High-Resolution Genetic Mapping With Ordered Arrays of *Saccharomyces cerevisiae* Deletion Mutants

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ABSTRACT

We present a method for high-resolution genetic mapping that takes advantage of the ordered set of viable gene deletion mutants, which form a set of colinear markers covering almost every centimorgan of the *Saccharomyces cerevisiae* genome, and of the synthetic genetic array (SGA) system, which automates the construction of double mutants formed by mating and meiotic recombination. The Cbk1 kinase signaling pathway, which consists minimally of *CBK1*, *MOB2*, *KIC1*, *HYM1*, and *TAO3* (*PAG1*), controls polarized morphogenesis and activation of the Ace2 transcription factor. Deletion mutations in the Cbk1 pathway genes are tolerated differently by common laboratory strains of *S. cerevisiae*, being viable in the W303 background but dead in the S288C background. Genetic analysis indicated that the lethality of Cbk1 pathway deletions in the S288C background was suppressed by a single allele specific to the W303 background. SGA mapping (SGAM) was used to locate this W303-specific suppressor to the *SSD1* locus, which contains a known polymorphism that appears to compromise *SSD1* function. This procedure should map any mutation, dominant or recessive, whose phenotype is epistatic to wild type, that is, a phenotype that can be scored from a mixed population of cells obtained by germination of both mutant and wild-type spores. In principle, SGAM should be applicable to the analysis of multigenic traits. Large-scale construction of ordered mutations in other model organisms would broaden the application of this approach.

SYNTHETIC genetic array (SGA) analysis automates the systematic construction of double mutants in the budding yeast *Saccharomyces cerevisiae*, enabling a comprehensive and high-throughput analysis of genetic interactions (Tong *et al.* 2001). In this system, a marked query mutation is first crossed to the set of ~5000 viable haploid gene deletion strains and then a series of robotic arraying procedures allows selected growth of double-mutant meiotic progeny. Because these haploid double mutants are formed by meiotic recombination and because the set of viable gene deletions represents a colinear series of mapping markers covering almost every centimorgan of the yeast genome, SGA analysis also provides a method for high-resolution genetic mapping. Here, we apply the SGA mapping (SGAM) method to identify a suppressor of the lethality associated with defects in the Cbk1 signaling pathway.

Budding yeast Cbk1 is a member of the Cot-1/Orb6/Ndr1/Warts family of serine-threonine protein kinases, which may have conserved roles in the control of cell polarity and morphogenesis (Yarden *et al.* 1992; Justice *et al.* 1995; Millward *et al.* 1995; Verde *et al.* 1998; Durrenberger and Kronstad 1999; Zallen *et al.* 2000). Cells deleted for *CBK1* have a number of morphological abnormalities, including defects in bipolar budding, polarized morphogenesis, and the separation of mother and daughter cell walls following cytokinesis (Dorland *et al.* 2000; Racki *et al.* 2000; Bidlingmaier *et al.* 2001). The cell separation defect is due to an inability to activate the Ace2 transcription factor, which localizes specifically to the daughter cell nucleus in a Cbk1-dependent manner (Racki *et al.* 2000; Bidlingmaier *et al.* 2001; Colman-Lerner *et al.* 2001). Ace2 activates a daughter-cell-specific transcription program including *CTS1*, which encodes a chitinase required for cell separation. A conserved signaling pathway encompassing Cbk1 has been uncovered by disparate genetic screens (Sullivan *et al.* 1998; Dorland *et al.* 2000; Racki *et al.* 2000; Bidlingmaier *et al.* 2001; Colman-Lerner *et al.* 2001; Du and Novick 2002; B. Nelson and C. Boone, unpublished results). This pathway is composed minimally of Cbk1 and a set of four other highly conserved proteins, Mob2, Tao3 (Pag1), Hym1, and Kic1, a kinase whose catalytic region resembles that of PAK-like kinases (B. Nelson and C. Boone, unpublished results). In the W303 genetic background, deletion mutants of *CBK1*, *MOB2*, *TAO3*,

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TABLE 1

Yeast strains

Strain name	Background	Relevant genotype	Source
W3031A	W303	*MAT***a** *ura3-1 leu2-3,112 his3-11 15 trp1-1 ade2-1 can1-100*	J. Hirsch
Y3576	W303	W3031A *cbk1Δ::URA3*	This study
Y1560	W303	W3031A *hym1Δ::URA3*	This study
Y3732	W303	W3031A *mob2Δ::natR*	This study
Y3400	W303	W3031A *kic1Δ::URA3*	This study
Y3577	W303	W3031A *tao3Δ::URA3*	This study
Y1748	W303	W3031A *MAT*α *cbk1::kanR*	This study
BY4741	S288C	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Brachmann *et al.* (1998)
Y3068	S288C	*MAT*α *can1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Tong *et al.* (2001)
Y3655	S288C	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Tong *et al.* (2001)
Y2806	S288C	*MAT*α *can1Δ::MFA1pr-HIS3 leu2Δ0 his3Δ1 lys2Δ0*	Tong *et al.* (2001)
Y3717	W303/S288C hybrid	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3 leu2 his3 lys2Δ0 cbk1Δ::URA3*	This study
Y3716	W303/S288C hybrid	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3 leu2 his3 lys2Δ0 hym1Δ::URA3*	This study
Y3968	W303/S288C hybrid	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3 leu2 his3 lys2Δ0 mob2Δ::natR*	This study
Y3715	W303/S288C hybrid	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3 leu2 his3 lys2Δ0 kic1Δ::URA3*	This study
Y3714	W303/S288C hybrid	*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3 leu2 his3 lys2Δ0 tao3Δ::URA3*	This study

HYM1, and *KIC1* are all viable but associated with defects in polarized morphogenesis and cell separation similar to those described above for *cbk1*Δ. In the S288C genetic background, deletion mutants of *CBK1*, *MOB2*, *TAO3*, *HYM1*, and *KIC1* are not viable. Genetic analysis revealed that an allele of an unknown gene functions as a W303-specific suppressor of the lethality caused by abrogation of the Cbk1 signaling pathway. We used the SGAM method to map the suppressor of the Cbk1 pathway to the *SSD1* locus.

MATERIALS AND METHODS

Yeast strains and media: Yeast strains used in this study are listed in Table 1. S288C-derived strains are congenic with BY4741 (Brachmann *et al.* 1998), except at indicated loci. W303-derived strains are congenic with W3031A (Hirsch *et al.* 1991), except at the indicated loci. YPD is 2% peptone, 1% yeast extract, 2% glucose, and 2% agar. Sporulation medium is 2% agar, 1% potassium acetate, 0.1% yeast extract, and 0.05% glucose, supplemented with uracil, histidine, and leucine. SC medium is 0.2% amino acid drop-out mix, 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 0.5% ammonium sulfate, 2% glucose, and 2% agar. Filter-sterilized solutions of L-canavanine (50 mg/liter; Sigma, St. Louis), G418 (200 mg/liter; GIBCO-BRL, Gaithersburg, MD), and clonNAT (100 mg/liter; Werner Bioagents) were added to cooled (<50°), autoclaved media where indicated. In cases where SC medium was supplemented with clonNAT or G418, the ammonium sulfate was replaced with 0.1% monosodium glutamate and the medium termed SC/MSG.

SGA analysis: SGA analysis was carried out as described (Tong *et al.* 2001) to identify viable gene deletions that show synthetic growth defects with deletions in Cbk1 pathway genes. Five starting strains for SGA analysis were constructed. These starting strains were to contain a marked deletion in a Cbk1 pathway gene, the W303-specific suppressor allele, as well as a mating-type reporter construct (*can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2*). First, the viable *cbk1Δ::URA3* (Y3576), *hym1Δ::URA3* (Y1560), *mob2Δ::natR* (Y3732), *kic1Δ::URA3* (Y3400), or *tao3Δ::URA3* (Y3577) deletion mutants in the W303 background were crossed to Y3068 (S288C background, *MAT*α *can1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) and *MAT***a** *can1Δ::MFA1pr-HIS3lys2Δ0* progeny carrying the *URA3*- or *natR*-marked deletion alleles were isolated by tetrad dissection. As these isolates were viable, they contained the W303-specific suppressor of deletions in the Cbk1 pathway genes. The W303/S288C hybrids were then backcrossed to another S288C strain to diminish the number of W303-specific alleles. Specifically, the W303/S288C hybrids were mated to the S288C strain Y3655 (*MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*), and viable *MAT*α *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2* progeny carrying the *URA3*- or *natR*-marked deletions were isolated by tetrad analysis. The resulting strains were one-fourth W303 and three-fourths S288C hybrids and were used as the *MAT*α starting strains for the subsequent SGA analysis.

For SGA analysis, the *MAT*α starting strains, *cbk1Δ::URA3* (Y3717), *hym1Δ::URA3* (Y3716), *mob2Δ::natR* (Y3968), *kic1Δ::URA3* (Y3715), or *tao3Δ::URA3* (Y3714) were mated to ~4700 individual *MAT***a** *xxxΔ::kanR* S288C haploid deletion strains. Yeast arrays were manipulated with a CPCA robot (Tong *et al.* 2001). Diploids were selected on SC-Ura-Lys plates or YPD plates supplemented with G418 and clonNAT for 1 day at 30° and then sporulated for 5 days at room temperature. To select for *MAT***a** *can1Δ::MFA1pr-HIS3::MF*α*1pr-LEU2* meiotic progeny, spores were germinated on SC-His-Arg medium supplemented with L-canavanine for 2 days at 30° and then transferred onto a fresh plate of the same medium for another day of growth at 30°. The resulting colonies were then transferred to SC/MSG-His-Arg supplemented with L-canavanine and G418 to select for *MAT***a** *xxxΔ::kanR* meiotic progeny. Finally,

cells were transferred to either SC/MSG-His-Arg-Ura supplemented with L-canavanine and G418 or SC/MSG-His-Arg supplemented with L-canavanine, G418, and clonNAT, to select for *MAT***a** double-mutant meiotic progeny. The final progeny were one-eighth W303 and seven-eighths S288C hybrids.

*MAT***a** double-mutant progeny with growth defects were identified by scoring colony size by eye on a scale of 1–5, where 1 is no colony and 5 is slightly decreased colony size. Gene deletions (*xxx*Δ*::kanR*) that consistently exhibited reduced colony size in wild-type control screens were eliminated from consideration. That is, finding genetic interactions with gene deletions (*xxx*Δ*::kanR*) that cause strong growth defects on their own is beyond the sensitivity of the current SGA methodology. SGA analysis was carried out three times on each starting strain and a synthetic interaction was scored if a gene deletion was synthetic lethal/sick in one or more of the three screens. Raw scores from SGA analysis are provided as supplemental data (available at http://www.genetics.org/supplemental/). While these data are expected to contain a substantial number of false positives that would not be confirmed by tetrad analysis (Tong *et al.* 2001), we anticipated that raw SGA data would be sufficient for mapping purposes.

To confirm some of the synthetic genetic relationships between the Cbk1 pathway deletions and gene deletions unlinked to *SSD1*, 31 deletions that appeared synthetic lethal in five out of five screens were mated to the *cbk1*Δ*::URA3* (Y3576) and *hym1*Δ*::URA3* (Y1560) starting strains and subjected to tetrad analysis. Seventeen deletions that showed synthetic interactions in four out of five screens and had known or suspected roles in cell polarity or structure were also subjected to tetrad analysis with *cbk1*Δ*::URA3* and *hym1*Δ*::URA3*. Spores were separated and germinated on YPD media. Nine out of the 48 (19%) interactions observed by SGA analysis that were not linked to *SSD1/YDR293C* could be confirmed by tetrad analysis. The relatively high false-positive rate may be due to the large number of loci (five) that must segregate to generate viable Nat^R (or Ura^+) Kan^R meiotic progeny, the sporulation defect associated with abrogation of the Cbk1 signaling pathway (B. Nelson and C. Boone, unpublished results), and/or the relatively low stringency used in scoring synthetic growth defects with each Cbk1 pathway gene deletion (see above; a synthetic interaction needed to be observed in only one-third of the SGA screens to be scored).

Algorithm for detecting significant groups of linked genetic interactions in SGA data: A list of gene deletions that were synthetic lethal/sick with at least four of the five Cbk1 pathway deletions *vs.* a list of ~4700 *kanR*-marked deletion mutants contained in our arrays was queried. First, the marginal probability (P_m) of a synthetic interaction was calculated from the total number of interactions divided by the total number of deletion strains screened. At each gene represented by a deletion strain, the number of synthetic interactions in the region surrounding that gene (five represented deletions in each direction) was calculated. The probability of observing this many interactions by chance alone was determined from a binomial distribution, assuming the probability of an interaction at any given gene is P_m. A Bonferroni correction was employed to compensate for the increased false-positive rate that results from performing multiple statistical tests. The Bonferroni correction increases the stringency of each significance test by dividing the *P*-value threshold by the number of tests performed.

Random spore analysis: Twenty-one strains carrying *kanR*-marked deletions in genes surrounding *SSD1*, in *SSD1* itself, and in four open reading frames (ORFs) lying at a great distance from *SSD1* on chromosome IV were mated to the *mob2*Δ*::natR* (Y3968) SGA starting strain. The heterozygotes were sporulated for 10+ days at room temperature and subjected to random spore analysis. Spores were released from asci by a 30-min incubation at 30° with 0.6 mg/ml zymolyase in 1 M sorbitol, followed by 1 min of vigorous vortexing. The separated spores were diluted in distilled water and spread on two SC/MSG-His-Arg plates supplemented with L-canavanine and clonNAT to select for the germination of *MAT***a** *can1*Δ*::MFA1pr-HIS3::MFα1pr-LEU2 mob2*Δ*::natR* spores. Spores that germinate and form colonies must contain the W303-specific suppressor of *mob2*Δ. Plates were incubated at 30° for 3 days. A total of 50–400 colonies were obtained per plate for each strain. Each plate was replicated onto SC/MSG-His-Arg supplemented with L-canavanine, clonNAT, and G418 and incubated for 2 days at 30° to determine what percentage of viable *MAT***a** *mob2*Δ*::natR* spore colonies were Kan^R.

Sequencing of *ssd1-d*: An ~4-kb fragment containing the entire *SSD1* open reading frame, as denoted in the Saccharomyces genome database (SGD), was amplified from genomic DNA derived from wild-type W3031A. PCR was performed with the Expand long template PCR system (Roche, Indianapolis) using the primers oMT1183/SSD1-UP (5′ GTCACTTTAATA TCGCAAAACAG) and oMT1182/SSD1-DOWN (5′ GGATA CTGAGGGGTGAAGC). Fifteen individual PCR reactions were pooled, the fragment purified with a Qiaquick PCR purification kit (QIAGEN, Chatsworth, CA), and the coding strand sequenced (ACGT Corporation). When compared to the *SSD1* sequence at SGD, which was derived from a S288C strain carrying the *SSD1-v* allele (Goffeau *et al.* 1996), no differences were detected in base pairs 1–2093 of the open reading frame. A single base-pair change was detected at nucleotide 2094, a C (SGD) to G (W303) mutation that creates a premature stop codon. The truncated *ssd1-d* gene product has a predicted molecular weight of ~77 kD. An identical mutation was found in the *ssd1-d* allele of a S288C-derived strain, JO371 (H. Friesen and B. Andrews, unpublished data).

Restoration of *cbk1*Δ lethality by *SSD1-v*: *cbk1*Δ*::kanR* (Y1748) and congenic wild-type (W3031A) strains in the W303 genetic background were transformed with plasmid P3765, a derivative of YEp24 (2μ, *URA3*) that houses a genomic fragment containing the *CBK1* gene. P3765 rescues both the "crusty" colony and cell-clumping phenotypes of Y1748 (data not shown). Individual Ura^+ transformants were subsequently transformed with either plasmid YEp13 (2μ, *LEU2*) or plasmid MTP251, a derivative of YEp13 that houses a 6-kb genomic fragment containing *SSD1-v*. Three Ura^+ Leu^+ transformants were picked and inoculated into SC-Ura-Leu and grown to saturation at 30°. Cultures were serially diluted (four 10-fold dilutions) in SC-Ura-Leu and then 5 μl of culture and each dilution were spotted onto SC-Leu and SC-Leu containing 0.1% 5-fluoro-orotic acid (5-FOA). Plates were photographed after 2 days at 30°.

RESULTS AND DISCUSSION

Mapping a common suppressor of lethality associated with *cbk1*Δ, *mob2*Δ, *tao3*Δ, *hym1*Δ, and *kic1*Δ deletion mutations: In principle, any allele leading to an epistatic phenotype, one identifiable within a mixed population of cells resulting from the germination of both mutant and wild-type spores, can be mapped with the SGAM methodology. Such phenotypes include but are not limited to: colony growth in an otherwise inviable genetic and/or environmental context, expression of chromophores, and morphogenetic phenotypes such as filamentous growth.

Consider the case of a haploid strain in which a suppressor mutation (*s*) in an unidentified gene (*S*) rescues

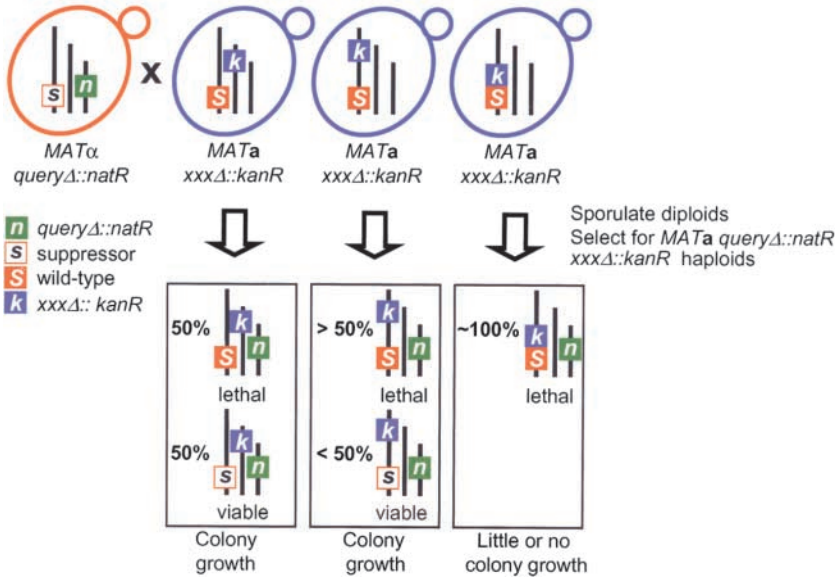

FIGURE 1.—Logic behind SGA mapping. In the example shown, SGA analysis is used to map the location of a mutant suppressor allele (*s*, open red box), which suppresses the lethality of a *natR*-marked gene deletion mutation (*query*Δ*::natR*, green box). As described in MATERIALS AND METHODS, SGA analysis combines the *natR*-marked deletion mutation with ~5000 viable *kanR*-marked deletion mutations (*xxx*Δ*::kanR*, blue boxes) through mating, meiotic recombination, and germination of haploid *MAT***a** *query*Δ*::natR xxx*Δ*::kanR* spore progeny. In deletion strains where the wild-type allele (*S*, solid red box) is tightly linked to the *kanR*-marked deletion (far-right yeast), the low frequency of recombination between the suppressor allele (*s*) and the *kanR*-marked deletion will limit the recovery of viable Nat^R Kan^R double-deletion progeny.

the lethality associated with deletion of an essential gene (*query*Δ*::natR*) marked with the dominant *natR* selectable marker (Figure 1). The viable double-mutant starting strain (*query*Δ*::natR s*) is crossed systematically to each of the ~5000 viable haploid deletion strains (*xxx*Δ*::kanR*) and the double-deletion meiotic progeny (*query*Δ*::natR xxx*Δ*::kanR*) are selected. If, for a given *kanR*-marked deletion strain, the wild-type allele (*S*) and the deletion (*xxx*Δ*::kanR*) are unlinked, 50% of the double-deletion meiotic progeny (*query*Δ*::natR xxx*Δ*::kanR*) will contain the suppressor mutation (*s*) and germinate to form a colony. In strains where the wild-type allele (*S*) is tightly linked to the deleted gene (*xxx*Δ*::kanR*), however, the low frequency of recombination between the suppressor allele (*s*) and the deleted gene (*xxx*Δ*::kanR*) will limit the recovery of viable double-deletion meiotic progeny. The general chromosomal location of the suppressor allele (*s*) can thereby be identified by the failure to observe colony growth for a linked set of double-deletion strains. In essence, to map the location of the mutation, SGAM employs a genome-wide and systematic set of two point crosses between the mutated allele and the marked deletion mutations.

On the basis of this logic, a number of advantages of the SGAM approach are evident:

1. Although the mutation is mapped by linkage to nonessential genes, it may lie in either nonessential or essential genes. To map mutations in essential genes, the allele must not cause lethality at the screening temperature.
2. Both dominant and recessive mutations can be mapped.
3. As the size of the linked group will be inversely proportional to the frequency of meiosis in the parent diploid colonies, the assay could be fine tuned by modulating sporulation efficiency. For instance, hampering sporulation by manipulating temperature and/or incubation times should extend the linked region.
4. In principle, multigenic traits in which several alleles are required to confer the mutant phenotype can be mapped, as linked groups lacking the mutant phenotype will form around each requisite allele.
5. Sequencing of the gene(s) lying within the center of the linked group should reveal the mutation. Once the candidate gene is mapped, subsequent molecular genetic analysis can then be carried out to prove that the mapped mutation is coincident with the suppressor mutation.

We applied the SGAM method to study of the Cbk1 signaling pathway. Deletion of *CBK1*, *MOB2*, *HYM1*, *KIC1*, or *TAO3* genes in the S288C genetic background, in which the complete set of yeast deletion strains is constructed, is lethal, whereas the equivalent deletion in the W303 background is not (WINZELER *et al.* 1999; DU and NOVICK 2002; B. NELSON and C. BOONE, unpublished results). Tetrad analysis of crosses between the viable W303 deletion mutants and an S288C strain revealed that 50% of the spores carrying a deletion mutation would germinate to form a colony, indicating that a single W303-based suppressor allele rescued the lethality of each deletion (data not shown). To map the suppressor allele, we first created appropriate starting strains for SGA analysis by crossing W303 strains that carried *URA3*- or *natR*-marked deletions of *CBK1*, *MOB2*, *HYM1*, *KIC1*, and *TAO3* with an S288C strain that carried the SGA mating-type-specific haploid selection reporter (*can1*Δ*::MFA1pr-HIS3::MF*α*1pr-LEU2*). SGA analysis was carried out with *cbk1*Δ, *mob2*Δ, *hym1*Δ, *kic1*Δ, and *toa3*Δ as query mutations against ~4700 viable *xxx*Δ*::kanR* deletion strains, and colony growth defects in the resultant double-deletion meiotic progeny were scored. We performed the strain manipulation robotically; however,

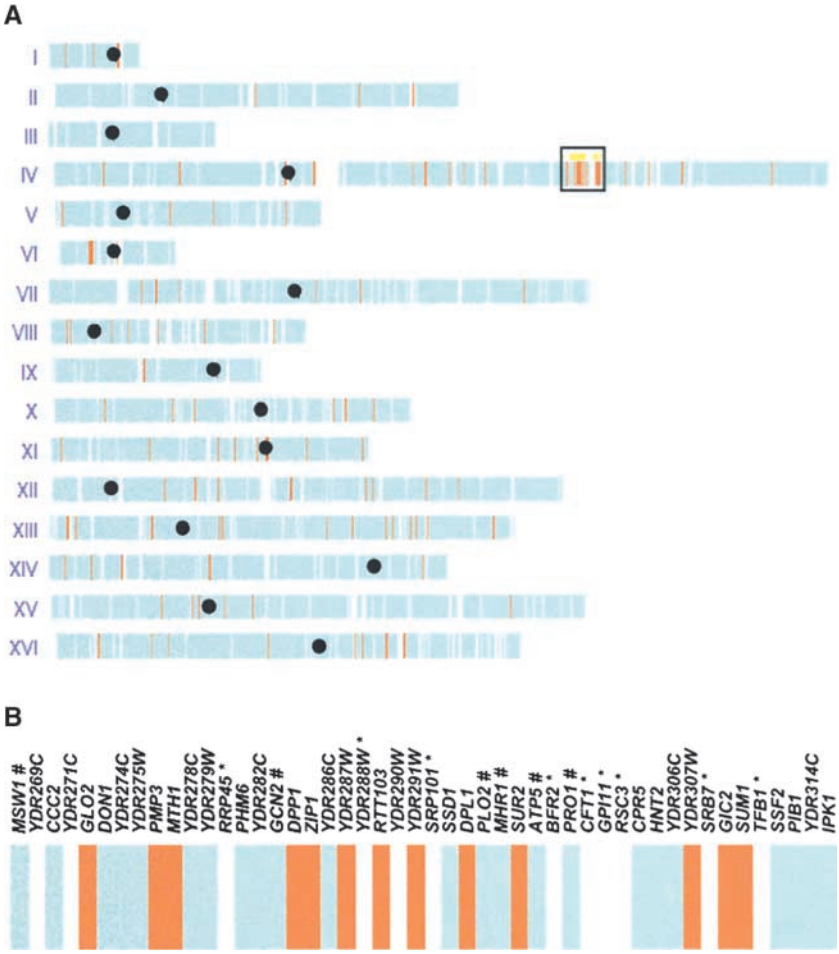

FIGURE 2.—(A) SGA analysis with gene deletions of Cbk1 pathway components revealed dispersed synthetic interactions as well as an apparent set of colinear synthetic interactions on chromosome IV. Starting strains deleted for *CBK1*, *MOB2*, *HYM1*, *KIC1*, and *TAO3* were subjected to SGA analysis *vs.* a near complete set of ~4700 *kanR*-marked, viable haploid deletion strains. Gene deletions included in this set are represented as blue lines and cover nearly the whole genome from chromosome I to XVI. Gene deletions that consistently exhibited synthetic interactions with deletions in Cbk1 pathway genes, found in at least four out of five screens, are represented as red lines and were found scattered throughout the genome. Centromeres are represented as a solid black circle. A set of genetic interactions in an ~90-kb region of chromosome IV (boxed) was identified as statistically significant by an algorithm that measures the density of synthetic interactions over a sliding window of 11 genes (see MATERIALS AND METHODS). The boxed region localizes the W303-specific suppressor of Cbk1 pathway deletions. (B) Magnification of the boxed ~90-kb region of chromosome IV. Genes are represented as in A. Essential genes are marked with * and are not represented by haploid deletion mutants. Gene deletions that consistently caused slow growth in SGA analysis *vs.* wild-type control strains were beyond the sensitivity of the assay and are marked with #.

SGA analysis can be conducted easily by hand pinning, as described previously (TONG *et al.* 2001).

Because it was likely that a single W303-derived suppressor mutation rescued the lethality of each deletion, we pooled the resultant genetic interactions and examined those that occurred for at least 4 of the 5 Cbk1 pathway deletions. In total, 31 deletions interacted with all five of the pathway members and 85 deletions interacted with four of the pathway members (see supplementary table at http://www.genetics.org/supplemental/ for results of all screens). Using a novel algorithm (see MATERIALS AND METHODS), we identified one chromosomal region that had a statistically significant enrichment of genetic interactions, which presumably mapped the location of the W303-derived suppressor (Figures 2 and 3). In this colinear set of 48 essential and nonessential genes, spanning an 89.6-kb (~28 cM) region of chromosome IV (MAPS), we identified 13 genetic interactions. This same region could also be identified from the primary data collected for three of the individual screens (*hym1*Δ, *kic1*Δ, and *tao3*Δ; see Figure 3), but pooling the data from all of the screens provides a method for removing false positives associated with the high-throughput analysis.

Mutations in *SSD1* suppress the lethality associated with deletions in Cbk1 pathway genes: Random spore analysis enabled us to determine the frequency of recombination between the putative Cbk1 pathway suppressor and the *kanR*-marked gene deletions in the mapped region of chromosome IV. The *mob2*Δ*::natR* SGA starting strain (Y3968) was mated to each *kanR*-marked deletion strain in this region. Viable *MAT***a** *mob2*Δ*::natR* meiotic progeny were selected for and replica plated onto media containing G418 to determine the percentage of viable *MAT***a** *mob2*Δ*::natR xxx*Δ*::kanR* recombinants (Figure 4A). For gene deletions such as *dpp1*Δ*::kanR* and *sur2*Δ*::kanR*, <10% of the viable *MAT***a** *mob2*Δ*::natR* meiotic progeny also grew on the G418 medium, as expected if a W303-based suppressor was linked to these deletion alleles (Figure 1). In contrast, for *ssd1*Δ*::kanR*, ~50% of the viable *MAT***a** *mob2*Δ*::natR* meiotic progeny also grew on the G418 medium. This result suggests that *ssd1*Δ*::kanR* suppresses the lethality associated with the *mob2*Δ*::natR* deletion. Indeed, unlike other deletions in this region, *ssd1*Δ*::kanR* showed no synthetic interactions with any of the Cbk1 pathway members by SGA analysis (Figures 2 and 3 and supplementary data at http://www.genetics.org/supplemental/).

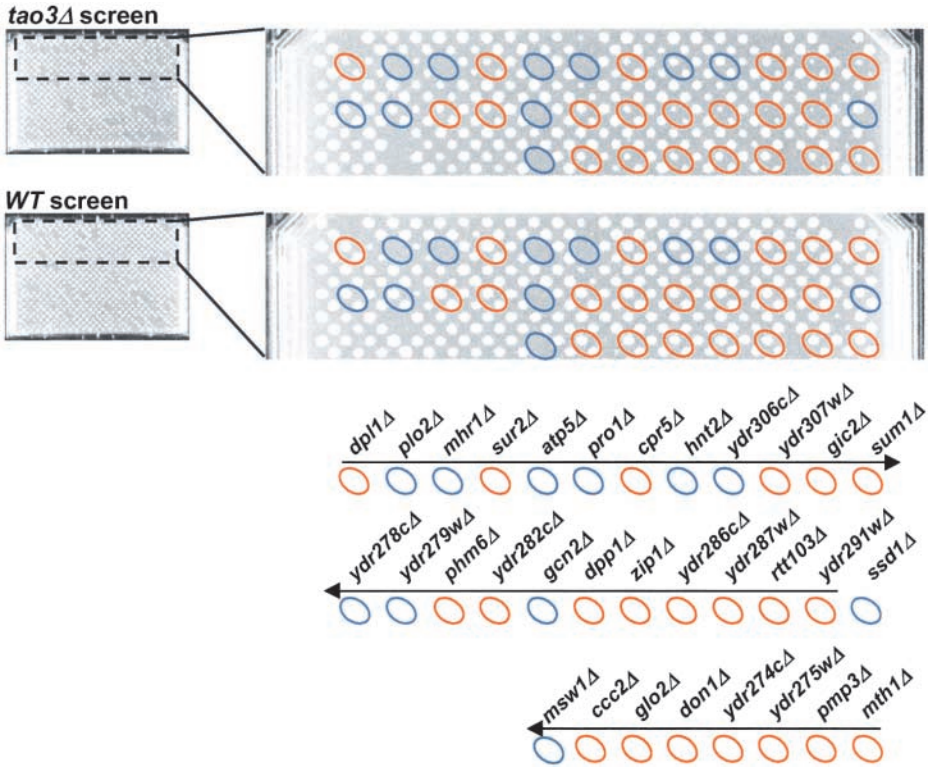

FIGURE 3.—A linked region of inviable or slow-growing double mutants surrounded the *SSD1* locus when SGA analysis was carried out with deletions of Cbk1 pathway components. In the final step of SGA analysis, double-mutant meiotic progeny were selected by growth on SC/MSG-His-Arg-Ura plates supplemented with L-canavanine and G418 (see MATERIALS AND METHODS). Of the 16 plates of arrayed double-mutant meiotic progeny, one contained the *ssd1*Δ*::kanR* strain and most of the deletions surrounding the *SSD1* locus in the top half of the plate (dashed lines); this plate and region are shown for a wild-type control screen (Y2806) and a *tao3*Δ*::URA3* (Y3714) screen. Each deletion strain is represented twice on the plate, located immediately diagonal to one another. In almost all cases, the size of the two colonies is identical, although there are exceptions (*e.g.*, only one of the *ssd1*Δ*::kanR* colonies grew up in the *tao3*Δ*::natR* screen, probably because of a pinning error). The *ssd1*Δ*::kanR* strain and deletions linked to the *SSD1* locus are outlined; red indicates *tao3*Δ*::URA3 xxx*Δ*::kanR* double deletions (top) that showed reduced colony size relative to the *xxx*Δ*::kanR* deletion alone (wild-type control screen, middle) while blue indicates roughly equal colony size in the two screens. The presence of blue circles, indicating equal growth in the presence and absence of *TAO3*, in the middle of the linked group of synthetic genetic interactions (red circles) can be explained (i) by growth defects, even in the presence of *TAO3* (*e.g.*, *gcn2*Δ, *atp5*Δ), which prevent the synthetic genetic interaction from being observable (see Figure 2 legend), and (ii) by weak or uncertain interactions (*e.g.*, *hnt2*Δ). The identities of each of the *xxx*Δ*::kanR* deletions is shown at the bottom. The deletion strains are arrayed on the plates in the same order as the corresponding genes on chromosome IV. Arrows indicate the topology of the genes with respect to the *SSD1* locus, with the arrowhead pointing away from the *SSD1* locus. Because Figure 2 presents the cumulative data from SGA analysis carried out against deletions in all five components of the Cbk1 signaling pathway, the synthetic interactions listed in Figure 2 differ somewhat from those observed in this single screen *vs. tao3*Δ*::URA3*.

When *cbk1*Δ, *mob2*Δ, *hym1*Δ, *kic1*Δ, and *toa3*Δ strains in the W303 background were crossed to *ssd1*Δ in the S288C background, tetrad analysis revealed that all of the spores containing a Cbk1 pathway gene deletion were viable (Figure 4B and data not shown). Therefore, not only does the deletion of *SSD1* suppress the loss of the Cbk1 pathway, but also the W303-derived suppressor must be either within or tightly linked to the *SSD1* locus. While this work was in progress, DU and NOVICK (2002) identified mutations within *SSD1* as suppressors of *cbk1*Δ and *tao3*Δ by a transposon insertion mutagenesis approach.

SSD1 encodes a putative RNA-binding protein implicated in the regulation of cell polarity and cell integrity that appears to physically interact with Cbk1 (RACKI *et al.* 2000; HO *et al.* 2002). It has been well established that *SSD1* is polymorphic in laboratory strains of *S. cerevisiae*: most S288C strains carry the dominant *SSD1-v* allele, while W303 strains usually harbor the recessive *ssd1-d* allele. The suffixes "v" in *SSD1-v* and "d" in *ssd1-d* are derived from SUTTON *et al.* (1991) and refer to the fact that deletion mutations in the *SIT4* gene are *v*iable in the presence of *SSD1-v* but *d*ead in the presence of *ssd1-d*. The opposite relationship appears to be true for deletion mutations in the Cbk1 signaling pathway, which appear to be viable in the presence of *ssd1-d* but dead in the presence of *SSD1-v*. We sequenced the *ssd1-d* allele in the W303 background and compared it to the *SSD1-v* allele sequenced as part of the yeast genome project (GOFFEAU *et al.* 1996). A point mutation at base-pair 2094 (C to G) of the *SSD1* open reading frame creates a premature stop codon in the W303 *ssd1-d* allele, resulting in a truncated protein with a predicted molecular weight of ~77 kD (data not shown). These findings are in general agreement with the observation that *ssd1-d* is expressed as a truncated ~83-kD protein (UESONO *et al.* 1997).

To confirm that the *ssd1-d* allele present in the W303 genome suppresses loss of the Cbk1 signaling pathway, we used a plasmid loss assay. *cbk1*Δ and congenic wild-type cells in the W303 background were transformed with a plasmid containing the *CBK1* gene and main-

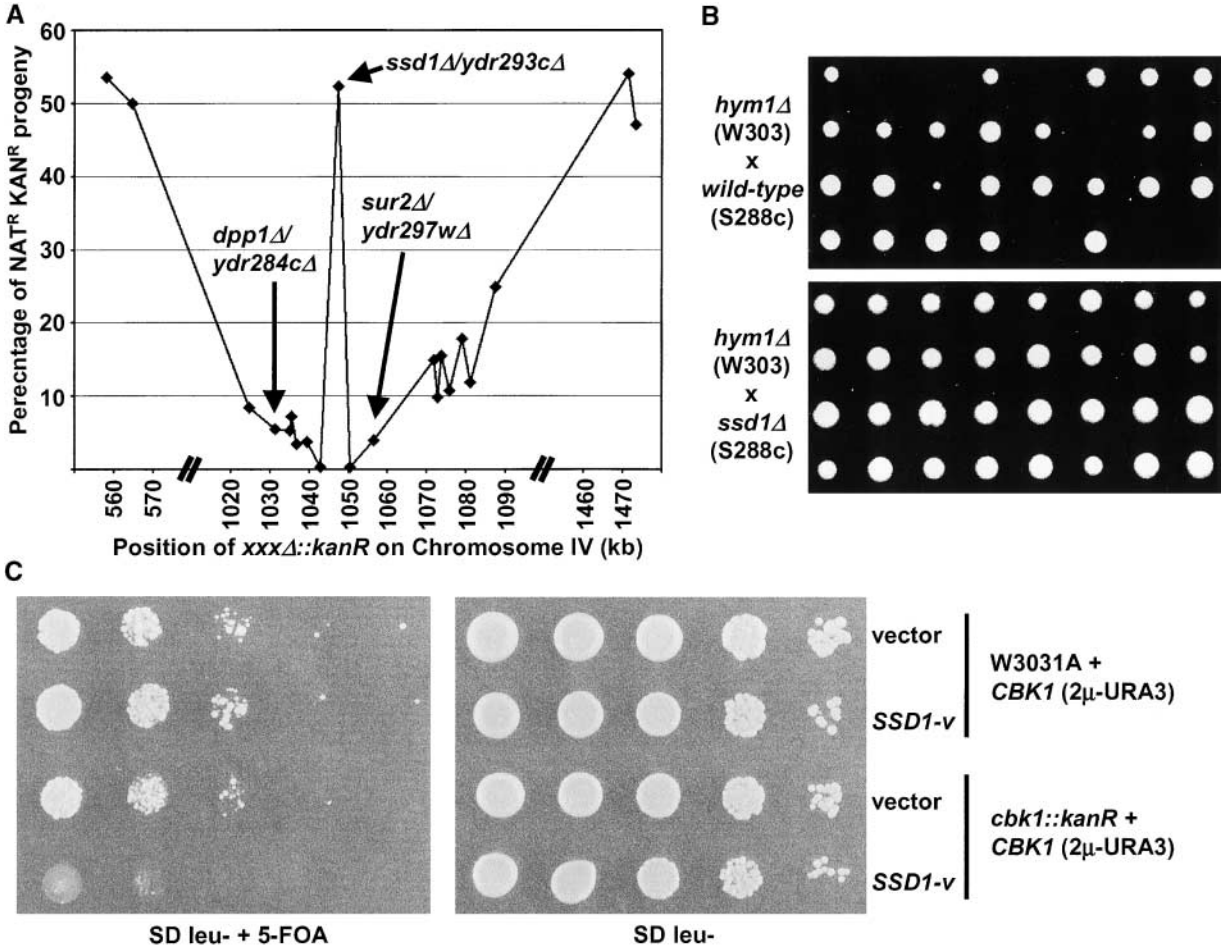

FIGURE 4.—Mutations in *SSD1* suppress the lethality associated with deletion of genes coding for Cbk1 pathway components. (A) Random spore analysis was carried out as described in MATERIALS AND METHODS to determine the frequency of recombination between the W303-specific suppressor of *mob2*Δ*::natR* and *kanR*-marked deletions on a section of chromosome IV in the vicinity of the *SSD1* locus. The percentage of recombinant progeny was plotted *vs.* position on chromosome IV, with the end of the ORF closest to the central *SSD1* locus determining the plotted base pair. As *kanR*-marked deletions approached *SSD1*, the percentage of recombinant progeny approached zero. At loci far removed from *SSD1*, the percentage of recombinant progeny returned to ~50%. The ~50% recombinant progeny seen for *ssd1*Δ*::kanR* is actually due to suppression of the lethality associated with *mob2*Δ*::natR* by *ssd1*Δ*::kanR*. (B) Tetrad analysis confirmed that *ssd1*Δ*::kanR* suppresses the lethality associated with deletion of *HYM1*. When a viable W303-derived *hym1*Δ*::URA3* is crossed to a wild-type S288C strain, a single suppressor segregates with the viability of the *hym1*Δ*::URA3*; *i.e.*, 50% of the *hym1*Δ*::URA3* spores germinate to form a colony (top). When the W303-derived *hym1*Δ*::URA3* was crossed to an S288C-derived *ssd1*Δ*::kanR* strain, all *hym1*Δ*::URA3* spores were viable (bottom). This result demonstrates that *ssd1*Δ*::kanR* suppresses the *hym1*Δ-associated lethality and that the W303-derived suppressor of the *hym1*Δ-associated lethality is tightly linked to the *SSD1* locus. Identical results were obtained for *cbk1*Δ, *mob2*Δ, *tao3*Δ, and *kic1*Δ (data not shown). (C) When expressed in the W303 background, *SSD1-v* restored the lethality associated with *CBK1* deletion. Congenic wild-type and *cbk1*Δ strains were sequentially transformed with a *URA3*-marked YEp24 plasmid containing the *CBK1* gene (P3765) and either a *LEU2*-marked YEp13 plasmid carrying *SSD1-v* (MTP251) or the YEp13 empty vector. These strains were grown to saturation in SC-Leu-Ura, serially diluted (four 10-fold dilutions), and the culture and each dilution were spotted on SC-Leu and SC-Leu containing 5-FOA, a drug that is toxic to yeast expressing the *URA3* gene. In the presence of *SSD1-v*, the *cbk1*Δ strain could not form colonies on SC-Leu containing 5-FOA.

tained with the *URA3* selectable marker. The plasmid rescued several *cbk1*Δ phenotypes (see MATERIALS AND METHODS). These strains were then transformed either with a *LEU2*-marked plasmid housing the *SSD1-v* allele or with the empty vector. The four Ura^+ Leu^+ genetic combinations were cultured and plated on SC-Leu and SC-Leu containing 5-FOA. On both types of plates, the absence of leucine maintained the *LEU2*-marked plasmids, but on the plates containing 5-FOA, cells possessing *URA3*-marked plasmids were selected against. In wild-type transformants, in either the presence or the absence of *SSD1-v*, some of the plated cells formed colonies on SC-Leu containing 5-FOA (Figure 4C). These cells were able to grow in the absence of the *URA3*-marked plasmid. Similarly, *cbk1*Δ cells transformed with the *LEU2*-marked empty vector formed colonies on SC-Leu

TABLE 2

Gene deletions that show synthetic growth defects with *cbk1*Δ *ssd1-d* and *hym1*Δ *ssd1-d*

Gene name	ORF	Functional information
BEM1	YBR200W	Bud site assembly and cell polarity, SH3-domain protein that binds Cdc24, Ste5, Ste20, Rsr1
BEM2	YER155C	Bud site assembly and cell polarity, member of the family of Rho-type GTPase-activating proteins
BEM4	YPL161C	Bud site assembly and cell polarity; interacts with several Rho-type GTPases
BST1	YFL025C	Vesicular transport; postulated to negatively regulate COPII vesicle formation
GAS1	YMR307W	Cell wall maintenance; regulates β-1,6-glucan crosslinking; null mutant has cell separation defects
GOS1	YHL031C	Vesicular transport, SNARE protein in endoplasmic reticulum to Golgi docking complex
KRE1	YNL322C	Cell wall maintenance; needed for β-1,6-glucan assembly
NBP2	YDR162C	Signal transduction, cell polarity
SAC7	YDR389W	Cell structure and actin assembly, GTPase-activating protein for Rho1

Tetrad analysis confirmed that these genes are synthetic lethal or sick with *cbk1*Δ *ssd1-d* and *hym1*Δ *ssd1-d*. Gene functions were obtained from the Saccharomyces genome database and the yeast proteome database.

containing 5-FOA. However, *cbk1*Δ cells transformed with the *LEU2*-marked vector housing *SSD1-v* did not form colonies on SC-Leu medium containing 5-FOA (Figure 4C), indicating that in the presence of *SSD1-v*, cells lacking *CBK1* (as carried by the *URA3* plasmid) were not viable. When introduced into the W303 background, the *SSD1-v* allele restored the lethality associated with deletion of *CBK1*.

Synthetic lethality with genes encoding Cbk1 pathway components: The SGA screens used for mapping the Cbk1 pathway suppressor also provided many candidate synthetic genetic interactions at loci unlinked to *SSD1* (Figure 2A). Here, we directly examined a subset of the strongest synthetic genetic interactions (see MATERIALS AND METHODS). Tetrad analysis confirmed that nine deletions unlinked to *SSD1* are important for growth in haploid *cbk1*Δ*::URA3 ssd1-d* cells and *hym1*Δ*::URA3 ssd1-d* cells (Table 2). Four of these genes are involved in reorganization of the cortical actin cytoskeleton (*BEM1*, *BEM2*, *BEM4*, and *SAC7*) and play key roles in bud emergence and development. Another gene, *NBP2*, has been generally implicated in cytoskeletal organization because *nbp2*Δ is synthetically lethal with several genes involved in spindle orientation and actin assembly (TONG *et al.* 2001). Moreover, the Nbp2 ortholog in the fission yeast *Schizosaccharomyces pombe*, Skb5, appears to bind and activate the PAK-like kinase Shk1(YANG *et al.* 1999) and *shk1*Δ shows synthetic lethality with a deletion of the gene encoding the *S. pombe* Cbk1 ortholog, Orb6 (VERDE *et al.* 1998). Two other genes (*GAS1* and *KRE1*) are directly involved in cell wall maintenance and polarized growth through β-1,6-glucan crosslinking and assembly. Finally, two of the genes (*BST1* and *GOS1*) are involved in vesicular transport. All of these genes function directly or indirectly in polarized cell growth, which could explain their essential role in *ssd1-d* cells defective for Cbk1 pathway signaling.

Ordered arrays as mapping tools: Ordered arrays of marked yeast deletion strains provide an inherently powerful tool for high-resolution genetic mapping. When combined with SGA methodology, this mapping method can be automated and carried out in high throughput. Although the test case shown here demonstrated the mapping of the suppressor of an essential gene deletion mutation, SGAM can be applied to any allele that leads to an epistatic phenotype that S288C strains do not normally display. These phenotypes include suppression of conditional alleles of essential genes, cytotoxic drug resistance, and filamentous growth. SGAM may be of particular use for rapid mapping of dominant mutations, which can be challenging to clone with standard techniques. Furthermore, we expect that the rapid exploration of all null phenotypes, as allowed by the yeast deletion sets, will eventually lead researchers to a fuller exploration of genetic space, as provided by screens for dominant alleles. In theory, several alleles responsible for complex, multigenic quantitative traits, such as the high-temperature growth phenotype of pathogenic *S. cerevisiae*, could be mapped in a single round of SGA analysis (MCCUSKER *et al.* 1994; STEINMETZ *et al.* 2002). To further the mapping process, the unique oligonucleotide bar-code tags built into each yeast deletion strain may be exploited for quantitative analysis of growth phenotypes of meiotic progeny by hybridization to DNA microarrays (SHOEMAKER *et al.* 1996).

Other methods for rapid mapping of mutations have been devised. For instance, thousands of markers provided by the allelic variation between different strains of *S. cerevisiae* have been detected with high-density oligonucleotide arrays and exploited for mapping novel mutations (WINZELER *et al.* 1998; STEINMETZ *et al.* 2002). When the mutant phenotype is amenable (see above), mapping with SGA analysis may be preferable to mapping with oligonucleotide arrays because of the poten-

tial for higher resolution. In the first stage of SGA mapping, a starting strain is crossed to ~4700 deletion stains to identify a chromosomal region(s) centered around the mutation. In the example presented here, a 89.6-kb (~28-cM) region was detected (Figure 2). This mapping resolution is similar to that obtained in test applications of high-density oligonucleotide arrays (Winzeler *et al.* 1998), which detected 11- to 64-kb regions surrounding four known loci. However, in the second stage of SGA mapping, random spore analysis of the meiotic progeny derived from deletion strains in the identified chromosomal region pinpoints the gene(s) tightly linked to the mutation. While *S. cerevisiae* genetics is particularly suited for automation and large-scale genetic analysis, the construction of ordered arrays of gene deletion mutants would enable this type of approach to be applied to other model organisms (Coelho *et al.* 2000), especially those with high rates of meiotic recombination.

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