Corrections

BIOCHEMISTRY. For the article “Kernel energy method: Application to insulin,” by Lulu Huang, Lou Massa, and Jerome Karle, which appeared in issue 36, September 6, 2005, of Proc. Natl. Acad. Sci. USA (102, 12690–12693; first published August 24, 2005; 10.1073/pnas.0506378102), the authors note an error in a funding acknowledgment: “L.M. thanks the National Institutes of Health for National Institutes of General Medical Sciences Grant M01RR016463 and National Center for Research Resources Grant RR00856 and the National Science Foundation for Centers of Research Excellence in Science and Technology grant support. This investigation was supported by Research Centers in Minority Institutions’ Award RR03079 and the National Science Foundation for National Institute of General Medical Sciences Grant MBRS SCORE5 S06GM606654 and the National Science Foundation for Centers of Research Excellence in Science and Technology grant support.” The correct acknowledgment is: “L.M. thanks the National Institutes of Health for National Institutes of General Medical Sciences Grant M01RR016463 and National Center for Research Resources Grant RR00856 and the National Science Foundation for Centers of Research Excellence in Science and Technology grant support. This investigation was supported by Research Centers in Minority Institutions’ Award RR03079 and the National Science Foundation for National Institute of General Medical Sciences Grant MBRS SCORE5 S06GM606654 and the National Science Foundation for Centers of Research Excellence in Science and Technology grant support.”

The correct, best-fit value for the prefactor $R_0$ is 1.927$^{+0.271}_{-0.238}$ Å (bounds represent 95% confidence intervals). We apologize for any inconvenience this error may have caused. This error does not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0507559102

BIOPHYSICS. For the article “Random-coil behavior and the dimensions of chemically unfolded proteins,” by Jonathan E. Kohn, Ian S. Millett, Jaby Jacob, Bojan Zagrovic, Thomas M. Dillon, Nikolina Cingel, Robin S. Dothager, Soenke Seifert, P. Thiyagarajan, Tobin R. Sosnick, M. Zahid Hasan, Vijay S. Pande, Ingo Ruczinski, Sebastian Doniach, and Kevin W. Plaxco, which appeared in issue 34, August 24, 2004, of Proc. Natl. Acad. Sci. USA (101, 12491–12496; first published August 16, 2004; 10.1073/pnas.0403643101), the authors note the following: “Due to a mathematical error in our analysis, we incorrectly reported the prefactor ($R_0$) for the Flory scaling relationship observed between the experimental $R_G$ of chemically denatured proteins and their sequence length:

$$R_G = R_0 N^v.$$ 

The correct formula is:

$$R_G = R_0 N^v.$$ 

The correct, best-fit value for the prefactor $R_0$ is 1.927$^{+0.271}_{-0.238}$ Å (bounds represent 95% confidence intervals). We apologize for any inconvenience this error may have caused. This error does not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0507472102

EVOLUTION. For the article “The application of statistical physics to evolutionary biology,” by Guy Sella and Aaron E. Hirsh, which appeared in issue 27, July 5, 2005, of Proc. Natl. Acad. Sci. USA (102, 9541–9546; first published June 24, 2005; 10.1073/pnas.0501865102), the authors note the following: “The stationary distribution of fixed genotypes (Eq. 9) was previously derived (1, 2) in the context of the evolution of transcription factor binding sites, by using detailed balance of the substitution dynamics (Eq. 3). The formula appears slightly different between refs. 1 and 2 and ref. 3, because of slight differences in assumptions; in refs. 1 and 2, continuous time is assumed, whereas in ref. 3, discrete time is assumed. Furthermore, in refs. 1 and 2, fitness is parameterized in terms of molecular distance from an optimum sequence. These authors also discuss a linear combination of fitness and a quantity they call ‘mutational entropy’; when the system is at equilibrium, this combination is very similar to our free fitness.”


Corrections

www.pnas.org/cgi/doi/10.1073/pnas.0507361102

GENETICS. For the article “Loss and gain of chromosome 5 controls growth of Candida albicans on sorbose due to dispersed redundant negative regulators,” by M. Anamal Karib, Ausaf Ahmad, Jay R. Greenberg, Ying-Kai Wang, and Elena Rustchenko, which appeared in issue 34, August 23, 2005, of Proc. Natl. Acad. Sci. USA (102, 12147–12152; first published August 11, 2005; 10.1073/pnas.0505625102), on page 12152, the first sentence of the last paragraph in the left column, “If each region encompasses at least one CSU gene, CSU51–CSU55, then three more genes in regions B, C, and 139 are expected” should read: “If each region encompasses at least one CSU gene, CSU51–CSU55, then four more genes in regions B, C, and 139 are expected.” The conclusions of the article remain unchanged.

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Loss and gain of chromosome 5 controls growth of Candida albicans on sorbose due to dispersed redundant negative regulators

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Communicated by Fred Sherman, University of Rochester School of Medicine and Dentistry, Rochester, NY, July 6, 2005 (received for review May 24, 2005)

A reversible decrease or increase of Candida albicans chromosome copy number was found to be a prevalent means of survival of this opportunistic pathogen, under conditions that kill cells or inhibit their propagation. The utilization of a secondary carbon source, L-sorbose, by reversible loss of chromosome 5, serves as a model system. We have determined that an ~209-kbp portion of the right arm of chromosome 5 contains at least five spatially separated, functionally redundant regions that control utilization of L-sorbose. The regions bear no structural similarity among themselves, and four of them contain sequences that bear no similarity with any known sequence. We identified a regulatory gene in region A that encodes a helix–loop–helix protein. Most important, the multiple redundant regulators scattered along chromosome 5 explain, in a simple, elegant way, why the loss of the entire homologue is usually required for growth on sorbose. Thus, an entire chromosome acts as a single regulatory unit, a feature not previously considered. Our finding appears to be a paradigm for the control of other phenotypes in C. albicans that also depend on chromosome loss, thus implying that C. albicans genes are not distributed randomly among different chromosomes.

Candida albicans is an important opportunistic fungal pathogen that is normally found in healthy humans. C. albicans is a natural diploid, containing eight pairs of chromosomes, that does not have mating between haploids but can perform a parasexual cycle in vitro, starting from matching the diploids and finishing with the tetraploids randomly losing chromosomes and subsequently returning to the diploids (1). It was established that the C. albicans population gains genetic variability in vitro because of high-frequency chromosome instability (reviewed in ref. 2). Most unusual, however, was the finding that C. albicans uses specific alterations of various specific chromosomes to survive and adapt to different environments in which cells are killed or their propagation is hampered. Among specific alterations, reversible change of chromosome copy number is a prominent, albeit unusual, means of regulation. For example, utilization of the secondary carbon sources L-sorbose (Sou+) and D-arabinose (Aru+) depends on chromosome-5 and chromosome-6 monosomy, respectively, or, alternatively, chromosome-2 trisomy, Aru− (3–5). Primary resistance to the antibiotic fluconazole and 5-fluoro-orotic acid depends on chromosome-4 monosomy and chromosome-4 trisomy, respectively (6, 7). Control of sorbose utilization was studied in detail, because it involves the loss of only chromosome 5, which can be reversed by duplication of the remaining homologue (2–4, 8, 9). Growth on sorbose depends on the SOU1 gene that encodes NADPH-dependent sorbose reductase, which converts L-sorbose to D-sorbitol, leading to the production of fructose. The loss of one copy of chromosome 5 up-regulates SOU1, which resides on another chromosome. During Sou−→Sou+ transition, the SOU1 transcript increases several-fold, although both SOU1 copies and its upstream sequences remain intact, suggesting that the copy number of chromosome 5 controls the copy number of a (CSU) gene, encoding a negative regulator residing on this chromosome. The ratio between CSU and SOU1, thus, determines the Sou phenotype (3, 8–11).

Here, we demonstrated that chromosome 5 apparently contains at least five functionally, but not structurally, redundant negative regulators, CSU51–CSU55, in five spatially separated regions within an ~209-kbp segment. We identified the first gene CSU51, which encodes a protein of the helix–loop–helix type. The loss of one copy of all five regions, or some of them in particular combinations, allowed growth on a sorbose plate, mimicking the Sou+ phenotype because of the loss of one chromosome 5. Multiple redundant regulators explain, in a simple, elegant way, why the entire chromosome needs to be lost in Sou+ mutants.

Materials and Methods

Strains, Plasmids, Primers, Media, Growth Conditions, and Software.

The C. albicans Ura− Sou− strain CAF4–2 and prototrophic Sou− strain 3153A and their Ura− Sou+ mutant Sor19, lacking one chromosome 5, and the exceptional Sou+ mutant Sor5, respectively, were reported in refs. 3, 8, 9, and 12. Escherichia coli strain XL-1 blue (13) and Saccharomyces cerevisiae strain B-6929 were used for the preparation of plasmids and for cloning purposes.

All plasmids used in this work are derivatives of pSFU1, pSF11 (14, 15), pABTEL, pAK45, pAK104 (16), and pCA88 (10), and pRC3915 was published in ref. 17. All primers are presented in Table 3, which is published as supporting information on the PNAS web site.

Yeast extract/peptone/dextrose, synthetic dextrose (SD) (18), L-sorbose media (5), yeast carbon base/BSA-rich medium, and SD medium containing the mycophenolic acid at 8 μg/ml are described in ref. 15. To prepare solid medium, 2% (wt/vol) agar was added. Uridine (50 μg/ml) was added when needed. The growth and handling of cells are described in refs. 6, 10, and 19.

DNA and protein sequences were analyzed with the software available on the sites of the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov), the Stanford Genome Technology Center (www-sequence.stanford.edu), the National Research Council Biotechnology Institute (Montreal) (http://candida.bri.nrc.ca/candida/index.cfm?page=CaGeneSearch), the Softberry protein database (www.softberry.com/berry.phtml), and ExpASy (http://expasy.hcuge.ch/index.html) and the programs MATIND and MATINSPECTOR (20).

Abbreviations: CSU, control of sorbose utilization; PFGE, pulsed-field gel electrophoresis. Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. DQ068774).

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Phenotypic Assay on L-Sorbose Medium. Either quantitative or spot dilution assays were carried out as described in ref. 7.

Pulsed-Field Gel Electrophoresis (PFGE). PFGE is described in refs. 3, 16, 19, 21, and 22.

Fragmentation of Chromosome 5. Various fragmentation cassettes were made in pAK104 carrying URA3, as described in ref. 16. Also, a 0.5-kbp HindIII/SacI fragment carrying a C. albicans telomere was removed from pABTEL and inserted in pSFU1 containing URA3 flipper (14) that was digested with SacI/NotI. Different fragmentation cassettes were prepared in the resulting pAK197 by subcloning ~1-kbp individual sequences of chromosome 5 or mapping sequences into a KpnI/XhoI site. Also, a 2.5-kbp XbaI fragment carrying the mutated IMH3 gene for resistance to mycophenolic acid was removed from pSFU1 and inserted in the XbaI site of pUC19. The resulting plasmid was linearized with SalI/PstI and ligated with a 0.5-kbp SalI/PstI fragment from pABTEL carrying a C. albicans telomere, thus creating pAK105. A 1.0-kbp chromosome sequence adjacent to fragmentation site 3 was amplified by using total genomic DNA of CAF4-2 and was subcloned into pAK195 linearized with SacI/BamHI, thus creating pAK195, which was used to fragment chromosome 5 at site 3 in double constructs.

Internal Deletion. Various deletion plasmids were made in pAK45 carrying URA3, as described in ref. 16. Also, various flanking sequences, either 0.5 kbp or 1.0 kbp long, were amplified by PCR from total genomic DNA of CAF4-2 and subcloned into pKpnI/XhoI and SacI/NotI sites of pSFU1 containing a URA3 flipper cassette, thus creating different deletion plasmids.

Standard Verification of the Desired Genetic Change. The site of chromosomal integration of the deletion cassette was confirmed with two pairs of primers, such that the PCR products were amplified across the 5’ and 3’ junctions, with one primer in each pair corresponding to a portion of the marker sequence. A single pair of primers, amplifying across the end of the fragmentation cassette opposite the telomere, was generated similarly. The eviction of the deletion cassette was confirmed by using a pair of primers that amplify across the eversion junction. Fragmented chromosomes or larger deletions were additionally visualized by using PFGE (16).

Results

Approaches. In this work, the Sou− strain CAF4-2 was used to identify CSU genes on chromosome 5. An initial screen for candidates was performed with a chromosome-5 DNA library prepared in replicative plasmid pCA88. In this plasmid, a chromosome fragment was coexpressed with SOU1, which confers a Sou− phenotype (ref. 10; and see Supporting Materials and Methods, which is published as supporting information on the PNAS web site). When a plasmid insert carried a putative CSU gene for a negative regulator, SOU1 was repressed, and the Sou− phenotype of the strain CAF4-2 was restored. The final identification of the sequences that carry CSU genes came from deletions on chromosome 5 that shifted the phenotype from Sou− to Sou+, similar to the loss of one homologue of chromosome 5. Independently, the phenotype shift from Sou− to Sou+ was obtained because of chromosome-5 telomere-mediated fragmentation. The coexpression system was also used to analyze truncated or mutated portions of the original chromosome sequence.

Multiple Deletions. One means of deleting spatially separated sequences on chromosome 5 was to remove, in one step, large portions of the chromosome by telomere-mediated fragmentation. This method allows us to deliberately break the chromosome and is based on homologous recombination between so-called mapping sequences, carried on the chromosome, and a plasmid. The plasmid also contains a selectable marker and a C. albicans telomere, which stabilizes and allows propagation of one of two expected fragments (ref. 16; and see Fig. 4B, which is published as supporting information on the PNAS web site). The sequences were also removed selectively, a process that required either two internal deletions or an internal deletion and a fragmentation. For two sequential manipulations, cells were transformed with either a URA3 flipper cassette (14) that was subsequently recycled on yeast carbon base BSA medium or with two cassettes carrying URA3 and IMH3 for mycophenolic acid resistance (15), respectively. The latter technique did not require an eversion step. It was irrelevant, for our purposes, whether one copy of the different sequences was sequentially deleted on the same or different chromosome-5 homologues.

Confirmation Strategies. Relating manipulations with chromosome 5 and phenotypic changes depended on the correct integration of deletion and fragmentation cassettes. We confirmed each site of cassette integration or eversion by PCR. The fragmented chromosomes and their sizes were additionally confirmed with PFGE, as published in ref. 16. Only constructs with the desired alteration(s) and no other visible change in chromosome patterns, as confirmed by a full electrokaryotype of each construct, were finally assayed on L-sorbose plates. To ensure that the introduced genetic change confers a certain phenotype, up to four constructs of the same type were prepared independently.

Cloning Nine Candidate Sequences and Assigning Three of Them Within the ~395-kbp Terminal Portion in the Right Arm of Chromosome 5, Which Controls the Sou Phenotype. We prepared a chromosome-5 DNA library in replicative vector pCA88 that carries SOU1 and confers growth on sorbose, Sou+, (see Approaches). A recipient Sou− strain CAF4-2 was transformed with the library, and 480 transformants were obtained. A total of nine plasmids that contained inserts, denoted 133–135 and 137–142, were finally confirmed to repress SOU1, causing no growth on sorbose plates and, thus, recovering the original Sou− phenotype of CAF4-2 (data not presented).

Further analysis included the exceptional Sou− mutant Sor5 that was derived from C. albicans strain 3153A on an L-sorbose plate (8, 9). In contrast to the majority of Sou− mutants (which lost one homologue of chromosome 5), Sor5 acquired a large deletion on homologue 5a, 5a−Δ, which presumably caused its phenotype (Fig. 1A and C). According to Southern blot analysis, this deletion encompassed genes HIS3, ADH1, and CAG1 near the right telomere (Fig. 2A) (23). This result prompted us to determine a continuous sequence in the right arm of chromosome 5. We aligned four contigs, 19-10194 (contains telomere), 19-10171, 19-10093, and 19-2472, from assembly 19 of the C. albicans genomic sequence (Stanford Genome Technology Center), and we also determined positions of two more contigs, 19-10155 and 19-10105 (16). These contigs are designated as 1–6 in this article (Table 1 and Fig. 2A).

Six of nine cloned inserts, 133–135 and 140–142, were excised from the plasmids used to prepare probes, which were hybridized to a chromosome blot of the mutant Sor5. As shown in Fig. 1B, two probes representing sequences 140 (6.892 kbp) and 135 (4.340 kbp) did not show signals with the truncated chromosome 5a−Δ, suggesting that these sequences map within the deletion. The other probes hybridized with both chromosomes 5b and 5a−Δ (data not presented). The ends of all nine inserts, 133–135 and 137–142, were sequenced and used to search assembly 19 of the Candida genome. Sequence 140 mapped in contigs 3 and 135, and an additional sequence, 139 (4.547 kbp), mapped in contig 1 (Fig. 2C). Taken together with the loss of HIS3, ADH1, and
CAGI1, this result suggested that the Sor5 deletion extends to at least \( \approx 356 \) kbp, encompassing contigs 1 and 2 and a portion of 3 (Fig. 2A). This result also suggested that sequence 139 maps into the deletion. Furthermore, as anticipated, the control terminal deletion of \( \approx 395 \) kbp between site 12 and the right telomere (Fig. 2B) on one homologue of chromosome 5, conferred a Sou\(^+\) phenotype, confirming the results with the Sor5 mutant (Table 1) (see Approaches for chromosome fragmentation). In contrast, another terminal deletion of \( \approx 88 \) kbp proximal to the left telomere (on another arm) did not change the phenotype (data not presented). The remaining six sequences, including the four that were mapped outside the Sor5 deletion, were distributed along chromosome 5 outside contigs 1–6. Importantly, each sequence was represented only once.

These results demonstrated that a terminal deletion of \( \approx 395 \) kbp on the right arm causes the Sou\(^+\) phenotype, similar to the loss of one homologue of chromosome 5.

Identifying Functional Redundancy Between Sequences 140 and 135 or Between Sequences 140 and 139. Systematic truncation of one homologue of chromosome 5 showed that Sou\(^+\) and Sou\(^-\) phenotypes are generated according to the size of the removed portion. The larger truncations, of \( \approx 345–378 \) kbp at sites 7–11, consistently caused a Sou\(^+\) phenotype, whereas strains with the shorter truncations, at sites 3–6, remained Sou\(^-\) (Fig. 2B and Table 1). This result was consistent with the finding that the \( \approx 395 \)-kbp terminal portion of chromosome 5 was important for regulation (see above).

Unexpectedly, the deletion of one copy of the intervening sequence of \( \approx 0.566 \) kbp between sites 6 (Sou\(^-\)) and 7 (Sou\(^+\)), which, presumably, contained the CSU gene and which, remarkably, fell within sequence 140 (Fig. 2B and C), did not produce Sou\(^-\). Similarly, Sou\(^+\) was not produced by the two larger deletions that overlapped the intervening sequence and removed either one copy of 140 (6.892 kbp) or one copy of the extended 78.5-kbp sequence between the deletion sites 4 and 9, the latter designated as deletion VII (Fig. 2D, Table 2). Also, the Sou\(^-\) phenotype of strain CAF4-2 did not change upon deletion of a single copy of either sequence 135 or 139 (Table 2).

Furthermore, pairwise deletions revealed redundancy of regulatory elements. The Sou\(^+\) phenotype was produced upon concomitant removal of sequences 140 and 135 or sequences 140 and 139 (Fig. 2E, lines 1 and 2) but not sequences 135 and 139 (see fragmentations in Fig. 2B and Table 2). Thus, all three sequences, 135, 139, and 140, are implicated in negative control of the Sou phenotype, representing independent functional regions, but regions 135 and 139 are not redundant. Clearly, growth on sorbose requires a concomitant removal of particular regions that are spatially separated on the right arm of chromosome 5.

**Fig. 1.** Determining fragments 135 and 140 within the deletion of the Sou\(^+\) mutant Sor5. (A) The three short chromosomes, indicated with a bracket, of the strain 3153A and its mutant Sor5, as separated with the orthogonal-field-alternation gel electrophoresis (OFAGE) apparatus. The remaining chromosomes are clustered together at the top of the gel. The bands for the chromosomes 6a, 6b, and 5a-\(\Delta\) are indicated with arrows. Note that 3153A has a homologue 6b shorter because of a deletion (6). (B) Signals obtained with the filter corresponding to the gel in A and with the probe prepared from the chromosome fragment 135. (C) Schematic representation of chromosomes 6a, 6b, and 5a-\(\Delta\), which are shown in A. A single chromosome is indicated with a dotted line. Two comigrating chromosomes, 6b and 7a, are indicated with a continuous line.

**Fig. 2.** The five functional regions [140 (encompassing A and B), 135, C, and 139] that contain CSU57 and the putative CSU52-CSU55 genes. (A) The schematic representation of chromosome 5, including contigs in the right arm, designated 1–6, as indicated by the open bars (16). For the contig nomenclature, see Table 4, which is published as supporting information on the PNAS web site. The tentative positions, but not the alignment, of contigs 5 and 6 have been determined. The gap between contigs 4 and 3 has been sequenced, and the sizes of two other gaps (between contigs 3 and 2 and between contigs 2 and 1) were deduced. The approximate positions of the SfiI site, CAGI, ADH1, and HIS5 genes and cloned fragments 133, 134, 137, 138, 141, and 142 that are distributed along chromosome 5 outside contigs 1–6 are indicated. The following are indicated in parentheses as kbp: chromosome-5 length; a total length of contigs 1–4, including gaps; and a suggested deletion in the Sor5 mutant. (B) The hemizygous truncation of chromosome 5 at sites 7–12, but not at sites 3–6, conferred the Sou\(^+\) phenotype. The positions of fragmentation sites 1–12 and the associated Sou phenotypes are presented above contigs 1, 3, and 4. (C) The five functional regions are shown as filled boxes, with their positions and sizes relative to contigs and fragmentations in B. (D) The large deletion VII of \( \approx 78.5 \) kbp on one chromosome 5, which is presented in scale with B and C, does not change the Sou\(^+\) phenotype. (E) The four types of hemizygous constructs that conferred the Sou\(^+\) phenotype: (i) 140–\(\Delta\) 135–\(\Delta\), (ii) 140–\(\Delta\) (by deletion VII) C-\(\Delta\) 139–\(\Delta\) (by fragmentation 3), (iii) A-\(\Delta\) C-\(\Delta\) 139–\(\Delta\) (by fragmentation 3), (iv) csu51–\(\Delta\) C-\(\Delta\) 139–\(\Delta\) (by fragmentation 3). The internal deletions and truncations are presented in scale with B–D. (F) Identification of CSU57 in the 953-bp sequence of region A. The fragmentation sites 6–8, within and flanking region A, are shown as boxed numbers with the corresponding Sou phenotypes. The nucleotide positions of the five ORFs are indicated. Asterisks designate the approximate positions of the frameshift mutations that were introduced in each ORF by site-directed mutagenesis. (G) The Sou phenotype of five strains transformed with pCA88-derived plasmids that coexpress SOU1 and differently mutated region A, as described above. Each of orf1–orf5 designates a mutated ORF in region A. Two control strains that carry either pCA88 with SOU1 alone (pCA88) or the normal sequence of region A (Reg. A), are also shown. Note that only destruction of ORF3 (circled asterisk in F) allowed expression of SOU1, leading to a Sou\(^+\) phenotype on an L-sorbose plate.
Table 1. Sou phenotype of strains in which one chromosome 5 is fragmented at various sites

<table>
<thead>
<tr>
<th>Fragmentation site*</th>
<th>12</th>
<th>11</th>
<th>10</th>
<th>9</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sou+ colonies, %</td>
<td>34; 57</td>
<td>16; 34; 37; 53; 85</td>
<td>82; 68; 42</td>
<td>59; 61</td>
<td>52; 42</td>
<td>62</td>
<td>0.0</td>
<td>0.2</td>
<td>1.8</td>
<td>0.0; 0.2</td>
</tr>
</tbody>
</table>

*The phenotypes for fragmentation at sites 1 and 2 were determined in combination with deletion of Region A (see Table 2). See Table 4 for the site positions and the values of the control strains.

Identifying a 0.953-kbp Functional Region A Within Sequence 140. Sequence 140 was conveniently coexpressed with SOU1 on the vector pCA88 (see Approaches) and systematically truncated. We found that the loss of a 0.953-kbp portion, which has been designated region A, abolished the repressive properties of SOU1 (see Fig. 2F). Region A, which maps on the chromosome between sites 6 and 8 (Fig. 2F), overlaps with the previously determined intervening sequence of 0.566 kbp between sites 6 and 7 (see above for systematic chromosome truncation). The functionality of region A was corroborated by an analysis of chromosome deletions, as described below.

confirm the above overexpression analysis and suggested that region A contains CSU. On the other hand, the constructs A-Δ135, against expectation and unlike the Sou+ constructs 140-Δ135, did not become Sou+. This result revealed that region A is not equivalent to region 140. We are confident that the phenotypic differences are not due to the method for deleting the regions, because the parallel constructs were similarly prepared, that is, A-Δ135-A and 140-Δ135-A combined internal deletions. On the other hand, A-Δ139-A and 140-Δ139-A combined internal deletion of region 140 and region A, with terminal deletion at site 3 (for example, Fig. 2E, line 1–3, and Table 2). The inequality of regions A and 140 revealed at least one more region, region B, in the remaining portion of sequence 140. It is clear that a combined deletion of regions A, B, and 135 confers growth; however, uncertainties remain whether deletion of both regions A and B or deletion of only region B is required. We would like to point out that, unlike a preliminary identification

Table 2. Sou phenotype of Ura+ and Ura− constructs lacking one copy of various regions 140 (A and B), A, 135, C, or 139 on chromosome 5

<table>
<thead>
<tr>
<th>Abbreviated genotype</th>
<th>Sou+ colonies, %</th>
<th>Sou− colonies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>140-Δ 135-Δ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion VII (140-Δ)</td>
<td>16; 12; 17</td>
<td></td>
</tr>
<tr>
<td>A-Δ fragment 3 (C-Δ 139-Δ)</td>
<td>43; 27</td>
<td></td>
</tr>
<tr>
<td>csu51-Δ fragment 3 (C-Δ 139-Δ)</td>
<td>73; 66; 70; 32; 78; 49</td>
<td></td>
</tr>
<tr>
<td>A-Δ fragment 1 (139-Δ)</td>
<td>0.2; 0.4</td>
<td></td>
</tr>
<tr>
<td>Ura− Sou− original CAF4−2</td>
<td>0.8; 0.8</td>
<td></td>
</tr>
<tr>
<td>Ura− Sou− derivative of CAF4−2, ER305</td>
<td>0.4; 0.1; 0.2; 0.1</td>
<td></td>
</tr>
<tr>
<td>Ura− Sou− mutant Sor19 lacking one chromosome 5</td>
<td>67; 61; 79</td>
<td></td>
</tr>
</tbody>
</table>

*For explanation, see Table 1. The Sou+ phenotype is indicated in bold.

†Integrative plasmid pRC3915 (LEU2 URA3) inserted at LEU2 locus in strain CAF4−2. The following strains (abbreviated genotypes) are presented in this table: ER1705, ER1792, ER1793 (140-Δ 135-Δ); ER1291, ER1292 [Deletion VII (140-Δ) fragment 3 (C-Δ 139-Δ)]; ER1463, ER1606, ER1644 [A-Δ fragment 3 (C-Δ 139-Δ)]; ER1826, ER1827, ER1833 [A-Δ fragment 2 (C-Δ 139-Δ)]; ER2119 [csu51-Δ fragment 3 (C-Δ 139-Δ)]; ER469, ER470, ER472 (140-Δ); ER1266, ER1271, ER1276, ER1277 [Deletion VII (140-Δ)]; ER514, ER515, ER517, ER518 (135-Δ); ERF06, ER2016, ER2017, ER2018, ER2112 (csu51-Δ); ER1314, ER1313, ER1312 (A-Δ); ER1668, ER1970, ER1971 (A-Δ 135-Δ); ER1963, ER1964, ER1965 (A-Δ C-Δ); ER1484, ER1485 [A-Δ fragment 1 (139-Δ)].
of region A by coexpression of the corresponding portions of region 140 with SOU1 on pCA88 (see above), region B could not be identified. The simple explanation is that splitting region 140 outside region A into two fragments destroyed the relevant CSU1.

We extended the analysis of the Sou phenotype due to concomitant deletion of regions A and 139 by combining internal deletion of region A with terminal deletions at sites 1 and 2. We found that combination with the shorter truncation at site 1 does not change the Sou− phenotype. However, in constructs with the truncation at site 2, which removed an ~9-kbp-longer portion, the Sou− phenotype shifted to Sou+, similar to the constructs A-Δ139-Δ containing fragmentation 3, as exemplified in Fig. 2E, line 3 (see also Table 2). This result revealed that there is at least one more functional region of 9 kbp, region C, between sites 1 and 2.

We thus initially cloned three regions, 140, 135, and 139, from the library, followed by confirmation using chromosome deletions. Two more regions, B and C, were determined by chromosome deletions.

Computational Analysis of Regions 140, 135, 139, and C. Nucleotide sequences of all regions were compared to the Candida genome databases and the whole genome database of NCBI. One large ORF was identified in each of regions A, B, and 139, and none of them was similar to any known sequence. Five ORFs were identified in region C; two of them were not similar to any known sequence, and the other three corresponded to the SFC1 identification in region C; two of them were not similar to any known sequence, and the other three corresponded to the sequence, and the other three corresponded to the CSU51 in Region A. We sequenced and compared the

Sequence Analysis of CSU51. The nucleotide sequence of the 255 bp of CSU51 encodes a deduced protein of 84 amino acids, with a proposed molecular mass of 9.24 kDa and a high content of serine (16%), alanine (21%), and glycine (15%) (Fig. 3). ExPASy tools predicted that Csu51p has a helix-loop-helix structure, with helix 1 and helix 2 spanning the regions between amino acids 2 and 3 and 17–20 and between 63–67 and 82–84, respectively. However, there is no significant similarity between the helix 1 and 2 regions of Csu51p and other helix–loop–helix proteins (25). Csu51p apparently localizes to the nucleus, according to the Softberry protein database, implying that Csu51p might be a transcription factor. It remains to be seen whether CSU51 binds to the SOU1 promoter, preventing an efficient transcription, or forms heterodimers with other transcription factors.

Discussion

We uncovered five separate regions, A, B, C, 135, and 139, within ~209 kbp of the right arm of chromosome 5 that are functionally,
but not structurally, redundant. These regions repress growth on sorbose medium, presumably through repression of a metabolic gene
SOU1 on another chromosome. We speculate that five regions belong to two redundant regulatory pathways, one including regions A and B and another including regions 135, C, and 139. Clearly, hemizygous deletion of the following did not produce Sou− phenotype: any single region; the pair of regions A and B; and the three regions 135, C, and 139. However, Sou− strains became Sou+ because of hemizygous deletion of all five regions or hemizygous deletions that combined the regions from two putative pathways: A, B, and 135; A, 135, C, and 139; and A, C, and 139. We have previously reported a weak CSU on chromosome 1, an essential gene BMHI, deletion of one copy of which increased SOU1 transcript by twofold (10). In addition, three other potential weak regulators, CSU2, CSU5, and CSU6, from different chromosomes, were partially characterized (G. Janbon, Y.-K.W., and E.R., unpublished data). The complexity and redundancy of the negative regulation could reflect a general redundancy of metabolic and regulatory pathways in the cell that now becomes increasingly obvious (26, 27).

If each region encompasses at least one CSU gene, CSU51–CSU55, then three more genes in regions B, C, and 139 are expected. The question is, what is a total number of CSUs on chromosome 5? Most informative in this respect is the fact that each of nine fragments of chromosome 5 (133–135 and 137–142) that we cloned from the chromosome-5 DNA library was represented only once. This is especially important because of the small size of the library versus the relatively large number of cloned fragments. It is possible that all or some of these fragments also contain CSUs. Furthermore, some additional CSUs could be uncovered in still unidentified portions of the chromosome, including the inside of the right arm. One explanation for the growth on sorbose due to the loss of one copy of CSUs carried in regions A, B, C, 135, and 139 but not all CSUs, could be that these genes represent all redundant pathways.

Multiple redundant regulators explain, in a simple, elegant way, why the entire chromosome needs to be lost in Sou− mutants. It remains to be seen whether chromosomes 4 and 6, which are lost in fluconazole-resistant and Ara− mutants, respectively, also contain multiple negative regulatory genes. These findings would imply that multiple groups of C. albicans genes are distributed nonrandomly among the different chromosomes. The diminution of the dose of multiple negative regulatory genes through the loss of an entire chromosome 5 of C. albicans appears to involve the same mechanism that produces the diminution of the dose of multiple tumor-suppressor genes in humans. For example, bladder cancer can arise through the loss of chromosome 9, which contains tumor suppressors in at least four spatially separated regions (28).

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