Meiotic recombination frequencies are affected by nutritional states in *Saccharomyces cerevisiae*

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Meiotic recombination in the yeast *Saccharomyces cerevisiae* is initiated by programmed double-strand breaks at selected sites throughout the genome (hotspots). α-Hotspots are binding sites for transcription factors. Double-strand breaks at α-hotspots require binding of transcription factor but not high levels of transcription *per se*. We show that modulating the production of the transcription factor Gcn4p by deletion or constitutive transcription alters the rate of gene conversion and crossing-over at *HIS4*. In addition, we show that alterations in the metabolic state of the cell change the frequency of gene conversion at *HIS4* in a Gcn4p-dependent manner. We suggest that recombination data obtained from experiments using amino acid and other biosynthetic genes for gene disruptions and/or as genetic markers should be treated cautiously. The demonstration that Gcn4p affects transcription of more than 500 genes and that the recombinationally ''hottest'' ORFs tend to be Gcn4p-regulated suggest that the metabolic state of a cell, especially with respect to nitrogen metabolism, is a determinant of recombination rates. This observation suggests that the effects of metabolic state may be global and may account for some as yet unexplained features of recombination in higher organisms, such as the differences in map length between the sexes.

Meiotic recombination in the yeast *Saccharomyces cerevisiae* is a regulated process. Both the timing and the positions of the initiating double-strand breaks are controlled. The enzyme catalyzing the initiating double-strand break, Spo11p, is induced after the meiotic program has started, and makes breaks only in DNA that has been replicated in meiotic S phase (1, 2). The distribution of double-strand breaks is controlled by local features such as chromatin structure (3–8) and is correlated with larger-scale features such as chromosome size (9).

Genetic analyses of gene conversion and crossing-over in yeast have often hinged on genes of interest disrupted with one or another selectable insertion such as *LEU2, TRP1*, or *URA3*. Furthermore, the effects of disruption on recombination have typically involved changes in conversion and/or crossing-over of nutritional markers. Nutritional changes introduced by both the disruptions and the markers have generally been assumed to be neutral with respect to their effects on recombination. However, elucidation of the nature of the initiation sites for recombination raises doubts as to the validity of this assumption.

The sites at which double-strand breaks initiate recombination fall into three categories, α-, β-, and γ-hotspots, a common feature of which seems to be locally open chromatin (7, 9–11). α-Hotspots have been defined by the observation that their high levels of recombination depend on the binding of transcription factors (12–15). β-Hotspots contain DNA sequences, such as $(CCGNN)_{12}$, that lead to nucleosome-free regions (7, 11). A number of artificial hotspots have also been shown to be nuclease hypersensitive (3–5). A γ-hotspot has been defined as a double-strand break site associated with high GC content based on a genomewide analysis (9, 10). There may be additional types of hotspots. For example, another genomewide analysis identified a 50-bp region with a poly(A) tract at its center, termed a ''CoHR,'' that has a high degree of correlation with known double-strand break sites (16). At *HIS4*, the transcription factors Rap1p, Bas1p, and Bas2p/Pho2p bind upstream of the ORF (17–19) and stimulate gene conversion 2- to 3-fold (13, 14, 20). How the transcription factors are stimulating recombination is not known, although it has been shown that they can recruit chromatin-remodelling factors such as histone acetyltransferases (21, 22). This, combined with the observation that all hotspots characterized to date are hypersensitive to DNase digestion of chromatin *in vitro* (3–5, 7, 8, 23), led to the suggestion that transcription factors make the DNA more accessible to Spo11p (10, 24). If this view is correct, then transcription factors with genomewide regulatory roles and the factors that modulate their activities are central to our understanding of the control of meiotic recombination.

Gcn4p is a basic leucine zipper transcriptional activator (25) affecting more 500 genes (26). These include genes involved in the biosynthesis of purines and vitamin cofactors, in addition to the amino acid synthetic genes previously shown to be regulated by Gcn4p (reviewed in ref. 27). Other classes of ORFs regulated by Gcn4p include peroxisomal genes, mitochondrial carrier proteins, amino acid transporters, transcription factors, protein kinases, and genes involved in glycogen metabolism, demonstrating that Gcn4p plays a central role in regulating the metabolic activity of yeast cells.

Production of Gcn4p itself is regulated by amino acid, purine, or glucose availability, being repressed under nonstarvation conditions and derepressed when glucose is limiting or when one or more amino acids or purines is lacking. The loss of ability to synthesize certain amino acids as well as other conditions leading to imbalances in the amino acid pool derepress *GCN4*, leading to a 10-fold increase in Gcn4p levels (27). For example, high levels of leucine lead to feedback inhibition of the isoleucine/valine biosynthetic pathways. In the absence of exogenous isoleucine and/or valine, this results in derepression of *GCN4* (27). Other stresses, such as DNA damage induced by methylmethanesulfate, also affect Gcn4p production (26). Gcn4p is regulated by a control system that senses the level of uncharged tRNAs (28). Under nonstarvation conditions, the 40S ribosomal unit initiates and terminates translation in the region of the four μ-ORFs contained in the 5′ leader sequence of *GCN4*, such that none of the 40S subunits reaches the start codon for Gcn4p. In contrast, under amino acid starvation conditions, accumulation of uncharged tRNAs, through complex downstream events, allows translation to begin at the correct start site for production of Gcn4p.

Previous studies of the role of Gcn4p in gene conversion at *ARG4* (6) and *HIS4* (12) failed to note a statistically significant effect of deletion of *GCN4*, though the *HIS4* data suggested a 25% decrease in gene conversion when *GCN4* was deleted. This observation, combined with puzzling results obtained for conversion frequencies at *HIS4* when certain auxotrophic markers

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Table 1. Strains used in this study

Strain	Relevant genotype*				
FAD 441, 444, 448, 496	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	
	HIS4	*LEU2*	*ade1*	*lys2–c*	
FAD 493, 495	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	
	HIS4	*leu2::kpn*	*ade1*	*lys2–c*	
FAD 446, 447, 492	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	
	HIS4	*LEU2*	*ade1*	*LYS2*	
FAD 445, 494, 498	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	
FAD 644, 645	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*LEU2*	*ade1*	*lys2–c*	*Δgcn4::kanMX*
FAD 648, 649	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*leu2::kpn*	*ade1*	*lys2–c*	*Δgcn4::kanMX*
FAD 642, 643	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*LEU2*	*ade1*	*LYS2*	*Δgcn4::kanMX*
FAD 646, 647	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	*Δgcn4::kanMX*
FAD 679, 680	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*GCN4*
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	*GCN4–URA3–GCN4^{c}*
FAD 681	*his4::xho*	*leu2::rI*	*ADE*	*lys2–d*	*GCN4*
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	*GCN4–URA3–GCN4*
FAD 491	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	
	HIS4	*LEU2*	*ade1*	*lys2–c*	
FAD 819	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	
FAD 820	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	
	HIS4	*leu2::kpn*	*ade1*	*lys2–c*	
FAD 847, 848	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	*Δgcn4::kanMX*
FAD 849, 850	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*leu2::kpn*	*ade1*	*lys2–c*	*Δgcn4::kanMX*
FAD 851, 852	*his4::xho*	*leu2::rI*	*ade1*	*lys2–d*	*Δgcn4::kanMX*
	HIS4	*LEU2*	*ade1*	*lys2–c*	*Δgcn4::kanMX*
FAD 410, 811, 812	*his4::cla*	*leu2::cla*	*ADE*	*lys2–d*	
	HIS4	*LEU2*	*ade1*	*lys2–c*	
FAD 516, 813, 814	*his4::cla*	*leu2::cla*	*ADE*	*lys2–d*	
	HIS4	*LEU2*	*ade1*	*LYS2*	
FAD 515, 816	*his4::cla*	*leu2::cla*	*ADE*	*lys2–d*	
	HIS4	*leu2::kpn*	*ade1*	*LYS2*	

*All strains are also *MAT***a**/*MATα*, *TRP5*/*trp5–1*, *ura3*/*ura3*, *CAN1*/*can1*, *MET13*/*met13*, and *cyh2*/*CYH2* (except FAD 495 and 496, which are *cyh2*/*cyh2*).

were varied in otherwise isogenic strains, has led us to reexamine the role of the metabolic state of the cell and Gcn4p in controlling levels of meiotic recombination.

Materials and Methods

Strains. All haploid strains are derived from either H394 (*ura3–1 ade1 lys2-d met13-4 cyh2 leu2::rI MAT***a***)* or H330 (*ura3-1 ade1 can1 lys2-c met13-2 trp5-1 cyh2 leu2::kpn MATα* (29–31). Ade^{+}, Lys^{+}, Met^{+}, and Leu^{+} derivatives were created by transformation with wild-type DNA. Derivatives of opposite mating type were created by transformation with the *HO* gene on a replicating plasmid, followed by loss of the plasmid (32, 33). The *his4::xho, his4::cla, leu2::cla, leu2::rI, leu2::kpn* alleles and the wild-type *CYH2* allele were created by two-step gene replacement as described (29, 31, 34, 35). All experimental diploids contain one chromosome set from the H394 line and one from the H330 line. Diploids differ from each other only in their nutritional markers or at *GCN4*, with one exception where the *CYH2* locus is homozygous for the recessive allele. Complete genotypes are listed in Table 1. In many cases, two or more independent diploids were dissected. No significant differences were detected among isogenic sets of crosses, and the data were pooled. Strains are referred to by their relevant genotype or phenotype.

The wild-type *GCN4* gene was replaced with the kanMX4 cassette by using the short oligonucleotide PCR-based gene disruption technique with selection for G418 resistance (36). Transformants were confirmed by PCR (36). *GCN4^{C}*, a constitutive allele of *GCN4*, was created by integration of plasmid p139 (37). This plasmid contains the entire *GCN4* ORF and flanking sequences, but the four regulatory μ-ORFs in the leader sequence of the mRNA have been deleted. The integration results in a nontandem duplication of *GCN4* flanking *URA3* and pBR322. Integrants contain either one copy of *GCN4* that has the μ-ORFs deleted and one wild-type allele (*GCN4^{C}-URA3-GCN4*) or two wild-type copies of *GCN4* (*GCN4-URA3-GCN4*). Transformants containing the μ-ORF deletion were identified by PCR and confirmed by sequencing. A transformant in which there were two wild-type copies of *GCN4* was used as a control. As discussed above, the multiple upstream AUG codons mediate translational control of *GCN4*, and their absence results in constitutively high production of Gcn4p (37). A heterologous gene cassette, natMX4, encoding resistance to the drug nourseothricin was placed ≈3,500 nt telomeric to *HIS4* by using PCR-

Table 2. Effect of Gcn4p on conversion at *HIS4*

	Percent conversion at *HIS4* ± SE (events/total tetrads)			
	Leu$^+$ Lys$^-$	Leu$^-$ Lys$^-$	Leu$^+$ Lys$^+$	Leu$^-$ Lys$^+$
Ade$^+$				
GCN4/GCN4	27% ± 1.7*‡ (195/719)	21% ± 1.7*§ (126/588)	19% ± 1.5†‡ (127/682)	13% ± 1.5†§ (72/534)
Δ*gcn4*/Δ*gcn4*	12% ± 1.9¶ (37/300)	10% ± 1.8¶ (28/274)	13% ± 1.9¶ (40/314)	10% ± 1.7 (33/313)
GCN4–URA3–GCN4/GCN4	ND	ND	ND	13 ± 2.4 (25/191)
GCN4^c–URA3–GCN4/GCN4	ND	ND	ND	48 ± 2.1‖ (94/197)
*Ade$^-$***				
GCN4/GCN4	40% ± 3.5 (78/196)	37% ± 3.6 (64/175)	ND	37% ± 3.6 (69/185)
Δ*gcn4*/Δ*gcn4*¶	29% ± 3.4 (52/180)	23% ± 3.1 (42/181)	ND	23% ± 3.6 (32/140)

ND, not determined.
*Prototrophy for leucine elevates conversion frequency significantly in lysine auxotrophs.
†Prototrophy for leucine elevates conversion frequency significantly in lysine prototrophs.
‡Auxotrophy for lysine elevates conversion frequency significantly in leucine prototrophs.
§Auxotrophy for lysine elevates conversion frequency significantly in leucine auxotrophs.
¶Conversion frequency is significantly lower in Δ*gcn4* strains than in *GCN4*.
‖Constitutive expression of Gcn4p elevates conversion frequencies significantly.
**Auxotrophy for adenine elevates conversion frequency significantly (independently of phenotype for leucine or lysine).

based gene insertion with selection on 200 μg/ml nourseothricin (38).

Genetic Analysis. Parents of diploids to be dissected were mixed on rich medium (yeast extract/peptone/dextrose; YEPD) and allowed to mate overnight at 30°C (39). Sporulation was induced at 22°C by transfer to synthetic complete medium (see below) containing 2% potassium acetate and 0.5% glucose. Ascal walls were removed by digestion with 0.25 mg/ml zymolase for 30 min at 37°C. The four spores of each tetrad were separated from each other, germinated on YEPD, and allowed to grow for 72 h at 30°C before scoring for genetic markers. Δ*gcn*4 and *GCN4^c* spore clones were allowed to grow for an additional 2 days before scoring. Non-Mendelian segregations (6:2, 2:6, 5:3, and 3:5 marker segregations) and reciprocal crossing-over were scored only in tetrads with four viable spores. *HIS4*, *TRP5 ADE1*, *MET13*, and *LEU2* alleles were scored by replica plating to synthetic complete media lacking the appropriate nutritional supplement. Synthetic complete medium is made by supplementing minimal medium (39) with the following nutrients: adenine sulfate, uracil, L-trptophan L-histidine-HCl, L-arginine-HCl and methionine, each at 31.6 mg/liter; tyrosine, L-leucine, and L-lysine-HCl, each at 47.5 mg/liter; L-phenylalanine at 79 mg/liter; L-glutamic acid and L-aspartic acid at 158 mg/liter; and threonine at 316 mg/liter. Segregation of the natMX4 cassette was scored on YEPD containing 200 μg/ml nourseothricin (38).

Map distances were calculated from the frequencies of tetratype (TT) nonparental di-type (NPD) and parental di-type (PD) asci by using the formula (1/2)(TT + 6NPD)/(TT + NPD + PD) (40).

Map distances (X) were compared as described on Stahl Lab Online Tools (http://www.groik.com/stahl). Differences are considered significant when $2\sqrt{(\mathrm{Var[X1] + Var[X2]})} < |\mathrm{X1 - X2}|$. Comparisons of frequencies of gene conversions were performed by using a standard normal test (the *z* statistic, http://faculty.vassar.edu/lowry/VassarStats.html). *P* values < 0.05 were considered significant.

Global Hotspot Map and *GCN4* Expression Array. Expression profile data from an array analysis of Gcn4p-regulated ORFs was obtained from http://www.rii.com/publications/mcb2001Marton.htm (26). The 303 ORFs designated as "hot" by virtue of being adjacent to a double-strand break (9), along with their associated expression profiles (26), were extracted from the total data. Both the total array data and the hot ORFs were then analyzed for the frequency of ORFs exhibiting Gcn4p-dependent induction. An ORF was included if there was significant increase in transcription in a starved *GCN4* strain as compared with a starved Δ*gcn4* strain. The significance of the difference between the proportions was determined by using the standard normal test.

Results

Metabolic State of the Cell Affects Conversion Frequencies. The frequency of gene conversion of the *his4*::*xho* allele (31) was measured in an isogenic set of strains that differed only in their genotype at *LEU2*, *LYS2*, or *ADE1* (Table 2). Lysine auxotrophy elevates gene conversion at *his4*::*xho* ≈1.4- to 1.6-fold. Auxotrophy for adenine elevates conversion from various values that depend on leucine and lysine phenotype, to a value of ≈38%, that is independent of phenotype for leucine or lysine. In contrast, prototrophy for leucine elevates gene conversion at *his4*::*xho* ≈1.4 fold. Prototrophy for leucine also elevates conversion at a second allele of *HIS4*, *his4*::*cla*, from 9.7% (30 of 309) to 15.1% (56 of 372). These differences are statistically significant. Gene conversion frequencies were also measured at four other loci that might be expected to respond to Gcn4p, *LEU2*, *TRP5*, *MET13*, and *CAN1*, and one that would not be expected to respond, *CYH2* (26). No statistically significant differences were detected at any of these loci except for *LEU2*, where auxotrophy for lysine increased gene conversion significantly from 6.2% (23 of 372) to 10.1% (42 of 414).

Although previous studies failed to demonstrate an effect of Δ*gcn4* on gene conversion frequency at either *HIS4* (12) or *ARG4* (6), two loci known to be regulated by Gcn4p, our studies at *HIS4* show a reduction in gene conversion under conditions expected to repress synthesis of Gcn4p. This, combined with the fact that amino acid auxotrophies modulate Gcn4p expression in a complex fashion, led us to test the effect of modulating Gcn4p expression on gene conversion at *HIS4* more directly. We compared two constructs affecting Gcn4p expression, a deletion and one that expresses Gcn4p constitutively at high levels (37). As can be seen in Table 2, deletion of *GCN4* reduces gene conversion to the level seen in Leu$^-$ Lys$^+$ Ade$^+$ strains. Overexpression of Gcn4p elevates gene conversion 4-fold above this level. No effects were seen at *LEU2*, *TRP5*, *MET13*, *CAN1*, or *CYH2*.

In Ade$^-$ strains, deletion of Gcn4p leads to a 1.5-fold decrease in conversion (Table 2). However, the level in the absence of Gcn4p is still significantly (2-fold) higher than it is in Ade$^+$ strains deleted for Gcn4p (Table 2).

Table 3. Effect of Δ*gcn4* on map distance in Lys⁻ diploids

	Genetic interval							
	Nat–*LEU2*				*MET13–CYH2*			
Gene	PD	NPD	TT	cM*	PD	NPD	TT	cM
GCN4								
All tetrads	201	6	148	25.9 ± 2.3†	257	0	114	15.4 ± 1.2
Conversion tetrads‡	30	4	45	43.7 ± 7.2	55	0	24	15.2 ± 2.6
Nonconversion tetrads	171	2	103	20.8 ± 3.7	202	0	90	15.4 ± 1.4
Δ*gcn4*								
All tetrads	245	2	140	19.6 ± 1.6†	265	1	130	17.2 ± 1.4
Conversion tetrads§	19	0	25	28.4 ± 3.7	28	0	16	18.2 ± 3.6
Nonconversion tetrads	226	2	115	18.5 ± 1.7	237	1	114	17.0 ± 1.5

PD, parental di-type; NPD, nonparental di-type; TT, tetratype; cM, centimorgan(s).
*Map lengths ± one standard error.
†Map lengths for *GCN4* and Δ*gcn4* are significantly different.
‡Conversion frequency = 22.3%.
§Conversion frequency = 11.4%.

Deletion of Gcn4p Reduces Crossing-Over. To assess whether Gcn4p expression was affecting reciprocal crossing-over as well as gene conversion, we inserted a drug resistance marker on the telomeric side of *HIS4*. Crossing-over in an interval that includes *HIS4* (natMX4-*LEU2*) was measured in wild-type and Δ*gcn4* strains (Table 3). A statistically significant decrease in crossing-over was detected on deletion of *GCN4*. We calculated the natMX4–*LEU2* map distance separately for tetrads with or without a gene conversion at *HIS4* (Table 3). Tetrads with no conversion were not significantly enriched for crossovers. No change in map distance was detected for the *MET13* to *CYH2* interval on chromosome VII.

Gcn4p-Regulated and Double-Strand Break-Associated ORFs. As described in *Materials and Methods,* we determined the number of ORFs showing regulation by Gcn4p in the total genome and in the recombinationally hot ORFs identified by Gerton *et al.* (9). After eliminating the ORFs for which data are unavailable, we could analyze 5,793 of the 6,222 total ORFs and 289 of the 303 hot ORFs. If an ORF's transcript increased on starvation only in wild-type cells and not in Δ*gcn4* cells, it was said to be regulated by Gcn4p. Of all ORFs, 17.5% meet this criterion, whereas 33.9% of the recombinationally hot ORFs are induced. Thus, the hot ORFs are significantly enriched for Gcn4p-regulated ORFs ($P < 0.05$).

Discussion

Gcn4p Influences Meiotic Recombination at Some but Not All Loci. The data presented demonstrate that both direct (gene disruption, overexpression) and indirect (metabolic state of the cell) modulation of Gcn4p levels affect frequencies of gene conversion and crossing-over at susceptible sites. We suggest that auxotrophic diploids sporulating on synthetic media experience metabolic imbalances, resulting in derepression of Gcn4p and consequent increased recombination caused by Gcn4p binding at the *HIS4* hotspot. Although the medium is supplemented with the relevant nutrients, the amounts may not be sufficient to compensate for the auxotrophic state of the cell. Indeed, the sporulation medium widely used (39, 41) is supplemented with amino acids at between 12% and 25% of the level used here. Neither medium is supplemented for isoleucine or valine, which is likely to exacerbate the amino acid imbalance in Leu⁺ cells, leading to Gcn4p induction.

Some Nutritional Effects on Conversion Are Independent of Gcn4p. A number of observations support the hypothesis that metabolically controlled transcription factors other than Gcn4p affect conversion frequencies. The observation that gene conversion at some Gcn4p-regulated loci is unaffected by the phenotypes that affect conversion at *HIS4* indicates that other trans- or cis-acting factors may mask or modulate the effect of Gcn4p. The observation that starvation for adenine elevates conversion at *HIS4* more than do either lysine auxotrophy or leucine prototrophy and that only a fraction of this elevation is Gcn4p-dependent suggest that adenine starvation influences other transcription factors that affect conversion at *HIS4*. For example, the transcription factors Bas1p/Bas2p and Rap1p are known to affect recombination at *HIS4*, and their deletion seems to have a greater effect on conversion at *HIS4* than does deletion of *GCN4* (12, 13). Recent studies of the regulation of transcription factors have shown that the interaction of Bas1p with Bas2p is inhibited by adenine (42). This study also demonstrated that efficient binding of Bas1p/Bas2p to the *HIS4* promoter requires adenine limitation (42). These observations may explain why White and Petes (12, 13) found that deletion of *BAS1* or *BAS2* has a greater effect on conversion at *HIS4* in their *ade6* strains than did deletion of *GCN4*.

The mechanism by which these transcription factors regulate recombination is not related to increased transcription (12, 13), but rather to an affect on the DNase sensitivity of the chromatin and the level of double-strand breaks that occur at the hotspot (23, 43). Although we have not directly demonstrated an effect on double-strand breaks at either *HIS4* or *LEU2*, the phenotype for growth on leucine affects both gene conversion frequency and the level of meiotic double-strand breaks at *LEU2*. Both conversion and double-strand breaks are at least 2-fold higher in Leu⁺ strains as compared with isogenic Leu⁻ strains (M. J. Lichten, personal communication). We suggest that, similarly to Rap1p, Gcn4p acts by participating in remodeling chromatin at the hotspot sequence, making it more accessible to the recombination machinery, and/or by directly recruiting the recombination machinery to the hotspot (10). Gcn4p has been reported to recruit histone acetyltransferase complexes to nucleosomes (10, 21, 22).

Meiotic Map Distance Is Affected by Gcn4p. The map distance in the interval embracing *HIS4* (natMX–*LEU2*) is decreased ≈30% by deletion of *GCN4*. At least half of the crossing-over in this interval can be attributed to the *HIS4* hotspot, as can be seen from the 1.5- to 2-fold enrichment in crossing-over among tetrads preselected for conversion at *HIS4* (Table 3). Modulation of Gcn4p can have interval-specific effects on crossing-over, because the Δ*gcn4* mutation does not change the map distance in the *CYH* to *MET* interval.

Implications for Studies of Meiotic Recombination. That the phenotype of yeast with regard to amino acid or purine metabolism can alter gene conversion frequencies and crossing-over at some loci has consequences. Many recombination studies have used *LEU2, TRP1, LYS2*, or *URA3* genes to disrupt a gene of interest, assuming that any resulting change in cellular metabolism was neutral with respect to the recombination phenotype being studied (for examples see refs. 44–49). The data presented here demonstrate that this assumption is invalid, particularly with regard to studies using *HIS4* as a "reporter" and using the *LEU2, ADE1*, or *LYS2* genes to make disruption mutations.

In a limited survey of the literature we have found instances where variations in recombination frequencies could be explained by differences in phenotype for leucine metabolism rather than disruption of the gene being tested. For example, in heteroallelic crosses, an approximately 2-fold higher frequency of histidine prototroph conversions was obtained in return-to-growth experiments when *DMC1* was disrupted with *LEU2* as compared with a *URA3* disruption (48). A *zip3*::*LEU2* disruption strain gave more His^+ prototrophs than the *leu2 ZIP3* control (49). In the case of the *DMC1* disruption, the conclusion that *dmc1* was reduced for recombination was not affected by this difference, although the authors were puzzled by the observation. On the other hand, Chua and Roeder (49) concluded that gene conversion was elevated by disruption of *ZIP3*. In the absence of data with a neutral disruption allele, this conclusion should be treated with caution. There is at least one case where an erroneous conclusion was drawn. Hunter and Borts (44) examined the effects of mutation in *MLH1*, a major mismatch repair gene (46, 50, 51), on crossing-over and conversion (all non-Mendelian segregation). When *LEU2* was used to disrupt *MLH1* in a Leu^- background, there was an increase in conversion for all of the alleles tested at *HIS4*. Increase in conversion independent of distance from the double-strand break site was unexpected. Based on their result, Hunter and Borts postulated that Mlh1p controlled total levels of meiotic gene conversion, perhaps by controlling the termination of heteroduplex DNA formation. We have since determined the frequency of non-Mendelian segregation at *HIS4* by using a kanMX4 disruption of *MLH1*. These experiments indicated that total conversion at *HIS4* is not affected by deletion of *MLH1* (unpublished data). Thus, Hunter and Borts were mislead by their use of a disruption mutation that altered the nutritional state of the cell. No other result in that paper is compromised by the use of a *LEU2* disruption.

The Effect of Gcn4p, and Hence Metabolic Factors, on Recombination Is Probably Genomewide. An analysis of the ORFs identified by Gerton *et al.* (9) as being adjacent to hotspots for recombination indicates that they are 2-fold enriched for regulation by Gcn4p over the genome average. This result suggests that analysis of the effects of mutation on recombination-dependent phenotypes such as meiotic spore viability and nondisjunction rates are also compromised by nonisogenicity for metabolic genes. Interval-specific effects of gene disruptions are also suspect, because intervals may vary in the density of ORFs that are regulated by Gcn4p. Thus, conclusions drawn from recombination data obtained where isogenicity for cellular metabolism genes was not maintained should be re-evaluated. Of particular concern are studies where decreases in conversion caused by the gene disruption might be masked by the elevation in conversion due to *LEU2* insertion and studies where the wild-type levels of recombination are stimulated by starvation for the amino acid that, in the mutant, is alleviated by the gene disruption.

The Role of Transcription Factors in Recombination Is Not Limited to *S. cerevisiae*. The *M26* hotspot of *Schizosaccharomyces pombe* is characterized by a DNA sequence (5′-ATGACGT(GA)-3′) that binds the basic leucine zipper transcription factors Atf1 and Pcr1, required for hotspot activity (15). Binding of the Atf1-Pcr1 heterodimer resulted in chromatin remodeling and elevated meiotic recombination (K. Ohta and W. Wahls, personal communication). Recently, Fox *et al.* (52) found that the variant sequences (C/T/G)TGACGT(A/C) can also bind Atf1-Pcr1 and function as meiotic hotspots in *S. pombe*, suggesting another link between binding of transcription activators and meiotic recombination. The equivalent factor in humans, AP-1 (53), has binding sites at a number of the known human recombination hotspots, including MS32 (54) and TAP2 (55). Indeed, hormonal control over transcription factors might possibly account for sex differences in recombination rates and location of crossovers.

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