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Supporting Online Material for

Aneuploidy and Isochromosome Formation in Drug-Resistant *Candida albicans*

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Science Supporting Online Material

Materials and Methods

Comparative genome hybridization arrays
CGH was performed as described previously (*S1*). All test strains were compared to the reference control strain, SC5314. Briefly, digested genomic DNA from the test strain and SC5314 were differentially labeled with Cy3-dUTP and Cy5-dUTP (Roche), respectively. DNAs were mixed in a 1:1 ratio and co-hybridized to a whole genome *C. albicans* microarray printed in our lab. Fluorescence intensity ratios (mean log 2 values) were obtained and analyzed using GenePixPro 5.1 and GeneTraffic 3.1.

Chromosome_map plots
A Matlab program was used to plot all microarray data points as function of their chromosome location as described previously (*S1*). All CGH data were plotted on a log2 scale and clipped to the range corresponding to 1-4 gene copies. Expression data (Fig. 1C) were not clipped but instead plotted as a running average over 5 sequential ORFs (*S2*).

Fluconazole susceptibility testing
Minimal inhibitory concentrations (MIC) were determined using fluconazole E-test strips (0.016–256 µg/ml, AB Biodisk) on Casitone-agar plates. Overnight YPAD cultures were diluted in sterile 0.85% NaCl to an OD_{600} of 0.01 and plated using a sterile cotton swab. Etest strips were applied and plates were incubated at 35ºC for 48 hours. The susceptibility endpoint was read at the first growth-inhibition ellipse. Strains with MIC values of 4 µg/ml and above were considered resistant to fluconazole.

Contour-clamped homogenous electric field (*CHEF*) *electrophoresis*
Whole-chromosome analysis was done as described previously (*S1*). Sfi I–digested chromosomes (*S3*) were separated on a 1% agarose gel (Megabase Agarose, Bio-Rad) in 0.5X TBE, using a CHEF DRIII (Bio-Rad) apparatus with the following conditions: 7- to 100-s switch, 4.5 $V \cdot cm^{-1}$, 120°angle, for 21 hours, followed by 80- to 400-s switch, 3.5 $V \cdot cm^{-1}$, 120° angle, for 21 hours. Southern hybridization was done as described below.

Southern hybridization
Genomic DNA (10 µg) was digested overnight with the restriction enzymes Eco NI and Sac I. Samples were run on a 0.8% agarose gel. DNA was transferred to Magnacharge nylon membranes (Osmonics), which were then probed and detected as described previously (*S1*). PCR probes (Table S2) were labeled with DIG-11-dUTP according to manufacturer's instructions (Roche).

Sequence analysis of Chromosome 5
To sequence the gap between contig 19.10170 and 19.10202, genomic DNA from two strains homozygous for chr5, 4448 and 4449 (*S4*), was amplified using primer pairs 2003/1990 and 1991/1915 (Fig. S1 and Table S2). All primers were within unique, non-repetitive DNA. Sequencing of PCR products was carried out at the AGAC Sequencing Facility at the University of Minnesota. Sequence analysis and alignment of allelic sequences was performed using Sequencher software, version 4.2 (Gene Codes Corp., Ann Arbor, MI).

Fig. S1. Sequence spanning the gap between contigs 19.10170 and 19.10202 in Assembly 19 of the *C. albicans* genome sequence (*S5–S7*), including 50 bp on either side of the gap. Polymorphic base positions for the alleles are designated as follows: Y = C/T, W = A/T, R = A/G.

GTTTATTGTGGCCCATTTAACTGAACCAAAGCGTGTACAGACTTGGCA
TGTGTGGATTTGCAGATTTGAATTTCGGGTTGTTACTCAACCGTTAACT
GAGACAATGCTGCTAACGAGTATTTCCCCCGATCCAGTGTGAATGAAA
AGTGTGGAAAATTTTGTTTCAGTTGGTAGAAGTGGTACTGTTAGCTAA
GGCAAGATGTTGTGAAAYCATCAATACCATAGGGAAGACTGATAGCT
GGAGATGTTTYCAATTGGTCTTGATGATTGCAGCTATCTCAATAATTAT
CTAGGTATCAGTCCAAGAAAAGTTYTCTTAAGGAAGATGGGGTGGAG
AACGAGTAGCTCGGCTTTGARATGAACTTGCAATGTTTCATGGTCGCT
TTTTGTCTTAAAGAAATACTCTCATCTTGCTTCTCAATTATGTATGGAA
TGYRGATTTTGTTGAACTGCTGCTCATTGATTTTGACCGACTTGCTGTT
ATTCTGAGTAGARAAATTTACCAGCAATTGTGTGTGTGTCTGTGTGTGT
TTTTTTTTTGAAAATGTGAAGTTGGCTAGTTTCATTATTGCAGYGTCTT
GTCCTCGACATAAACAGCCTAGTGATAATAAGTGTGAAGAGTAGTAAT
TGAAGTTTGGTGTGTCTAGGTGGTGTTTAAGACTGTTTAGTATTTTCGC
AAGAATTTTGTTAACTTTGTGTGTATAAAATTCCTCAGCTACAGTTTCT
ACTTTTCCCACAGACAATAGCCCACTAGATTATCTCAATATTAACTCTA
AAACATTTTCTTCAGTTCTCACTATTAGACAAAGGGAAGTTTTCTCTTT
GATAAGYCCTTCTCCTGTGAAAAGTTTCGAGATGTAACGTTTCGCAGT
AATAGAGAGCCAGAATCCATTTTTGTGTACTACAGACAAATTCAGAAG
TTTCAACTGCTGCATATCGCCTTAAATGACTGTAGCATTCGTCCAAATT
GAGACCCTCAATTACATTTTGTCAAAAAAATTGGTCCCTAGTGTTGCT
ATCGATAACGAAGGTGAAGGCAGTTTAGCTTGGAGRCATTTAGAGAA
CTTAGTTACATCTCATCTTCCGTTTCGAGAAATCGTTGATTTACCGTGC
AGCGCTTATATTGATTGCTACTTGTTCCCAGCACCACAGCAATATAGC
AATCWTAAATAAATTGCCCCGCGGTTGACAGTGTATATCTTCGAGGAA
TGGCAACCTTTGCCCC

Table S1. Strains used in this study. Copy number of *MTL* alleles was estimated from CGH array data. MTL status: Among the 8 strains with i(5L) were two independent events in which *MTL* became homozygous. Notably, none of the i(5L) strains had equal numbers of *MTLa* and *MTL*α alleles. This suggests that i(5L) formation is accompanied by homozygosis of the *MTL* locus and, perhaps, homozygosis of other genes on chr5L as well. For consistency, MIC values were reassessed for all strains using E-test strips.

Strain number	Strain ID	MTL status	MIC (µg/ml)	Strain source
YJB2348	SC5314	a/α	0.75	(*8*)
4448	SC5314	a/a	0.75	(*4*)
4449	SC5314	α/α	0.75	(*4*)
YJB9613	T118(N0)	a/ α	0.25	(*9*)
YJB8741	N4-330	a/α	0.25	(*9*)
YJB8734	D8-330	a/α	4	(*9*)
YJB8735	D9-165	a/a/a/α	6	(*9*)
YJB8736	D9-330	a/a/α	64	(*9*)
YJB8737	D11-330	a/a/a/α	64	(*9*)
YJB8738	D12-165	a/α/α/α	8	(*9*)
YJB8739	D12-260	a/α	64	(*9*)
YJB8740	D12-330	a/α	4	(*9*)
YJB9309	DSY2285	a/α	0.25	(*10*)
YJB9311	DSY2286	a/α/α/α	16	(*10*)
YJB9175	S315	a/a	>64	(*11*)
YJB9180	P39	a/a/a/a	>256	(*11*)
YJB8638	FH1	a/α	2	(*12*)
YJB9185	FH5	α/α/α/α	>256	(*12*)
YJB8640	FH8	α/α/α/α	64	(*12*)

Table S2. Primers used in this study. F, Forward; R, reverse.

Primer ID	Primer description	Sequence (5´ to 3´)
Sequencing*		
2003	F	CAGTCGGGCGATTATCT
1990	R	TTGACGAACGTAGGGACCGT
1992	F	TGGCCGCAACGTAATCTATG
1915	R	CAGCCTTTGAGCCCTATT
Southern analysis		
1925	Probe L-F	GAATTGCCGTTCCGATTG
1926	Probe L-R	CCGCAAATGCTTGCTCTT
1910	Probe R-F	CCAGACCCCTGCCTCTTT
1909	Probe R-R	AGTTGGATGCGTTGGTCC
2251	Probe I-F	GGCGGTACACCTGCTGTT
2252	Probe I-R	TTCAGCGGCTTCAGGAAC
2010	Cse4-Probe-F	TACTTCTGGTCAACGAGGCT
2011	Cse4-Probe-R	GACTGACATCCGTACTATCG
2203	Telomere adjacent Probe-F	CCCATCGACCACCCATTA
2204	Telomere adjacent Probe-R	GGCGTCGATTTTGGAATG

*Primers used for sequencing across the chr5 gap.

Table S3. The presence of i(5L) isochromosome is correlated with maintenance of high levels of Flu^R. Independent isolates derived from YJB9185 (parent) were tested for fluconazole resistance using E-strip assays. Isolate 1 is YJB9517 shown in Fig. 3. The presence of i(5L) was detected on Southern blots of CHEF gels containing undigested chromosomes and probed for i(5L) as in Fig. 2C.

Isolate	MIC (µg/ml)	i(5L) present?
Parent	>256	Yes
1	8	No
2	>256	Yes
3	16	No
4	>256	Yes
5	>256	Yes
6	>256	Yes
7	>256	Yes
8	>256	Yes
9	16	No

References and Notes

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