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Frequencies of mutagen-induced coincident mitotic recombination at unlinked loci in *Saccharomyces cerevisiae*

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Abstract

Frequencies of coincident genetic events were measured in strain D7 of *Saccharomyces cerevisiae*. This diploid strain permits the detection of mitotic gene conversion involving the *trp5-12* and *trp5-27* alleles, mitotic crossing-over and gene conversion leading to the expression of the *ade2-40* and *ade2-119* alleles as red and pink colonies, and reversion of the *ilv1-92* allele. The three genes are on different chromosomes, and one might expect that coincident (simultaneous) genetic alterations at two loci would occur at frequencies predicted by those of the single alterations acting as independent events. Contrary to this expectation, we observed that *ade2* recombinants induced by bleomycin, β-propiolactone, and ultraviolet radiation occur more frequently among *trp5* convertants than among total colonies. This excess among *trp5* recombinants indicates that double recombinants are more common than expected for independent events. No similar enrichment was found among Ilv^+ revertants. The possibility of an artifact in which haploid yeasts that mimic mitotic recombinants are generated by a low frequency of cryptic meiosis has been excluded. Several hypotheses that can explain the elevated incidence of coincident mitotic recombination have been evaluated, but the cause remains uncertain. Most evidence suggests that the excess is ascribable to a subset of the population being in a recombination-prone state.
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Keywords: Mitotic recombination; Gene conversion; Coincident recombination; Bleomycin; β-Propiolactone; Ultraviolet light; Yeast; Simultaneous genetic events

1. Introduction

The yeast *Saccharomyces cerevisiae* is a eukaryotic microorganism that lends itself to the study of allelic recombination, occurring between related sequences on homologous chromosomes in a diploid cell, as well as of ectopic recombination between sites of homology located at any position in either a haploid or diploid cell [1]. Mitotic recombination in a diploid cell may be reciprocal or nonreciprocal [1,2]. Reciprocal recombination (crossing-over) entails the exchange of segments of chromatids between homologous chromosomes, whereas nonreciprocal recombination (gene conversion) is the change of an allele into that on the homologous chromosome by the unidirectional transfer of a DNA sequence between nonsister chromatids [1,3–6]. Gene conversion is responsible for the great majority of events of intragenic recombination [1]. Mechanisms of mitotic recombination have been covered in several excellent reviews [4–6].

Recombinational processes in mitotic cells maintain the integrity of the genome by repairing breaks in DNA and chromosomes and by circumventing the effects of

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chemical adducts in DNA. They are also implicated in the formation of some chromosomal rearrangements [1]. Because it can create homozygosity in a previously heterozygous cell, mitotic recombination is implicated in human somatic cell disease, most notably cancer [7–9]. The principal initiating lesion for mitotic recombination is a double-strand break in DNA [1,8]. Single-strand breaks also induce recombination, but they are thought to be processed into double-strand breaks before being subject to the enzymes that complete the process [1,8]. Other DNA structures that can trigger mitotic recombination apparently include single-stranded regions arising at sites of stalled replication [10]. Many chemical mutagens and radiation have been shown to induce both reciprocal and nonreciprocal mitotic recombination in yeast [11]. DNA damage may induce recombination by providing additional substrates (i.e., lesions) for the initiation of the recombinational process or by activating genes encoding enzymes required for recombination [1]. Split-dose experiments have failed to provide clear evidence for the latter, in that most results have been consistent with additivity. Even though more evidence supports the former mechanism than the latter, the two modes of induction need not be mutually exclusive [1].

The diploid strain D7 of *S. cerevisiae* was constructed by F.K. Zimmermann for the efficient detection of mitotic gene conversion at the *trp5* locus, mitotic crossing over and gene conversion at *ade2*, and point mutations causing reversion of the *ilv1-92* allele [3,12,13]. The genotype of D7 is shown in Table 1. D7 is heteroallelic at *trp5* (*trp5-12*/*trp5-27*), requires tryptophan supplementation for growth, and gives rise to tryptophan-independent (Trp^+) recombinants by mitotic gene conversion [12]. The *ade2-40* and *ade2-119* alleles exhibit interallelic complementation, making D7 an adenine prototroph that produces typical white colonies. Haploid or homozygous yeasts that express *ade2-40* produce red colonies, resulting from a blockage in the adenine pathway, whereas expression of the leaky *ade2-119* allele causes a pink phenotype. Mitotic crossing-over between the centromere and the *ade2* locus gives rise to sectored pink and red twin-spot colonies [3]. Gene conversion at *ade2* can produce red colonies, pink colonies, and red/white or pink/white sectored colonies, but not red/pink twin spots. A small fraction of red or pink altered colonies may arise from other genetic events, including point mutation, deletion, and chromosome loss, but these are minor contributors to the total following exposure to recombinagens. The *ilv1-92* locus is homozygous and permits the detection of mutations by selecting for isoleucine prototrophy (Ilv^+). Revertants arise by true back mutation and suppressor mutations [3,14]. The D7 assay is well characterized and responsive to diverse mutagens and carcinogens [11].

One would expect mutations or recombinational events involving unlinked genes to be independent of one another, such that the frequency of a genetic event involving one gene is not influenced by that involving other genes, other than as reflected in rates of mutation or recombination for the organism as a whole. Thus, if mutation occurs in gene A at a frequency of 10^{-6} and in gene B at 10^{-7}, one would expect the frequency of coincident mutation in both A and B to be 10^{-13}. The mutagenesis literature contains few analyses of coincident genetic events, perhaps because they occur at low frequencies and their measurement is prone to artifacts that can mimic the rare events of interest. Yeast strain D7 is well suited to investigating this subject, in that the indicator genes *trp5*, *ilv1* and *ade2* are on different chromosomes, and the spontaneous frequencies of the recombinational events are at least 100 times higher than typical mutation frequencies. Moreover, mutagen treatments can substantially increase the recombinant and revertant frequencies, making it possible to detect coincident genetic events at easily measurable frequencies.

Most of our experiments were conducted with bleomycin (BLM), which is a potent mutagen and recombinagen in strain D7. BLM is a glycopeptide

Table 1
Strains of *Saccharomyces cerevisiae*

Strain	Alternative designation	Source	Genotype
D7	–	[a]	MATa/α *ade2-40/ade2-119 trp5-12/trp5-27 ilv1-92/ilv1-92*
FZ1	ITO-3A	[a]	MATa *ade2-119 trp5-b ilv1-92 MAL SUC*
FZ2	ITO-6C	[a]	MATα *ade2-40 trp5-a ilv1-92 MAL SUC*
STX20-1C	ATCC#208008	[b]	MATa *lys2 gal2*
P49	ATCC#208009	[b]	MATα *lys2 gal2*
X2144-S19	ATCC#204590	[b]	MATa *met13 leu1 trp5-48 cyh2 aro2 lys5 ade5 gal*
X2144-S22	ATCC#204591	[b]	MATα *met13 leu1 trp5-48 cyh2 aro2 lys5 ade5 gal*

[a] F.K. Zimmermann, Technische Hochschule, Darmstadt, Germany.
[b] American Type Culture Collection/Yeast Genetic Stock Center (http://www.atcc.org).

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