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Global Analysis of the Relationship between the Binding of the Bas1p Transcription Factor and Meiosis-Specific Double-Strand DNA Breaks in *Saccharomyces cerevisiae*†

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In the yeast *Saccharomyces cerevisiae*, certain genomic regions have very high levels of meiotic recombination (hot spots). The hot spot activity associated with the *HIS4* gene requires the Bas1p transcription factor. To determine whether this relationship between transcription factor binding and hot spot activity is general, we used DNA microarrays to map all genomic Bas1p binding sites and to map the frequency of meiosis-specific double-strand DNA breaks (as an estimate of the recombination activity) of all genes in both wild-type and *bas1* strains. We identified sites of Bas1p-DNA interactions upstream of 71 genes, many of which are involved in histidine and purine biosynthesis. Our analysis of recombination activity in wild-type and *bas1* strains showed that the recombination activities of some genes with Bas1p binding sites were dependent on Bas1p (as observed for *HIS4*), whereas the activities of other genes with Bas1p binding sites were unaffected or were repressed by Bas1p. These data demonstrate that the effect of transcription factors on meiotic recombination activity is strongly context dependent. In wild-type and *bas1* strains, meiotic recombination was strongly suppressed in large (25- to 150-kb) chromosomal regions near the telomeres and centromeres and in the region flanking the rRNA genes. These results argue that both local and regional factors affect the level of meiotic recombination.

From comparisons of genetic and physical maps, it is clear that recombination events are unevenly distributed. Regions with relatively high and low levels of exchange are termed "hot spots" and "cold spots," respectively. As first shown for *Saccharomyces cerevisiae* (28), meiotic recombination events in many eukaryotes (including humans) are initiated by double-strand DNA breaks (DSBs) catalyzed by Spo11p, a topoisomerase II-related protein. In general, there is a good correlation between the frequency of DSBs and the rate of local meiotic recombination (36, 43). In the study described here, we use DNA microarrays to measure the rate of DSBs for all open reading frames (ORFs) and intergenic regions. We assume that these measurements will reflect the meiotic recombination activities near the DSB sites, although the nature of the later steps of recombination (strand invasion, extent of heteroduplex formation, etc.) could influence the recombination frequency.

From studies of individual hot spots in *Saccharomyces cerevisiae*, as well as from more global studies, several generalizations concerning hot spots and cold spots can be made. First, DSBs usually occur in intergenic regions rather than within genes (4, 22, 58), suggesting a connection between "open" chromatin and preferred sites for Spo11p-induced cleavage. Second, for some hot spots (α hot spots), binding of transcription factors is required for hot spot activity (56) and DSB formation (19). This requirement for transcription factor binding does not indicate a direct connection between transcription and hot spot activity, since deletion of a TATAA sequence, which substantially reduces transcription of the *HIS4* gene, has no effect on hot spot activity (55). Third, in addition to α hot spots, there are hot spots associated with nucleosome-excluding sequences (β hot spots) (29) and local high G+C base composition (γ hot spots) (22).

Hot spots share no obvious common sequence motif, and the mechanistic explanations of the associations described above are not clear. The simplest explanation of the observations is that hot spot activity is a function of a particular chromatin structure (43). In support of this explanation, mutations that affect chromatin influence hot spot activity (60, 61), although it has not been demonstrated whether these effects are direct or indirect.

The mechanism responsible for meiotic recombination cold spots in *Saccharomyces cerevisiae* is also not understood. Lambie and Roeder (33) showed that the centromere of chromosome III repressed meiotic crossing over and gene conversion. A reduction in the rate of DSB formation near the centromeres and telomeres of yeast chromosomes has been shown by Southern analysis of yeast chromosome III (4); by pulse-field gel studies of chromosomes I, III, and VI (30); and by a global analysis of DSB formation throughout the genome by using DNA microarrays (10, 22). In addition, the Ty retrotransposons have low levels of meiotic recombination (32), and

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TABLE 1. Genotypes of haploid and diploid strains

Strain	Parental strain(s)	Relevant genotype[a]	Reference[b]
DNY25	AS13	**a** *his4-lopc*	42
HF4	DNY25	**a** *his4-lopc rad50S::URA3*	19
PM227	HF4	**a** *his4-lopc rad50S::URA3 BAS1-ZZ::kanMX*	
JG105	DNY25	**a** *his4-lopc SPO11-ZZ::K.1.URA3*	
JG149	JG105	**a** *his4-lopc SPO11-ZZ::K.1.ura3*	
JG168	JG149	**a** *his4-lopc SPO11-ZZ::K.1.ura3 rad50S::URA3*	
PM214	JG168	**a** *his4-lopc SPO11-ZZ::K.1.ura3 rad50S::URA3 bas1::kanMX*	
MW79	AS13	**a** *arg4-tel*	56
HF7	MW79	**a** *arg4-tel rad50S::URA3*	19
HF7U	HF7	**a** *arg4-tel rad50S::ura3*	
MD332	HF7U	**a** *arg4-tel rad50S::ura3 SHM2-1*	
DNY107	AS4	α *rad50S::URA3*	42
PM228	DNY107	α *rad50S::URA3 BAS1-ZZ::kanMX*	
JG106	AS4	α *SPO11-ZZ::K.1.URA3*	
JG148	JG106	α *SPO11-ZZ::K.1.ura3*	
JG165	JG148	α *SPO11-ZZ::K.1.ura3 rad50S::URA3*	
PM213	JG165	α *SPO11-ZZ::K.1.ura3 rad50S::URA3 bas1::kanMX*	
MS81	AS4	α *arg4-tel*	56
HF8	MS81	α *arg4-tel rad50S::URA3*	19
HF8U	HF8	α *arg4-tel rad50S::ura3*	
MD337	HF8U	α *arg4-tel rad50S::ura3 SHM2-1*	
DNY26	DNY25 × AS4	**a**/α *his4-lopc/HIS4*	19
JG110	JG105 × JG106	**a**/α *his4-lopc/HIS4 SPO11-ZZ::K.1.URA3/SPO11-ZZ::K.1.URA3*	
JG169	JG168 × JG165	**a**/α *his4-lopc/HIS4 SPO11-ZZ::K.1.ura3/ SPO11-ZZ::K.1.ura3 rad50S::URA3/rad50S::URA3*	
PM216	PM214 × PM213	**a**/α *his4-lopc/HIS4 SPO11-ZZ::K.1.ura3/SPO11-ZZ::K.1.ura3 rad50S::URA3/rad50S::URA3 bas1::kanMX/bas1::kanMX*	
PM238	PM227 × PM228	**a**/α *his4-lopc/HIS4 rad50S::URA3/ rad50S::URA3 BAS1-ZZ::kanMX/BAS1-ZZ::kanMX*	
MD341	MD332 × MD337	**a**/α *arg4-tel/arg4-tel rad50S::ura3/rad50S::ura3 SHM2-1/SHM2-1*	

[a] All strains were derived by transformation from either AS4- or AS13-derived haploid strains (51). The genotype of AS13 is **a** *leu2 ade6 ura3 rme1*. The genotype of AS4 is α *trp1 arg4 tyr7 ade6 ura3 spt22*. Only mating type and markers that differ from those of the progenitor AS13 and AS4 strains are indicated.
[b] If no reference is given, the strain was constructed for this paper.

insertion of a Ty element near a hot spot results in a substantial reduction in the activity of the hot spot (5).

In previous studies, we have examined factors required for the hot spot activity associated with the *HIS4* gene (43). Four transcription factors bind upstream of *HIS4*: Bas1p, Bas2p, Gcn4p, and Rap1p (2, 3, 18, 52). Bas1p, Bas2p, and Rap1p are essential for hot spot activity (56, 57), and binding of Gcn4p has a stimulatory effect on recombination (1), although it is not absolutely required. Bas1p is a Myb-related transcription factor that is required for optimal levels of *HIS4* expression (3, 52). In conjunction with Bas2p, Bas1p is involved in transcriptional activation of a number of genes involved in regulation of AMP and histidine biosynthesis. The activating effects of Bas1p and Bas2p on their target genes are strongest when cells are starved for adenine, but Bas1p and Bas2p are also required for optimal basal levels of expression for many of these genes (14). In addition to genes involved in adenine and histidine biosynthesis, several genes involved in one-carbon metabolism (for example, *GLN1*, *SHM2*, and *MTD1*) are regulated by Bas1p and Bas2p (15, 21).

About 20 genes have been shown to be regulated by Bas1p (16). Thus far, *HIS4* is the only Bas1p-regulated gene for which the effect of Bas1p on meiotic recombination activity has been examined. To determine whether Bas1p stimulates meiotic recombination at all of its genomic binding sites, we have mapped all of the Bas1p binding sites in the genome and monitored the frequency of meiotic DSB formation for all yeast genes in both wild-type and *bas1* mutant strains. As described below, we found that the effects of Bas1p on meiotic recombination activity are context dependent.

MATERIALS AND METHODS

Strain construction. All strains used in this study are isogenic (except for changes introduced by transformation) with the previously described strains AS4 (α *trp1 arg4 tyr7 ade6 ura3 spt22*) and AS13 (**a** *leu2 ura3 ade6 rme1*) (51). A summary of the genotypes of haploid and diploid strains used in this study is in Table 1. Details of the constructions are in the supplemental material.

Identification of Bas1p binding sites in PM238. We monitored Bas1p binding by using PM238, a strain homozygous for *BAS1-ZZ::kanMX* and *rad50S*. Using the chromatin immunoprecipitation protocol of Lieb et al. (37) with minor modifications (described in the supplemental material), we isolated DNA associated with Bas1p from cells incubated in sporulation medium. The resulting DNA sample was PCR amplified, labeled with a fluorescent nucleotide, and mixed with a sample of genomic DNA that had been amplified and labeled with a different fluorescent nucleotide. This mixture was hybridized to a microarray containing double-stranded PCR products representing both ORFs and intergenic regions (22). The ratio of normalized signal intensity ($\log_2$ of P2/P1, with P2 representing the experimental sample and P1 representing the genomic control) reflects the relative Bas1p binding occupancy of each genomic interval. We acquired the microarray images with a GenePix 4000A scanner and analyzed the images with the GenePix Pro 5.0 software. The details of these procedures are given in the supplemental material.

Purification of hot spot-associated DNA from JG169 (wild-type) and PM216 (*bas1*) strains. The diploid JG169 and PM216 strains are homozygous for a gene encoding an epitope-tagged version of Spo11p (*SPO11-ZZ::K.1.URA3*) and *rad50S*. In *rad50S* strains, Spo11p is covalently attached to the DNA ends pro-

duced by the DSBs that initiate recombination (28). For preparation of the samples, strains were sporulated for 24 h. The Spo11p-associated DNA was prepared by immunoprecipitation using methods similar to those described by the Koshland lab (http://www.ciwemb.edu/labs/koshland/Protocols/Yeast/chipmod.html) with modifications described in the supplemental material. The Spo11p-enriched DNA was then used as a hybridization probe for the microarrays as described above, with ratios reflecting the relative recombination activity of each genomic interval.

Data analysis and data availability. The data from both the Bas1p binding studies and recombination activities were analyzed using the ChIPOTle version 1.0 software (13), which uses a sliding-window approach to identify and measure peaks of DNA binding activity. For each type of experiment, the input data for the ChIPOTle program were the median values of the $\log_2$ red/green (62) normalized ratio for each ORF or intergenic region. The motif search program MDscan (38) was used to identify the motif associated with Bas1p binding. Other details of the data analysis are described further in the supplemental material. Raw data are available from the University of North Carolina Microarray Database (https://genome.unc.edu/), and Excel spreadsheets for each microarray are in the supplemental material.

Southern analysis of DSB formation. DNA was isolated from meiotic and premeiotic cells by using a method described previously (42). Hybridization probes were prepared by PCR amplification of genomic DNA. The sequences of the primers used to prepare the probes are given in the supplemental material. As a control, all DNA samples were hybridized to the *YGR177C* probe, since this gene is a very strong recombination hot spot even in the *bas1* strain.

RESULTS

Comprehensive identification of Bas1p binding sites in meiotic cells. As described in the introduction, the activity of the *HIS4* recombination hot spot is dependent on the binding of the Bas1p transcription factor. In order to determine whether this relationship was conserved for other regions in the genome, we mapped all genomic Bas1p binding sites and then examined the effects of the *bas1* deletion on the meiotic recombination activities of genes adjacent to the Bas1p binding sites. The binding sites were mapped by chromatin immunoprecipitation (using an epitope-tagged version of Bas1p), followed by DNA microarray analysis (ChIP-chip) (12).

Our previous studies of meiotic recombination were done with diploids generated by mating two related haploids, AS4 and AS13 (51). All strains in the current study are isogenic to these strains, except for changes introduced by transformation (genotypes are given in Table 1). For the Bas1p binding studies, we used the diploid PM238, which is homozygous for the *BAS1-ZZ* allele, which encodes a Bas1p that is tagged at the C terminus with two immunoglobulin G binding domains (ZZ) from protein A of *Staphylococcus aureus*; proteins with the ZZ tag can be precipitated using beads with bound IgG. PM238 cells, incubated for 3 h in sporulation medium, were treated with formaldehyde to cross-link DNA to bound proteins, and the resulting Bas1p-DNA complexes were purified. Following reversal of the cross-links, the precipitated DNA was labeled and used as a hybridization probe for DNA microarrays that represented all of the yeast ORFs and intergenic regions (generally one spot per ORF or intergenic region).

The experimental samples were labeled with Cy3 or Cy5 fluorescent nucleotides, and a control sample of total genomic DNA was labeled with the opposite fluorescent nucleotide. The ratio of hybridization between the two probes was determined for each spot. Seven microarrays were analyzed using immunoprecipitates derived from independent meiotic cultures. This analysis was done with the ChIPOTle software (13). In brief, ratios derived from the microarray hybridizations

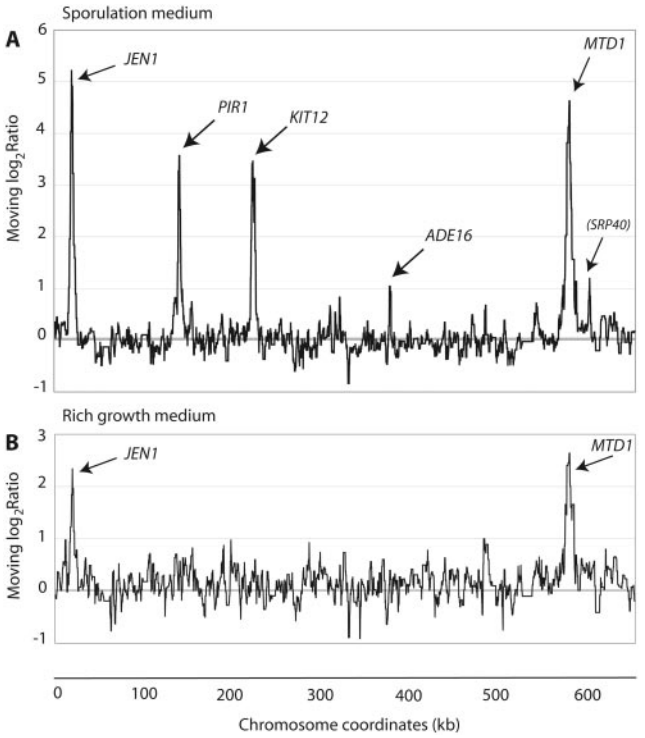

FIG. 1. Bas1p binding on yeast chromosome XI in cells incubated for 3 hours in sporulation medium (A) or grown in rich medium (YPD) (B). We performed chromatin immunoprecipitation using an epitope-tagged Bas1p and used Bas1p-associated DNA as a hybridization probe on a microarray. The resulting data were analyzed using a 1,000-bp window that was moved at intervals of 250 bp (ChIPOTle version 1.0). Bas1p binding is considerably stronger in cells incubated in sporulation medium (A) than in cells grown in rich medium (B). Neither *KIT12* nor *SRP40* has a consensus Bas1p binding site in the promoter region, although *KIT12* has a perfect match to the consensus within its coding sequence.

were used to calculate a moving average along each chromosome, using a 1-kb window that was moved 250 bp at a time. The statistical significance of the binding peaks produced by this analysis was determined as described in the supplemental material. We considered peak values associated with a P value of ≤ 0.0005 to be significant. An example of this analysis for chromosome XI is shown in Fig. 1A. In the samples derived from sporulating cells, 107 significant binding peaks (hereafter referred to as "sites") were detected (see Table S1 in the supplemental material); the complete data set for these experiments, including P values, is at https://genome.unc.edu/.

Identities and positions of DNA sequence motifs associated with meiotic Bas1p binding. For each of these binding sites, an ORF or intergenic region was assigned according to the location of the peak of the binding signal. We then used the MDscan motif search program (38) to look for conserved sequences associated with Bas1p binding. For this analysis, we examined the ORFs or intergenic regions assigned to each binding site, along with 500 bp of sequence to each side of the assigned region. This analysis identified the motif TGACTC T>C G>T (Fig. 2A). The core motif TGACTC is identical to that identified by Daignan-Fornier and Fink (14) as a Bas1p binding site. We examined the location of this motif in

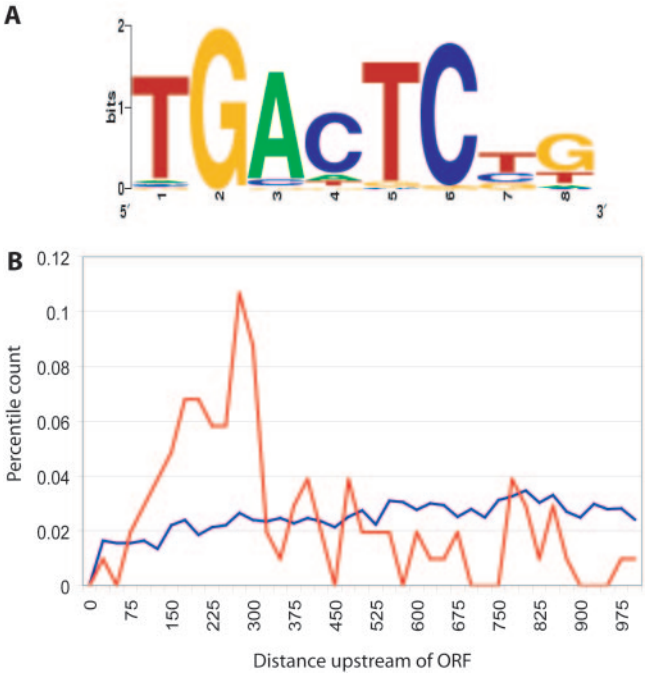

FIG. 2. Consensus Bas1p binding site and location of the binding site relative to the initiation codon of the target gene. (A) We used the MDscan program (38) and identified a sequence associated with Bas1p binding; the core motif is the same as that identified by Daignan-Fornier and Fink (14) as a Bas1p binding site. (B) We determined the location of the Bas1p binding motif in upstream regions identified as bound (red) and unbound (blue) by the microarray analysis. The distances shown on the *x* axis are given relative to the initiating codon of the target ORF.

a 1-kb window located upstream of putative target genes. There was a pronounced peak in the frequency of this motif 200 to 350 bp upstream of the initiating codon (Fig. 2B). When we examined regions upstream of genes that were not Bas1p targets, we found no preferred position (Fig. 2B).

Genes targeted by Bas1p in meiotic cells. We applied two additional criteria to reduce the probability of false positives among the Bas1p binding sites we identified (details are in the supplemental material). We disqualified from further analysis those sites in which the peak of binding was based upon only a single underlying high value and those with a relatively low value that upon manual inspection were revealed to be "shoulders" on peaks with high values. To identify genes whose expression was likely to be regulated by Bas1p binding, we also excluded sites that were not in intergenic regions located within 600 bp upstream of a potential target gene. After applying these criteria, then number of sites was reduced from 107 to 56 (Table 2). It is possible, of course, that some of the Bas1p binding sites excluded by various criteria (for example, the requirement that the Bas1p binding site be in an intergenic region) are nonetheless involved in gene regulation.

Table 2 shows the identified binding sites and the target genes potentially regulated by Bas1p binding. Since the binding motif is sometimes between two divergently transcribed genes, there are more potential target genes (71) than binding sites (56). In previous studies, 16 genes were shown to be regulated by Bas1p, including *ADE1*, *ADE3*, *ADE4*, *ADE5*,*7*, *ADE6*, *ADE12*, *ADE13*, *ADE17*, *HIS1*, *HIS4*, *HIS7*, *URA1*, *URA3*, *GLN1*, *MDT1*, and *SHM2* (15, 16, 50). All but three (*URA1*, *URA3*, and *GLN1*) of these genes are represented in our list. In addition to the previously identified genes, we found a number of other genes in the adenine and histidine pathways that are likely to be regulated by Bas1p, including *ADE2*, *ADE8*, and *HIS3*. When we analyzed the 71 putative target genes by using the SGD Gene Ontology Term Finder, we found very significant ($P < 0.00001$) overrepresentation of genes in a number of categories, including nucleotide biosynthesis, purine nucleotide metabolism, one-carbon compound metabolism, and amino acid biosynthesis.

Gelling et al. (21) found that glycine induces many yeast genes, some involved in one-carbon metabolism (such as *GCV1*, *GCV2*, and *GCV3*) and some involved in purine biosynthesis. The latter class included *ADE1*, *ADE2*, *ADE5*,*7*, *ADE13*, and *ADE17*, genes also regulated by Bas1p. Gelling et al. found that the Bas1p binding motif was overrepresented in the genes of the one-carbon regulon and that *GCV1* and *GCV2* have decreased transcription in *bas1* strains. In Fig. 3, we show the pathways connecting purine metabolism, histidine metabolism, and one-carbon metabolism. In sporulating cells, Bas1p binds to the upstream regions of a high fraction of these genes.

Genes targeted by Bas1p in vegetative diploid cells grown in rich medium. Previously, as part of a study involving 106 yeast transcription factors, Lee et al. (34) analyzed Bas1p binding sites by a ChIP-chip approach. We repeated this analysis for several reasons. First, the experiments of Lee et al. were done using haploid strains grown in rich growth medium. We were primarily interested in examining Bas1p binding in a diploid strain incubated in sporulation medium, although we also analyzed the diploid strain grown in rich medium. Second, the genetic background of the strain used by Lee et al. was different from that used in our meiotic recombination studies. Third, the Bas1p binding motifs identified in the study by Lee et al. (TTTTYYTTYTTKY-TY-T [scored by E value] and CS-CCAATGK—CS]) had no obvious relationship to the previously identified binding motifs of TGACTC (14), although a subsequent analysis by the same group reported TGACTC as the Bas1p binding motif (24).

Seven microarrays were analyzed using immunoprecipitates derived from independent vegetative cultures of PM238, grown in rich medium. We identified only 15 significant binding sites (*P* value of ≤ 0.0005). Using the same criteria described above, we determined that there were nine Bas1p binding sites likely to be involved in gene regulation, located upstream of 11 potential targets. In general, the hybridization signals observed for Bas1p binding sites in cells grown in rich medium were considerably weaker than those observed in sporulating cells (compare of Fig. 1A and B). Seven sites were upstream of single target genes, including *GCV1*, *GCV2*, *GCV3*, *ADE2*, *SHM2*, *MTD1*, and *HXT1*. Two sites were between the divergently transcribed genes *IES5* and *MET6* and the divergently transcribed genes *JEN1* and *SRY2*. Interestingly, eight of these nine Bas1p binding sites were identified as among the top binding sites (highest degree of enrichment) in the cells incubated in sporulation medium (Table 2). The simplest interpretation of this result is that the adenine starvation that occurs in sporulation medium results in quantitatively more Bas1p binding, although the most highly occupied binding sites in each

TABLE 2. Bas1 binding sites in sporulating yeast cells and associated meiotic recombination activities[a]

Gene	ORF	Bas1 binding[b]	Recombination activity[c]			Up/down[e]
			Wild type	*bas1*	*P*[d]	
GCV2	YMR189W	5.83	0.3423	0.3356	6.29E−01	NS
MRPS17	YMR188C		0.2934	0.9086	1.51E−06	Up
SHM2	YLR058C	5.47	0.8928	0.5832	7.98E−04	Down
JEN1	YKL217W	5.29	0.6104	0.5626	5.82E−01	NS
SRY1	YKL218C		0.2805	0.1789	1.29E−01	NS
GCV3	YAL044C	5.15	0.9804	0.9593	2.32E−01	NS
ADE2	YOR128C	5.14	0.3932	0.5558	4.50E−01	NS
GCV1	YDR019C	4.97	0.3302	0.3127	7.51E−01	NS
MTD1	YKR080W	4.79	0.7124	0.9018	1.52E−02	Up
ADE17	YMR120C	4.71	0.8001	0.728	5.12E−02	Down
ADE4	YMR300C	4.61	0.2683	0.1591	1.30E−01	NS
ADE12	YNL220W	4.54	0.8016	0.6784	5.18E−02	Down
POP4	YNL221C		0.387	0.3741	7.07E−01	NS
ADE13	YLR359W	4.43	0.3188	0.1615	3.23E−03	Down
HIS4	YCL030C	4.17	0.9883	0.7048	3.34E−04	Down
IES5	YER092W	4.16	0.922	0.9725	4.10E−03	Up
MET6	YER091C		0.6364	0.7354	5.28E−01	NS
ADE6	YGR061C	3.84	0.6342	0.6538	2.59E−01	NS
ADE1	YAR015W	3.77	0.8439	0.5801	4.57E−03	Down
ADE8	YDR408C	3.70	0.9199	0.8567	7.93E−01	NS
SIZ1	YDR409W		0.5909	0.5227	4.37E−01	NS
KTI12	YKL110C	3.67	0.5981	0.7345	6.13E−01	NS
HIS1	YER055C	3.61	0.2608	0.4573	5.66E−01	NS
PIR1	YKL164C	3.45	0.2007	0.1917	5.25E−01	NS
PIR3	YKL163W		0.2342	0.1932	5.17E−01	NS
CEM1	YER061C	3.29	0.0492	0.0743	8.56E−01	NS
STR3	YGL184C	3.25	0.4664	0.2535	2.58E−01	NS
MTO1	YGL236C	3.13	0.5968	0.4963	5.76E−01	NS
ADE5,7	YGL234W		0.3243	0.3601	9.81E−01	NS
SNO1	YMR096W	3.10	0.4866	0.6396	5.64E−01	NS
SNZ1	YMR095C		0.4541	0.4917	5.16E−01	NS
YPR004C	YPR004C	2.65	0.3017	0.1206	3.04E−01	NS
MNT4	YNR059W	2.37	0.3273	0.2185	4.50E−01	NS
GID8	YMR135C	2.23	0.8022	0.9216	7.44E−02	NS
GAT2	YMR136W		0.7893	0.8796	4.85E−01	NS
URA2	YJL130C	2.22	0.4102	0.343	4.78E−01	NS
SUT2	YPR009W	2.21	0.4404	0.0838	3.42E−03	Down
YDL025C	YDL025C	2.18	0.0923	0.052	1.44E−01	NS

Continued on following page

TABLE 2—*Continued*

Gene	ORF	Bas1 binding[b]	Recombination activity[c]			Up/down[e]
			Wild type	*bas1*	*P*[d]	
ICL1	YER065C	2.17	0.5214	0.5582	6.89E−01	NS
ADE3	YGR204W	2.14	0.9119	0.9321	3.81E−01	NS
ECM21	YBL101C	2.10	0.4269	0.3538	7.62E−01	NS
TRP2	YER090W	2.09	0.3769	0.3139	5.13E−01	NS
PTC2	YER089C		0.5264	0.4769	5.39E−01	NS
HPT1	YDR399W	1.88	0.8801	0.9009	4.15E−01	NS
ZRC1	YMR243C	1.82	0.5022	0.4775	2.09E−01	NS
MCH1	YDL054C	1.76	0.2461	0.1223	2.95E−02	Down
STF1	YDL130W-A	1.75	0.6078	0.7091	9.06E−02	NS
RSM10	YDR041W	1.71	0.8725	0.9192	4.94E−01	NS
YDR089W	YDR089W	1.68	0.918	0.8492	3.08E−02	Down
BNA6	YFR047C	1.59	0.9614	0.9271	3.50E−01	NS
RBA50	YDR527W	1.58	0.3439	0.3975	5.81E−01	NS
SER2	YGR208W	1.57	0.7779	0.8496	6.42E−01	NS
SEC20	YDR498C	1.45	0.3198	0.6891	9.95E−04	Up
LCD1	YDR499W		0.4536	0.5112	1.66E−01	NS
MIR1	YJR077C	1.42	0.9991	0.9969	2.66E−01	NS
BNA2	YJR078W		0.9935	0.9873	3.70E−01	NS
EPL1	YFL024C	1.41	0.5509	0.4508	3.02E−01	NS
BUD27	YFL023W		0.498	0.4501	7.99E−01	NS
FMP45	YDL222C	1.38	0.5218	0.4684	5.82E−01	NS
PET1	YOR201C	1.34	0.7455	0.7829	8.89E−01	NS
HIS3	YOR202W		0.8294	0.7422	2.92E−01	NS
ARG5,6	YER069W	1.29	0.2476	0.270	8.14E−01	NS
MOT3	YMR070W	1.26	0.9518	0.9575	6.64E−01	NS
VHT1	YGR065C	1.19	0.607	0.5704	3.81E−01	NS
PHO89	YBR296C	1.12	0.192	0.1499	2.40E−01	NS
MGA1	YGR249W	1.11	0.3056	0.2472	2.93E−01	NS
ECM22	YLR228C	1.08	0.9183	0.8374	1.90E−01	NS
ARO1	YDR127W	1.05	0.5358	0.6015	5.77E−01	NS
HIS7	YBR248C	0.98	0.4199	0.6099	4.36E−01	NS
GUA1	YMR217W	0.96	0.808	0.8398	3.36E−01	NS
SKY1	YMR216C		0.6778	0.8501	1.62E−01	NS

[a] As discussed in the text, we identified 56 Bas1p binding sites in sporulating cells. Fifteen of these sites were located between divergentally transcribed genes, resulting in 71 potential targets. We also measured the recombination activity of each ORF in the genome in both wild-type and *bas1* mutant strains.

[b] Highest value ($\log_2$ ratio of hybridization to Bas1p-enriched DNA/total genomic DNA) for a 1-kb "window" within the binding site peak.

[c] Recombination activities were calculated by determining the ratio of hybridization to two hybridization probes (Spo11p-enriched DNA sample/total genomic DNA) and ranking these ratios for all ORFs and intergenic regions; the values shown are the median ranking, with 1 representing the "hottest" and 0 representing the "coldest."

[d] Comparison of the rankings of samples in the wild-type (11 microarrays) and *bas1* (10 samples) strains by a two-tailed *t* test, assigning a probability to the difference between the samples.

[e] Based on the *p* value, we determined whether the recombination activity of the ORF was significantly reduced (down), significantly elevated (up), or not significantly affected (NS).

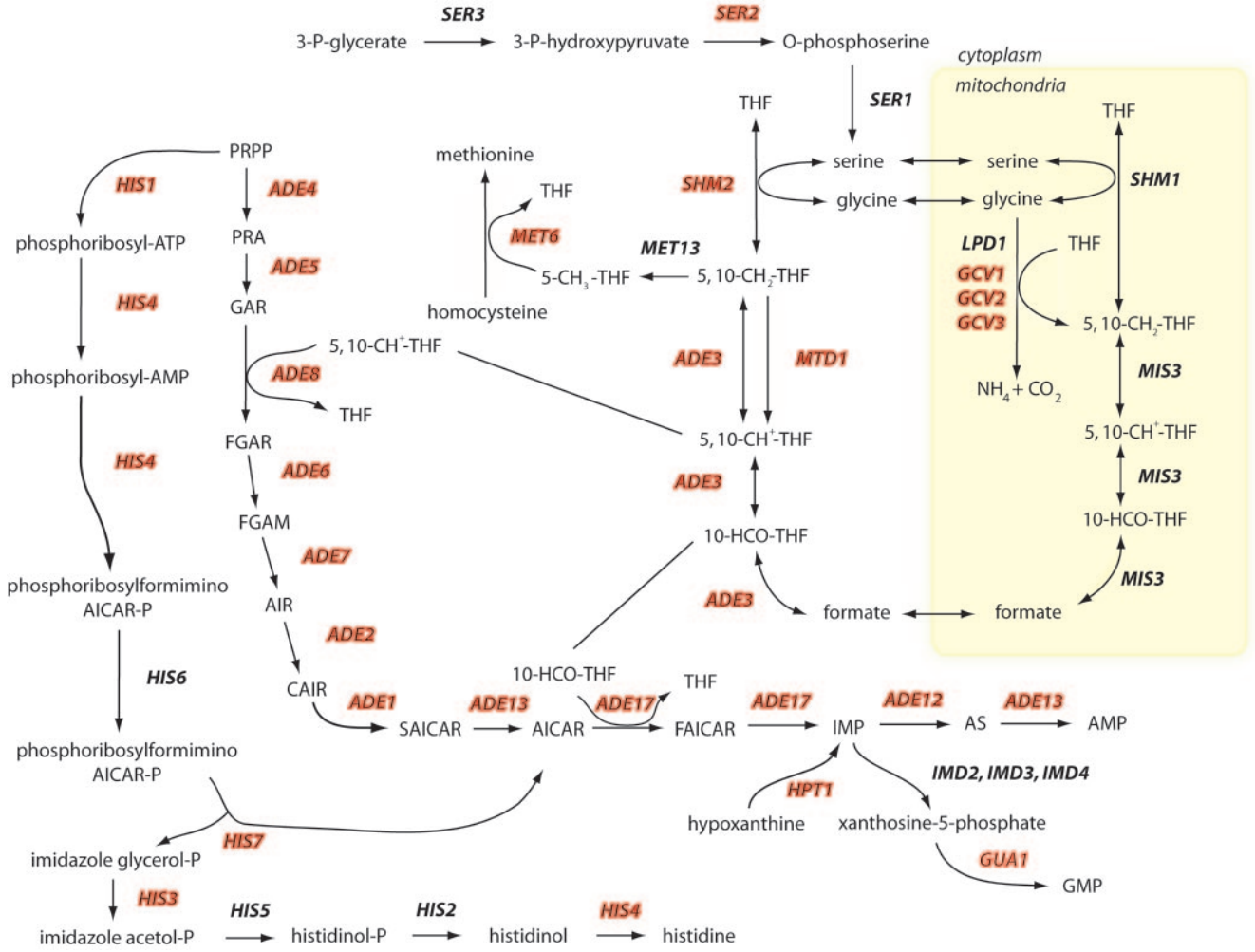

FIG. 3. Relationship between Bas1p binding and metabolic pathways concerned with histidine and adenine biosynthesis and one-carbon metabolism. This diagram is an extension of Fig.7 of reference 21. Genes identified in our study as binding Bas1p in the promoter regions are shown in red. The abbreviations in the adenine biosynthetic pathway are as follows: PRPP, 5-phosphoribosyl diphosphate; PRA, 5-phosphorybosylamine; GAR, 5′-phosphoribosylglycinamide; FGAR, 5′-phosphoribosyl-*N*′-formylglycinamide; FGAM, 5′-phosphoribosyl-*N*′-formylglycinamide; AIR, 1-(5′-phosphoribosyl)-5-aminoimidazole; CAIR, 1-(5′-phosphoribosyl)-5-aminoimidazole-4-carboxylate; SAICAR, 1-(5′-phosphoribosyl)-4-(*N*-succinocarboxyamido)-5-aminoimidasole; AICAR, 5-amino-1-(5′-phosphoribosyl)-imidazole-4-carboxyamide; FAICAR, 5-formamido-1-(5′-phosphoribosyl)-imidazole 4-carboxyamide; THF, tetrahydrofolate.

condition appear to be similar. Only one gene, *HXT1*, was identified in our mapping of the Bas1p binding sites in mitotic but not sporulating cells. Since *HXT1* is more than 75% identical to five other genes, the interpretation of this binding is unclear.

Global analysis of recombination hot spots in a wild-type and *bas1* yeast strains. Our study was motivated by the observation that the meiosis-specific DSB associated with the *HIS4* recombination hot spot is dependent on Bas1p. To determine whether this relationship was general, we examined the relationship of Bas1p binding with meiotic recombination activity (level of DSBs) throughout the genome in both wild-type (JG169) and *bas1* mutant (PM216) diploid strains. Both strains were homozygous for the *rad50S* mutation (allowing accumulation of Spo11p-associated broken DNA molecules [28]) and *SPO11-ZZ* (encoding a fully functional epitope-tagged Spo11p). For each strain, Spo11-ZZp-DNA complexes were isolated from cells sporulated for 24 h (details are in the supplemental material). The Spo11p-associated DNA was labeled with Cy3 or Cy5 fluorescently tagged nucleotides, and total genomic DNA from the same strain was labeled with the opposite nucleotide. Samples were then mixed and hybridized to microarrays containing ORFs and intergenic regions prepared as described by DeRisi et al. (17). Eleven microarrays were used for analysis of JG169 (each representing an independent sporulated culture), and 10 microarrays were used for PM216.

The data were analyzed by methods similar to those that we (22) and others (10) used previously (described in detail in the supplemental material). By comparing the ratio of hybridization of the DSB-enriched DNA (Spo11p-associated DNA) with the hybridization of the unenriched genomic DNA sample, in the wild-type strain we identified 605 ORFs that had significantly ($P < 0.001$) more DSB activity than the average ORF and 451 ORFs that were significantly "colder" than the

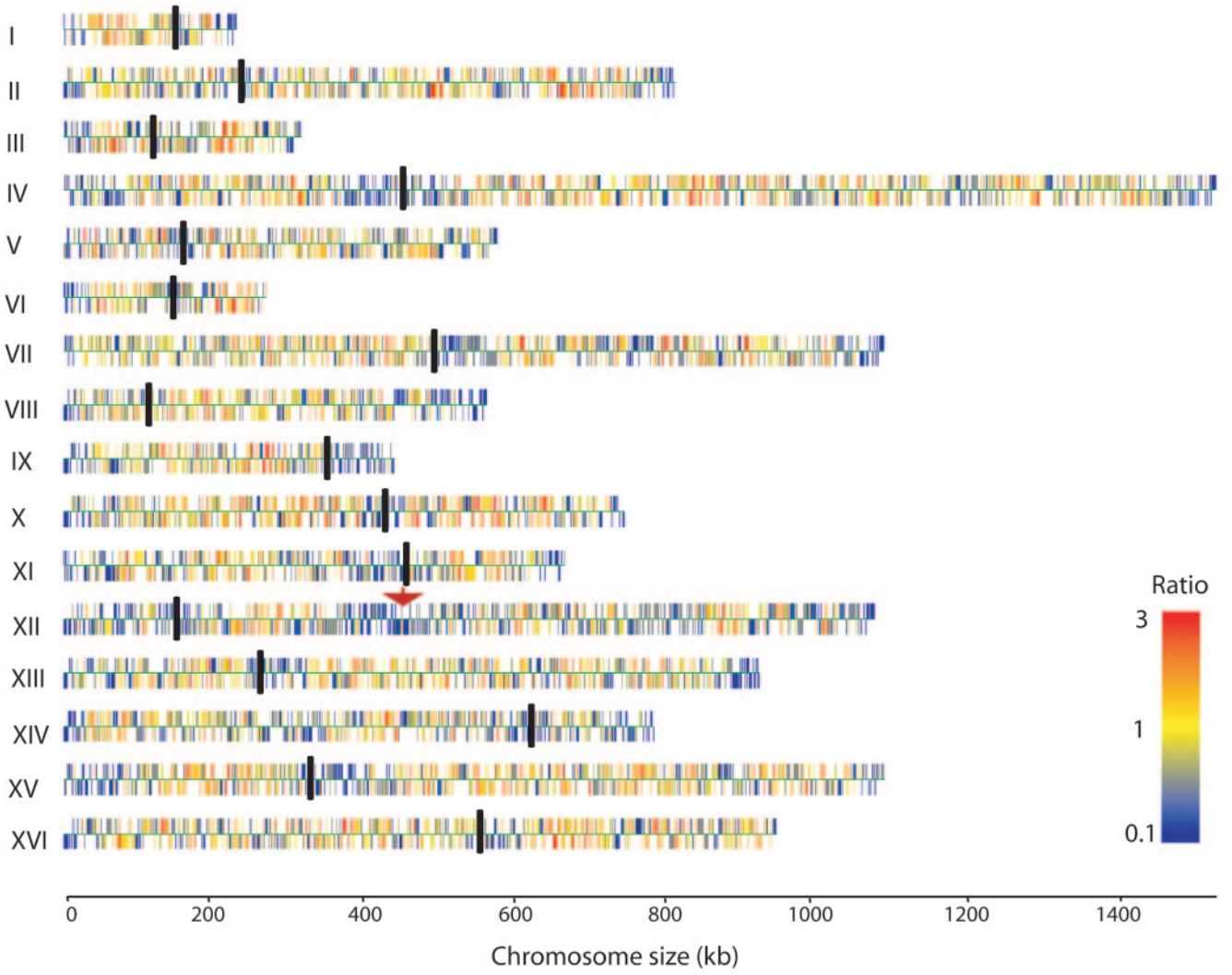

FIG. 4. Recombination activities of all ORFs in the wild-type strain JG169. We used GeneSpring software to show the relative recombination activities. The color code on the right indicates the median ratio of hybridization (DSB-enriched sample/total genomic DNA), with red representing hot spots and blue representing cold spots. No data were recorded for ORFs color coded as gray. The black ovals represent the centromeres, and the red arrow above chromosome XII indicates the position of the rRNA gene cluster; on the microarray, only 2 of the 100 repeats are represented. Thus, the suppression of recombination extends about 100 kb to each side of the cluster.

average. The median ratio ($\log_2$ [Spo11p-enriched DNA/total genomic DNA]) for all ORFs and intergenic regions in the wild-type strain is given in Table S2 in the supplemental material. A representation of the recombination activity for the whole genome is shown in Fig. 4.

In general, our measurements of the frequencies of DSB formation for yeast ORFs agreed well with our previous measurements (done using a slightly different procedure) (22) and those of Borde et al. (10). The details of this comparison and other features of the recombination patterns observed in wild-type strains are presented in the supplemental material. As we and others noted previously (8, 22), many recombination hot spots are in regions of high GC content, and the correlation between recombination activity and third-position GC content is even more striking (6). There is also a strong negative correlation between cohesin binding and recombination activity (see Fig. S1A and Table S3 in the supplemental material). Since cohesins bind at AT-rich regions, one interpretation of these results is that cohesin binding reduces the efficiency of Spo11p-mediated DSB formation (22). Another notable feature of the data was the large regions of suppressed recombination at the telomeres and centromeres, averaging 83 kb and 113 kb, respectively (Fig. 4; see Fig. S1 in the supplemental material). In addition, there was a very large (about 250-kb) region of suppressed recombination flanking the rRNA gene cluster (see Fig. S2 in the supplemental material). Although we previously noted that cold ORFs were often located near the centromeres and telomeres (22), the extent of suppression was less clear in our previous study. Borde et al. (10) also pointed out large regions of suppressed recombination at the telomeres, at the centromeres, and adjacent to the rRNA gene cluster. Since strains with a *sae2* (used by Borde et al.) or *rad50S* (used in our present study) mutation have reduced frequencies of DSBs in late-replicating regions (including the

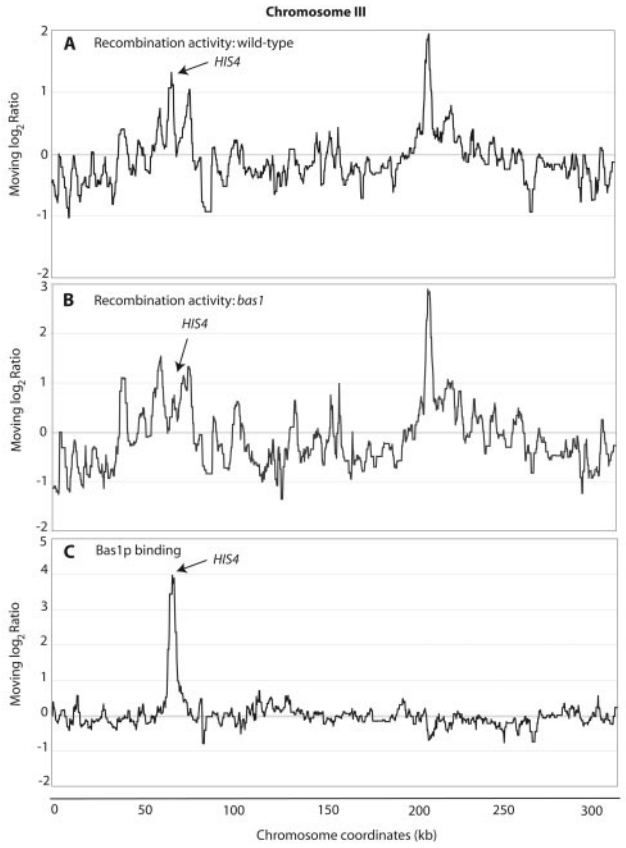

FIG. 5. Comparison of recombination activities on Bas1p targets on chromosome III in wild-type and *bas1* strains. As shown in panels A (wild-type strain) and B (*bas1* strain), the recombination activities of most regions are unaffected by the *bas1* mutation. One exception is the *HIS4* gene, where the recombination activity is significantly reduced, as expected from our previous study (56). In panel C, we show Bas1p binding (in meiotic cells) with a single chromosome peak at the position of the *HIS4* gene.

telomeres) relative to frequencies in wild-type strains (9), it is possible that the extent of suppressed recombination observed at the telomeres in both of these studies is greater than it would be in a wild-type strain.

Using the same procedures, we measured the recombination activities in PM216, an isogenic *bas1* derivative of JG169. The data for all ORFs and intergenic regions are in Table S2 in the supplemental material. In general, the patterns of hot spots and cold spots were very similar in the wild-type and *bas1* strains. Figure 5 shows recombination activity on chromosome III, indicating the reduction of hot spot activity near *HIS4*. The *bas1* strain had 569 hot ORFs, comparable to the 576 observed in the wild-type strain. A total of 431 of the ORFs that were hot in the wild-type strain were also hot in the *bas1* strain. Of the 145 that were hot in the wild-type, but not the *bas1*, strain, the average ranking was 0.836, indicating that these ORFs were only slightly below the cutoff for being hot. Clearly, most of the hot ORFs were not significantly affected by the *bas1* mutation, and those that were affected were usually on the border of our criteria for being hot.

We found that 153 ORFs had significantly ($P < 0.01$) different recombination activities in the wild-type and *bas1* strains (see Table S2 in the supplemental material). Of these ORFs, 68 had higher activities in the wild-type than in the *bas1* strain, and 85 had lower activities in the wild-type than in the *bas1* strain. When we analyzed the genes whose recombination activity was lowered in the *bas1* strain by using the SGD GO term find (Process), there was a significant excess of genes involved in purine metabolism ($P = 0.0003$), sterol transport (0.001), one-carbon metabolism (0.004), and energy pathways (0.006). Our analysis of Bas1p binding sites described above had shown that many of the genes bound by Bas1p were involved in purine metabolism or one-carbon metabolism.

Since Bas1p is a transcription factor that affects the transcription of multiple genes, the effects of the *bas1* mutation on recombination activity could be direct or indirect. The effects of Bas1p on recombination activity are more likely to be direct for those genes whose upstream regions are bound by Bas1p. Table 2 shows the 56 Bas1p binding sites that we have identified; these sites are potentially involved in the regulation of 71 genes, since some of the binding sites are located between divergently transcribed genes. Of these 71 genes, recombination rates were significantly reduced for nine genes (*SHM2*, *ADE17*, *ADE12*, *ADE13*, *HIS4*, *ADE1*, *YDR089W*, *MCH1*, and *SUT2*) and significantly elevated for four genes (*MRPS17*, *IES5*, *MTD1*, and *SEC20*).

As expected from our previous analysis, recombination activity at the *HIS4* locus was significantly reduced by the *bas1* mutation (Table 2 and Fig. 5). To confirm the microarray results, we analyzed meiosis-specific DSB formation by standard Southern analysis for the *HIS4*, *SHM2*, *ADE8*, and *MRPS17* genes. As predicted from the microarray data in Table 2, DSB formation was reduced by the *bas1* mutation for *HIS4* and *SHM2*, was unaffected by the *bas1* mutation for *ADE8*, and was increased by the mutation for *MRPS17* (Fig. 6A and B). For the *HIS4* locus, we showed previously that hot spot activity is lost as a consequence of the *bas1* mutation or deletion of the region containing the Bas1p binding site upstream of *HIS4* (19, 56). There are two Bas1p binding sites located upstream of *SHM2*, at positions −221 and −271. We constructed a diploid (MD341) that had the wild-type *BAS1* gene but was homozygous for mutations that altered the TGACTC binding sites to AAACTC; the allele with these upstream alterations is *SHM2-1*. As shown in Fig. 6C, the *SHM2-1* mutation resulted in loss of the *SHM2*-related DSB.

From these data, it is clear that the effect of Bas1p binding on recombination activity is complicated. The simple assumption that Bas1p binding would stimulate recombination for Bas1p-regulated genes is clearly incorrect. An alternative hypothesis is that there is no direct relationship between Bas1p binding and recombination activity for any ORF and that all of the observed effects of Bas1p on recombination are indirect. This hypothesis is very unlikely since point mutations in the Bas1p binding sites upstream of *SHM2* result in loss of local DSBs even in the presence of the wild-type *BAS1* gene. We discuss the context dependence of the effects of Bas1p binding on recombination rates further below.

DISCUSSION

Previously, we showed that the Bas1p transcription factor was required for formation of the meiosis-specific double-

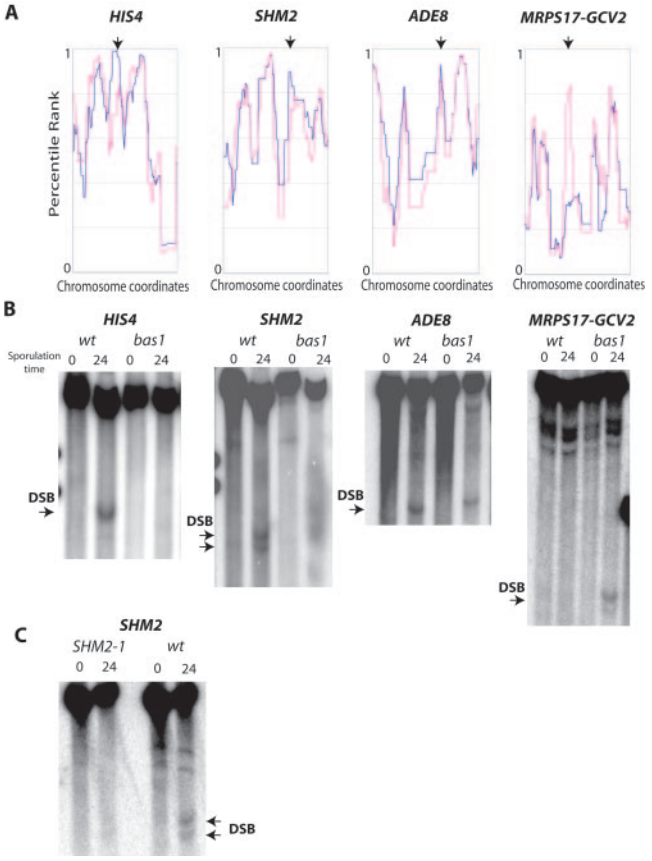

FIG. 6. Context-dependent effects of the *bas1* deletion on local meiosis-specific DSBs of four genes (*HIS4*, *SHM2*, *ADE8*, and *GCV2*) that have upstream Bas1p binding sites and effects of deletion of Bas1p binding sites upstream of *SHM2* on local meiosis-specific DSB formation. (A) Recombination activities of small chromosome regions containing the target gene, expressed as ranked values. Activities in the wild-type and *bas1* strains are represented with blue and red lines, respectively. The recombination activities of the *HIS4* and *SHM2* genes are significantly reduced by the *bas1* mutation, recombination of the *ADE8* gene is unaffected, and the recombination activity of the *MRSP17-GCV2* genes is increased. (B) Southern analysis confirming the microarray data for the same four genes. The positions of the meiosis-specific DSBs are indicated by arrows. The restriction enzymes used for the analysis of *HIS4*, *SHM2*, *ADE8*, and *GCV2*, respectively, were BglII, NcoI, EcoRV, and StuI. wt, wild type. (C) Southern analysis of a strain (MD341) in which the two Bas1p binding sites upstream of *SHM2* were altered (TGACTC to AAACTC). The strain homozygous for the resulting mutation (*SHM2-1*) lacked the meiosis-specific DSBs (indicated by arrows) upstream of *SHM2* that were present in the wild-type strain (EcoRI digest of genomic DNA). The filters were stripped and rehybridized to a probe derived from YGR177C (described in the supplemental material), a gene associated with a very strong DSB. Similar levels of DSBs were observed in both samples (data not shown).

strand DNA break that initiates recombination at *HIS4* (19). In the present study, we extended these observations by mapping Bas1p binding sites throughout the genome and monitoring DSB formation throughout the genome in wild-type and *bas1* strains. Of the 56 Bas1p binding sites identified in sporulating cells, Bas1p stimulates recombination at 9 of these sites, has no effect at 43 sites, and, unexpectedly, suppresses recombination at 4 sites.

Bas1p binding sites. When cells were grown in rich medium, we identified nine significant Bas1p binding sites corresponding to the target genes *GCV1*, *GCV2*, *GCV3*, *ADE2*, *SHM2*, *MTD1*, *HXT1*, *IES5*/*MET6*, and *JEN1*/*SRY2*. Fifty-six significant binding sites were observed in sporulating cells. The simplest explanation for the higher number of targets is that the sporulating cells are starved for adenine, a condition known to increase the transcription rates of many Bas1p-regulated genes (16). The transcription-stimulating effects of Bas1p require Bas2p, and the interaction between Bas1p and Bas2p occurs more efficiently in cells starved for adenine (47, 49, 63). Our results suggest that the Bas1p-Bas2p interaction increases the occupancy of these proteins at the promoter. Our list of Bas1p binding sites approximately doubles previous estimates of the number of genes likely to be regulated by Bas1p (15, 16, 21, 24, 50). It is possible, of course, that some of the genes that have Bas1p bound to their upstream regions are not regulated by Bas1p.

A microarray analysis of Bas1p binding has also been performed by Young and coworkers (24, 34). In the more recent publication from this group (24), Bas1p binding sites were examined in cells grown in rich growth medium (YPD) and in cells starved for amino acids. An analysis of their data indicates 27 significant ($P < 0.001$) Bas1p binding sites in cells grown in rich medium and 17 significant Bas1p binding sites in cells grown under conditions of amino acid starvation (24). For the cells grown in rich medium, eight of the nine sites that we identified are among the 27 sites detected by Harbison et al. (24). Of the 56 Bas1p binding sites that we identified in sporulating cells (a starvation condition), 8 were the same as those observed in amino-acid-starved cells (17 total sites) by Harbison et al. Thus, these studies agree reasonably well.

Although the effects of Bas1p on gene expression were first characterized for genes of the adenine and histidine biosynthetic pathways, some genes involved in the glycine, glutamine, and 10-formyl tetrahydrofolate pathways are also regulated by Bas1p and Bas2p (15, 21). There is a striking correspondence between the Bas1p binding sites detected in our study and the genes that are induced by glycine (21). Although we have not done expression studies, if most of the genes adjacent to the Bas1p binding sites are up-regulated in medium lacking adenine, these conditions would increase nucleotide production in several ways: (i) an increase in the enzymes of the adenine biosynthetic pathway, leading to AMP and GMP production; (ii) an increase in the enzymes of the histidine pathway, leading to increased levels of AICAR [5-amino-1-(5′-phosphoribosyl)-imidazole-4-carboxyamide] (one of the precursors in the production of AMP and GMP); and (iii) an increase in the production of 5,10-CH^+-tetrahydrofolate, a cofactor required in the adenine biosynthetic pathway. It should be noted, however, that the induction of several genes (for example, *GCV2*) by glycine is, at least in part, separable from the induction of the same genes by adenine starvation, since gene expression is elevated by glycine even in the absence of Bas1p (21).

Relationships between recombination activity and local and global features of chromosome structure. We attempted to correlate the hot spots with a large number of factors (association with repetitive chromosome elements, gene expression studies, transcription factor binding sites, gene expression in sporulating cells, nucleosome occupancy, etc.), as described in

the supplemental material. The strongest correlation was with local GC content, an association that we noted previously (22). As pointed out by Birdsell (6), the correlation is stronger for third-position GC-content than for total GC, although the correlation is striking for both (see Table S3 in the supplemental material). Birdsell (6) suggested that high recombination rates result in the formation of GC-rich regions as a consequence of GC-biased gene conversion. Since GC-rich sequences derived from bacterial plasmids often function as meiotic recombination hot spots in yeast (51, 59), we prefer the hypothesis that high GC content elevates recombination rates rather than vice versa. In addition, the recombination activities of a reporter gene integrated at multiple sites on chromosome III were strongly correlated with the GC content of the region flanking the insertion (11, 45). Despite the correlation between local GC content and recombination activity, it should be noted that all hot spots are not in regions of elevated G+C content, nor are all regions of elevated G+C content recombination hot spots.

Based on the observation that cohesins bind AT-rich regions (7, 23), we suggested previously (22) that hot spots might represent regions where the initiation of recombination was not prevented by cohesin binding. Another observation consistent with this hypothesis is that mitotic (23, 35) and meiotic (23) cohesins are preferentially located between convergently transcribed genes, regions that are negatively correlated with recombination hot spots. However, the observation that the correlation (see Table S3 in the supplemental material) between hot spots and third-position GC content is stronger than that between hot spots and binding of Rec8 (a meiosis-specific cohesin) argues that at least part of the effect of base composition is independent of cohesin binding. Another possibility is that regions of high GC content are associated with a chromatin modification that facilitates recombination (43). Relevant to this point is the observation that there is a very significant positive correlation between GC content of genes and their mRNA levels (40).

It is evident in this study, as in previous studies (4, 10, 22, 33, 44), that meiotic recombination near the centromeres, the telomeres, and the rRNA gene cluster is suppressed. Silencing of gene expression in *S. cerevisiae* is also associated with the telomeres and the rRNA genes, although not with the centromere (25). These effects, however, are much more local than the suppression of meiotic recombination. Although there is considerable telomere-to-telomere variation, the transcriptional silencing is usually limited to within a few kilobases of the chromosome end (48).

The absence of hot spots near the telomeres and centromeres argues that the frequency of recombination is controlled at least two levels. At the local level, recombination is affected by local GC content, transcription factor binding, and other factors. Superimposed on the local control is the suppression mediated by telomeres and centromeres. It is likely that both global and local controls are exerted by chromatin modifications, with the suppressing modifications being dominant. This type of model is consistent with the observation that hot spots moved to a novel chromosome context are often not hot spots (20).

Relationship between DSB formation and crossovers. In *S. cerevisiae*, about half of the DSBs are associated with crossovers and about half are repaired by mechanisms that do not lead to crossovers (46). Crossovers are measured in centimorgans (cM), with 1 cM representing 2% crossing over per tetrad. From comparisons of the genetic (measuring the frequency of crossovers) and physical maps, it is clear that the smaller chromosomes have more centimorgans per kilobase than the larger chromosomes (26). Assuming that all crossovers are initiated by DSBs, one could explain this result in two ways: either the smaller chromosomes have more DSBs per kilobase than the larger chromosomes, or the DSBs on smaller chromosomes are resolved as crossovers more frequently on the small than the large chromosomes. The recombination activity measured in our studies gives us the opportunity to address this question, because our data reflect the rate of DSB formation rather than the rate of crossovers.

To determine whether the ratio of DSBs to kilobases varies according to chromosome size, we summed the ratios of hybridization of the Spo11p-enriched sample/genomic DNA by using the ChIPOTle program (1-kb windows moved 250 bp at a time) for each chromosome; we refer to this sum as the "DSB activity" of the chromosome. These values were divided by the size of the chromosome. As shown in Table 3, the values for each chromosome (normalized to a value of 1 for the whole genome) were quite similar, varying between 0.99 and 1.12 for all chromosomes except chromosome XII (value of 0.70). The relatively small number of DSBs on chromosome XII reflects the 900-kb recombinationally silent rRNA gene locus of chromosome XII. In contrast, the normalized number of centimorgans per kilobase varied considerably, from 1.89 for chromosome VI (the second smallest chromosome) to 0.58 for chromosome XII (the largest chromosome). The negative correlation of chromosome size and crossovers per kilobase is very significant ($P < 0.0001$), even if chromosome XII is removed from the calculation ($P = 0.0005$). We also calculated the normalized crossovers divided by the normalized DSBs for each chromosome (Table 3). The four smallest chromosomes (I, III, VI, and IX) have the highest ratios. There is a very significant ($P < 0.001$) negative correlation between this ratio and chromosome size. A restatement of this conclusion is that a higher fraction of the DSBs are resolved as crossovers for the small chromosomes than for the large chromosomes.

What is responsible for this effect? Although a detailed molecular mechanism cannot yet be provided, it is likely to be a consequence of crossover interference. In yeast, as in most other eukaryotes, for a genetic interval of 50 cM or less, double crossovers are less frequent than expected based on the frequency of single crossovers (26, 39), a phenomenon termed chiasma interference. In contrast, conversion events that are unassociated with crossovers do not show interference (see reference 39 and references therein), indicating that interference does not primarily reflect competition between initiating DSBs. By doing experiments in which recombination was measured for the same physical interval on chromosomes of different sizes, Kaback et al. (26) showed that small chromosomes had less interference than large chromosomes.

On the basis of the data of Baudat and Nicolas (4), the frequency of DSB formation on chromosome III is about 1 DSB/100 kb. If we assume that a crossover reduces the probability of an adjacent crossover over an interval considerably greater than the average distance between initiation events,

TABLE 3. Recombination activity (DSB activity) and crossovers on chromosomes I to XVI

Chromosome	Size (kb)[a]	DSB activity[b]	DSB activity/kb[c]	Normalized DSB activity/kb[d]	cM[e]	cM/kb		Normalized crossovers/ normalized DSB activity[h]
						Corrected[f]	Normalized[g]	
I	230	879	3.82	1.07	115	0.63	1.75	1.64
II	813	3,083	3.79	1.06	246	0.32	0.89	0.84
III	317	1,217	3.84	1.08	152	0.54	1.50	1.39
IV	1,532	5,711	3.72	1.04	473	0.34	0.94	0.90
V	577	2,130	3.69	1.03	202	0.39	1.08	1.05
VI	270	1,067	3.95	1.11	133	0.68	1.89	1.70
VII	1,091	4,104	3.76	1.05	403	0.38	1.06	1.01
VIII	563	2,039	3.62	1.01	170	0.45	1.25	1.24
IX	440	1,663	3.78	1.06	198	0.52	1.44	1.36
X	746	2,983	4.00	1.12	224	0.34	0.94	0.84
XI	666	2,473	3.71	1.04	251	0.41	1.14	1.10
XII	1,869	4,689	2.51	0.70	383	0.21	0.58	0.83
XIII	924	3,424	3.71	1.04	321	0.38	1.06	1.02
XIV	784	2,774	3.54	0.99	292	0.41	1.14	1.15
XV	1091	4,112	3.77	1.06	360	0.38	1.06	1.00
XVI	948	3,503	3.70	1.04	275	0.33	0.92	0.88

[a] The ribosomal RNA gene cluster of about 900 kb was included in the size estimate for chromosome XII. The size of chromosome XII was corrected for the ORFs that were missing on the microarray.

[b] Sum for the whole chromosome of the hybridization ratios (Spo11p-enriched DNA/total genomic DNA) for a 1-kb window moved at 250-bp intervals.

[c] DSB activity divided by chromosome size.

[d] In this column, the data for DSB activity per kilobase were normalized by dividing them by 3.57. This value represents the average DSB activity per kilobase over the whole genome. It was calculated by dividing the sum of the DSB activities for each chromosome (45,857) by the sum of the chromosome sizes (12,861 kb).

[e] Size of the chromosome, based on the *Saccharomyces* Genome Database.

[f] Since the distance in centimorgans is calculated by genetic crosses, estimates of the genetic length of the chromosomes in the *Saccharomyces* Genome Database are based on the most distal genetic markers on the chromosomes. Here, the distance in centimorgans was divided by the number of kilobases between the most distal genetic markers (corrected kilobases).

[g] Centimorgans per kilobase (corrected) divided by the average centimorgans per kilobase (corrected) for the whole genome (0.36). The value of 0.36 was obtained by dividing the total centimorgans (4,198) by the sum of the corrected kilobases for the genome (11,593).

[h] Normalized centimorgans per kilobase for each chromosome divided by the normalized DSB activity per kilobase. This ratio is related to the fraction of the DSBs that are resolved as crossovers.

then the small chromosomes would have reduced interference relative to the larger chromosomes. Since interference is sometimes observed for intervals that include about 250 kb flanking a crossover (39), this assumption seems reasonable. Kaback et al. (26) suggested a similar model in which the larger chromosomes initiate crossovers earlier than the smaller chromosomes and, therefore, have a longer time interval in which to experience interference. In summary, our results demonstrate that the relatively elevated rates of crossovers on small yeast chromosomes are not a consequence of relatively high rates of DSBs and are likely to represent an interference-related phenomenon. The suggestion that the probability that a DSB will be resolved as a crossover or noncrossover is affected by chromosome context is also consistent with the observations that the association between gene conversion and crossing over varies considerably for different loci in yeast (27).

This conclusion is somewhat in conflict with the recent observations of Turney et al. (53). Using strains with translocations between chromosomes III and VII, they showed that the degree of interference for two sets of three linked markers was unchanged when the size of the chromosomes was altered by more than a factor of two. There are a number of possible explanations of this discrepancy. First, their genetic background was different from the one used in our study. Second, the chromosomal alterations used in their study may have had unintended effects caused by repositioning of the centromeres or telomeres. Third, interference may be a sequence-specific phenomenon (as suggested [53]), and the sequences predisposed to interference may be preferentially located on the smaller chromosomes.

Effect of Bas1p binding on recombination hot spot activity. Our expectation for these experiments was that Bas1p would be a positive activator of recombination, acting locally to promote recombination of the DNA flanking its binding site. Instead, we found relatively few genes whose recombination was affected by the *bas1* deletion. In our analysis, we concentrated on the genes whose upstream regions contained consensus Bas1p binding motifs, since the effects of the *bas1* deletion on recombination were more likely to be direct for these genes. Of the 56 binding sites identified, the recombination activity was reduced for 9 downstream target genes, was elevated for 4 genes, and was unaffected for 43 genes (Table 2). We found that the strength of Bas1p binding was related to the probability that recombination was affected by Bas1p. In Table 2, of the 13 ORFs whose recombination activity was significantly affected by Bas1p, 9 ranked in the top 15 for binding activities and none ranked in the bottom 15, a significant departure from the null hypothesis (P value of <0.01 by the Fisher exact test).

Of the nine genes that had significantly less recombination activity in the *bas1* strain, six (*SHM2*, *ADE17*, *ADE12*, *ADE13*, *HIS4*, and *ADE1*) were previously shown to be positively transcriptionally regulated by Bas1p binding (15, 16, 50), and three (*SUT2*, *MCH1*, and *YDR089W*) have not been shown to be Bas1p regulated. The mechanism by which Bas1p positively activates transcription is not completely clear. At the *HIS4* locus, and at all other loci thus far examined, Bas1p acts in

conjunction with Bas2p (52). This interaction is facilitated by a metabolite that accumulates in cells starved for adenine (49). An additional level of complexity is that *HIS4* expression is activated (upon amino acid starvation) by Gcn4p, a protein that binds to a site that overlaps the binding site of Bas1p, and Rap1p, a protein that binds to the *HIS4* upstream region (41). Rap1p binding is thought to displace nucleosomes, facilitating the interaction of Gcn4p and/or Bas1p/Bas2p with the binding site. Valerius et al. (54) argued that Gcn4p and Bas1p/Bas2p at the *HIS7* locus stimulate transcription through two different pathways, Gcn4p by interacting with the Swi/Snf complex to remodel chromatin and Bas1p/Bas2p by interacting with SAGA. Since the SAGA complex contains Gcn5p, a histone acetyltransferase (31), Bas1p and Bas2p may be functioning indirectly by affecting the level of histone acetylation at its target loci. It should be noted that deletion of $gcn5^+$ in *Schizosaccharomyces pombe* reduces the activity of the *M26* hot spot (60).

In the context of the possible role of Bas1p in transcription, Bas1p-dependent hot spots may be those in which the entry of the recombination machinery (Spo11p and associated proteins) is helped by acetylation of histones flanking the potential DSB site. If this model is correct, it is unclear why the recombination activity of some loci was elevated by the deletion of *BAS1*. One possible explanation is that, for some loci, the chromatin-remodeling activities of Gcn4p might facilitate the entry of recombination machinery better than the presumptive histone acetylation activity promoted by Bas1p. Since these two proteins bind to the same sequence, deletion of Bas1p could result in better binding of Gcn4p.

Many of the genes located near Bas1p binding sites had the same recombination activity in wild-type and *bas1* strains. This result demonstrates that Bas1p binding is neither necessary nor sufficient for recombination hot spot activity in otherwise wild-type cells. One explanation of this result is that the recombination activity of a region represents the integration of various chromatin-"loosening" modifications, with Bas1p being only one of the factors. At some loci, Bas1p might be the major contributor and, therefore, the absence of Bas1p would have a substantial effect of recombination activity; at other loci, the effects of other transcription factors would play a more substantive role. In addition, as indicated in Table 2, the relative affinity of Bas1p for its targets is not constant, and Bas1p would be expected to have more of an effect on genes located near the strongest binding sites (as discussed above). Another possibility is that there may be recombination-suppressing modifications (for example, histone methylation events) that preclude stimulation of recombination activity by Bas1p.

Summary. Our data indicate that Bas1p binding is capable of activating recombination, but this activation is context specific. The simplest interpretation of our data is that hot spot activity is governed by a balance between chromatin-loosening and -tightening activities that affect the accessibility of the DNA to the enzymes that initiate meiotic exchange (43). It is also possible, however, that the recombination machinery recognizes a specific histone "code." Further progress on this problem will require a detailed analysis of recombination activities in strains with various mutations affecting histone modifications and other chromatin-remodeling activities.

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