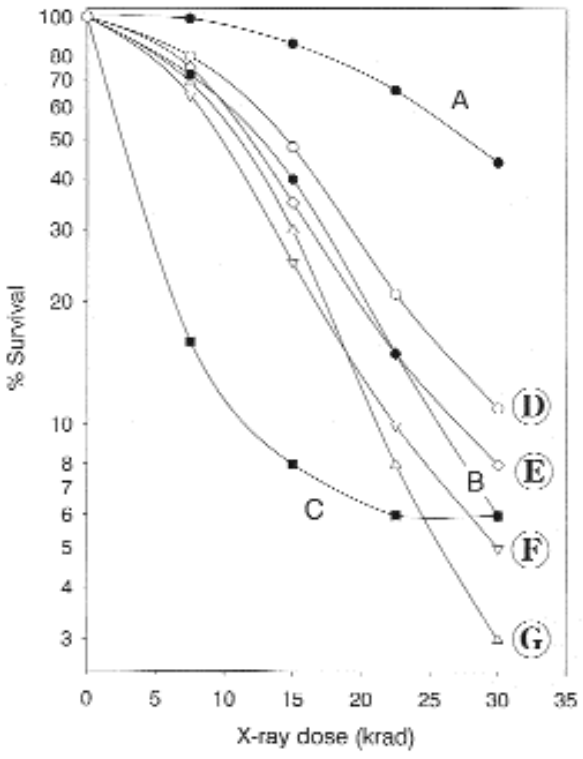

Figure 1. X-ray survival curves for the laboratory strains. A, B and C, *S. cerevisiae* B-8722 (*MAT***a**/*MAT*α), B-11961 (*MAT***a**/*MAT***a**) and B-6929 (*MAT***a**), correspondingly. D, E, F, and G, *C. albicans* FC18, 3153A, C9 and WO-1, correspondingly.

strain. This increased sensitivity of homozygous *MAT*a/*MAT*a diploid strains is due in part to the absence of the dimer protein Mat**a**1p-Matα2p, a product of the *MAT* locus that induces the repair genes belonging to the *RAD52* epistatic group [24].

All of the laboratory *C. albicans* strains showed survival curves remarkably similar to the X-ray survival curve of the diploid strain of *S. cerevisiae* homozygous at the *MAT* locus (Figure 1). In this regard, it is important to note that Hull *et al.* [25] analyzed the structures of the mating-type-like (*MTL*) loci, identified *MTL***a***1*, *MTL*α*1* and *MTL*α*2* alleles in the studied *C. albicans* strain, similarly to the *S. cerevisiae MAT* loci, and constructed hemizygous derivatives of the strain with either *MTL***a***1* or *MTL*α*1* and *MTL*α*2* alleles disrupted [26]. These genetically produced hemizygous strains mated on a plate with low frequencies [C. M. Hull, personal communication], and also mated at approximately 10^{-6} when the mixtures of cells were passed through a mouse [26]. Also, Magee and Magee [27] achieved low frequency of mating by crossing populations with opposite *MTL* loci, in which cells had mixed, hemizygous and homozygous, condition of *MTL* locus. In their experiments, hemizygosity was achieved by passing strains through L-sorbose medium, which induces the loss of either one of two chromosome 5 homologues carrying opposite *MTL* loci [13]. The subsequent exposure of mutated cells to the rich medium results in relatively frequent duplication of the remaining homologue of chromosome 5 [13], thus creating a mixture of cells with different condition of the chromosome 5 and *MTL* locus. The similarity of curves for *C. albicans* laboratory strains and *S. cerevisiae* strain homozygous at the *MAT* locus may reflect a different natural level of resistance of a different species or, in case the resistance of *C. albicans* could be

Table 2. UV and X-ray levels of sensitivity of *C. albicans* 3153A mutants having spontaneous and selected chromosomal alterations (see Figure 2).

Mutants	Sensitivity to	
	X-ray	UV light
m1 to m7, m9, m500, Sor1, Sor2, Sor4 to Sor6, Sor10, Ara3, 301-303	A, Normal	E,Normal
m8, m10 to m13, Sor7 to Sor9, Ara2, Ara4 to Ara15,	A, Normal	F
Sor3	B	E, Normal
300	C	E, Normal
Ara1	B	F
m10	D	F
m14	Variable	Variable

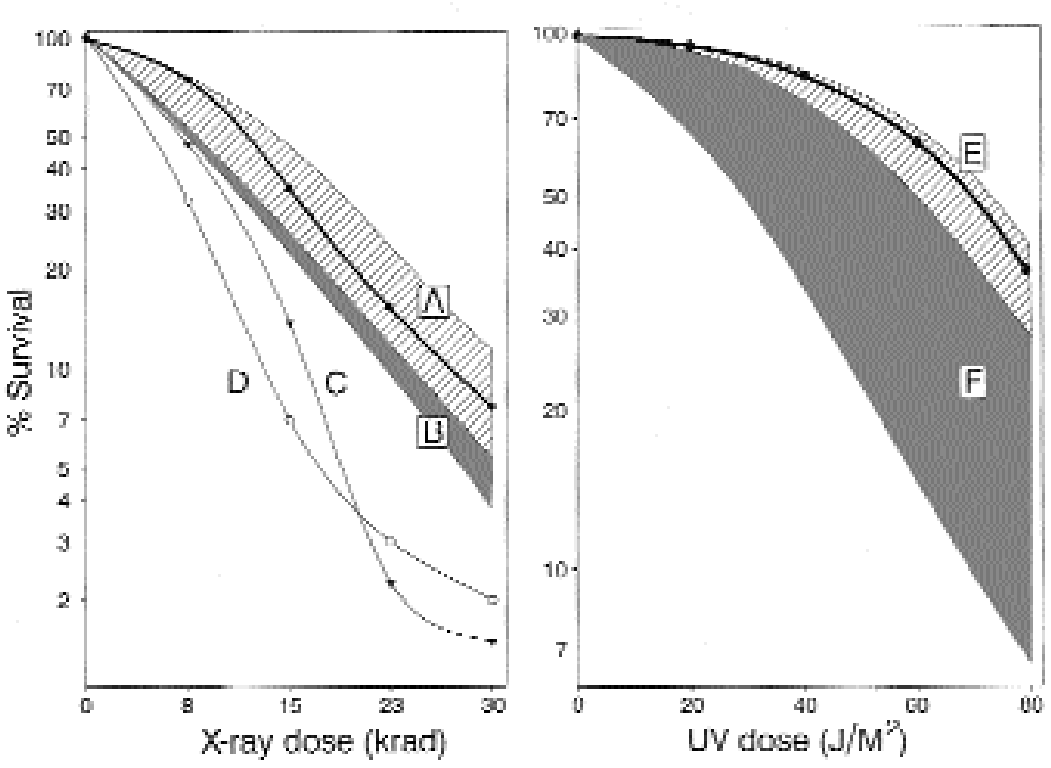

Figure 2. X-ray and UV survival curves for the mutants of *C. albicans* 3153A. A, *C. albicans* parental 3153A and mutants m1 to m9, m11 to m13, 301 to 303, Sor1, Sor2, Sor4 to Sor10, Ara2 to Ara15; B, Sor3 and Ara1; C and D, 300 and m10, respectively; E, *C. albicans* parental 3153A and mutants m1 to m7, m9, m500, 300-303, Sor1 to Sor6, Sor10, Ara3; F, m8, m10 to m13, Sor7 to Sor9, Ara1, Ara2, and Ara4 to Ara15.

potentially the same as of *S. cerevisiae*, reflecting certain deficiency of *MTL* locus in *C. albicans.* Because mating of *C. albicans* occurred at frequencies far below the frequencies observed with analogous strains of *S. cerevisiae*, the regular heterozygous strains of *C. albicans* may not have the properties of heterozygous diploid strains of *S. cerevisiae*. Perhaps there is certain deficiency of *MTL* locus in *C. albicans*, which probably is reflected in inefficient mating and lowered resistance to ionizing radiation (Figure 1).

Furthermore, the Sor1 - Sor10 mutants (Table 1) were obtained as monosomic for chromosome 5 [13] and can be considered hemizygous for either one of the mating type locus. Because these mutants were grown in rich medium prior to radiation experiments, they are represented by mix populations of cells, which are hemi-and homozygous by *MTL* locus (see above). It is noteworthy that the mix populations of Sor1 - Sor10 mutants by-in-large had the same X-ray sensitivity as the heterozygous parental strain, 3153A (Table 2, Figure 2), contrary to the expected difference by the analogy with *S. cerevisae*. Thus, in contrast to *S. cerevisae*, X-ray sensitivity does not appear to depend on the genetic constitution of the mating loci. It remains to be seen if the diminished mating and X-ray response are directly related.

In addition, the UV-survival curves of the *C. albicans* laboratory strains showed little variability (data not shown). In contrast, Suzuki *et al.* [28] reported variability among clinical isolates. It is important to note for our purpose that the radiation sensitivity of strain 3153A, from which the mutants were derived, appeared typical for the species. These results were highly reproducible and have been confirmed from the results of three independent experiments.

In this study, we have investigated the UV and X-ray sensitivity of two major classes of *C. albicans* mutants derived from 3153A, the spontaneous morphological mutants, and the selected positive mutants, which were comprised of the L-sorbose and D-arabinose utilizers. While the spontaneous morphological mutants have a wide range of altered karyotypes, the selected positive mutants constitute homogenous classes.

Many of the mutants of this study had increased sensitivity to radiation (Table 2 and Figure 2). Approximately 36% of the spontaneous morphological mutants were more sensitive to UV, one mutant was more sensitive to both UV light and X-rays, and approximately 14% were more sensitive solely to X-rays. Olaiya *et al*. [29] also reported a similar increase in UV sensitivity of two supposedly morphological mutants, which the authors isolated from strain 3153A for the inability to form germ tubes (see [3] for the equivalence of nongerminative and morphological mutants). In this regard, we would like to note that use of UV survival curves by Olaiya and Sogin [30], Olaiya *et al*. [29], and Suzuki *et al*. [28] to deduce ploidy is erroneous, and increased UV sensitivity reflects only a diminished repair capacity, similar to the mutants of our study. All arabinose positive mutants but one, Ara3, which has an exceptionally altered chromosomal pattern (see Table 1 and [14]), were more sensitive to UV, but not to X-ray. In addition, unlike the morphological and arabinose positive mutants, the sorbose positive mutants did not show any significant change. As described above, the X-ray survival curves were similar to the curve of strain 3153A (Figure 2, sector A), except for Sor3, which fell in sector B (Figure 2). Similarly, UV survival curves for Sor7, Sor8 and Sor9 were positioned slightly distant from the rest of the sorbose mutants, at the top of sector F (Figure 2). The broader spectrum of sensitivities in morphological mutants is consistent with this group large variety of chromosomal changes.

In contrast, selected positive mutants, although originated from different subclones of the parental strain, had changes in specific chromosomes. Namely, all sorbose utilizing mutants were predominately monosomic for chromosome 5, or were mixtures of cells monosomic for chromosome 5 or homozygous for one or another of the two chromosome 5 homologues. Arabinose utilizing mutants are represented by two groups, each having a specific alteration of either chromosome 6 or 4. Perhaps a link between sensitivity to UV light and D-arabinose positive phenotype can be established after a more thorough investigation. An alternative explanation would be that coincidentally both chromosomes 6 and 4 are implicated in UV sensitivity.

In this work, we determined the UV and X-ray sensitivity of laboratory and mutant strains of *C. albicans* and found the lack of expected resistance of diploid laboratory strains to X-ray. The increase in sensitivity to ionizing radiation may be related to inefficiency of mating. In addition, although the mechanism is unknown, our results clearly established that certain chromosomal alterations cause increased sensitivity to radiation, which is another feature among many others effected by chromosomal alterations. It is also unclear why such a high proportion of the mutants with chromosomal changes are sensitive to radiation, especially to UV light. Possibly the common change in chromosome copy number, which influences positive or negative regulators in *C. albicans* [11,13], is sufficient for affecting UV but not X-ray repair.

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