EFFECTS OF GLUCOSE REPRESSION ON THE TRANSMISSION AND RECOMBINATION OF MITOCHONDRIAL GENES IN YEAST (*SACCHAROMYCES CEREVISIAE*)

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ABSTRACT

Matings of a number of *Saccharomyces cerevisiae* stocks give different output ratios of mitochondrial genotypes depending on whether the cells are glucose-repressed or derepressed. The effects of glucose repression are independent of cellular mating type and mitochondrial genotype, and take place at least in part after zygotes are formed. An explanation is proposed in terms of changes in the relative numbers of mitochondrial DNA molecules contributed by the **a** and α parents, modified by selective replication or destruction of molecules inside the zygote.

THE most useful and versatile organism now available for the study of the transmission genetics of mitochondria is the yeast *Saccharomyces cerevisiae* (reviewed by BIRKY 1975). These facultative anaerobes are able to obtain sufficient energy for growth and reproduction by the fermentation of sugars such as glucose or galactose, under which conditions the mitochondrial functions of protein synthesis, electron transport, and oxidative phosphorylation are dispensable. These same mitochondrial functions are indispensable when the yeast are grown on nonfermentable carbon sources such as ethanol or glycerol. When glucose is present in the culture medium, respiration is not only dispensable but is repressed to low levels. This phenomenon, called catabolite repression, glucose repression, the Crabtree effect, or the inverse Pasteur effect, was first described clearly by SLONIMSKI (1955) and by EPHRUSSI *et al.* (1956), and has since been the subject of numerous investigations (see PERLMAN and MAHLER 1974 for pertinent references). Briefly, repressed cells show, relative to derepressed cells, (i) a greatly reduced activity of mitochondrial enzymes involved in electron transport, correlated with reduced oxygen uptake; (ii) a reduction in number of mitochondrial DNA molecules per cell (in some strains); and (iii) a smaller number of mitochondria which are larger and more highly branched (in some strains).

It seems likely that some or all of these changes in mitochondrial and cell physiology will affect the inheritance of mitochondrial genes. Yeast crosses begin with the mating of haploid cells each of which contains a large number of mitochondrial genophores; the resulting zygote likewise contains multiple copies of the mitochondrial genome and will be heteroplasmic (cf., heterozygous) for

mitochondrial genes for which the parents carried different alleles. Within ten generations or less, the vegetative segregation of mitochondrial genophores results in the diploid progeny being homoplasmic (cf., homozygous) at all mitochondrial gene loci (THOMAS and WILKIE 1968a; LINNANE *et al.* 1968). The percentages of homoplasmic diploid cells of different genotypes, called output ratios, are the parameters studied for clues to the events and mechanisms of mitochondrial transmission genetics. Such studies show that mitochondrial genes undergo recombination in heteroplasmic cells (THOMAS and WILKIE 1968b). Other phenomena seen in output ratios include bias (the preferential transmission of mitochondrial genes from one parent; AVNER *et al.* 1973); polarity (the preferential recovery of the ω^+ mitochondrial allele and of genes linked to it; BOLOTIN *et al.* 1971); and zygote heterogeneity (different zygotes from the same cross produce very different output ratios—COEN *et al.* (1970)—and may fall into several discrete classes, including uniparental classes—BIRKY (1975) and unpublished data).

The relevant subcellular phenomena and parameters which may affect these output ratios include (DUJON, SLONIMSKI and WEILL 1974; BIRKY 1975) the relative numbers of mitochondrial genophores of different genotypes in zygotes; the statistics of "mating" and recombination (reciprocal and nonreciprocal) in the population of genophores in heteroplasmic cells; the packaging of genophores into organelles; relative rates of replication of mitochondrial DNA molecules of different genotypes and from different parents; and possibly the enzymatic destruction of some molecules. In order to compare experimental results, it is essential to know whether the output ratios of mitochondrial genetic crosses are influenced by the carbon source used for growth or by the stage in the growth cycle of the cells. Moreover, glucose repression can be used to test the effects, on output ratios, of modifying some of the subcellular parameters (GOLDTHWAITE, CRYER and MARMUR 1974). In this paper I show that glucose repression modifies the direction and extent of bias and polarity and the types of zygote classes and that these effects cannot be completely explained by changes in the input ratios of mitochondrial genomes.

MATERIALS AND METHODS

Mitochondrial genes: Mitochondrial genes conferring resistance to chloramphenicol, erythromycin, or oligomycin are designated C^R, E^R, or O^R respectively; the wild-type alleles conferring sensitivity to these antibiotics are C^S, E^S, and O^S. Subscripts identify specific mutations. The C^R and E^R mutants used here represent the R_I and R_{III} loci of DUJON, SLONIMSKI and WEILL (1974), and are separated by about ten map units. The C^R mutants are very closely linked to the ω locus (BOLOTIN *et al.* 1971). The O_I mutant used here is unlinked in the sense of AVNER *et al.* (1973), i.e., is separated from both R_I and R_{III} by about 20% recombination.

Yeast strains: Saccharomyces cerevisiae or stocks derived from hybridization of *S. cerevisiae* with *S. carlsbergensis* (P. P. SLONIMSKI, personal communication) are used in these studies. Table 1 contains a list of strains, with their genotypes and sources.

Media: MMD, MMG, MME, and MMGal are minimal media after WICKERHAM (1946) but with 1 mg $CaCl_2$/liter, and contain 1% glucose (dextrose), 4% glycerol, 4% ethanol, or 2% galactose, respectively, as carbon sources. SSD, SSG, and SSGal are semisynthetic complete media identical to MMD etc., but with the addition of 0.2% Difco yeast extract and 75 mg/ml

TABLE 1

Strains used

Strain	Genotype: Nuclear	Genotype: Mitochondrial	Abbreviation	Source
D22–2(2)–3	**a** *ade-2*	$\omega+C^{S}E^{R}{}_{2}O^{S}$	D22	E^{R} spont. mut. in D22 from D. WILKIE
D6–2(16)–3	*α arg met*	$\omega+C^{S}E^{R}{}_{16}O^{S}$	D6	E^{R} spont. mut. in D6 from D. Y. THOMAS
DP1–1B/517–2	*α his try*	$\omega+C^{R}{}_{517}E^{S}O^{S}$	DP1	P. P. SLONIMSKI
55R5–3C/221–2	**a** *ura*	$\omega-C^{S}E^{R}{}_{221}O^{S}$	55R5	P. P. SLONIMSKI
IL16–10B–2	*α his*	$\omega-C^{R}{}_{321}E^{S}O^{S}$	IL16	P. P. SLONIMSKI
IL126–1B–2	*α his*	$\omega-C^{R}{}_{321}E^{S}O^{S}$	IL126	P. P. SLONIMSKI
D243–4a–1	**a** *ade-1 lys*	$\omega+{}^{S}E^{S}O^{R}{}_{4}$	D243	R. CRIDDLE
2–36–1	*α try-1*	$\omega+C^{R}E^{R}O^{S}P^{R}$	2–36	R. KLEESE
N123	**a** *his*	$\omega-C^{S}E^{S}O^{S}P^{S}$	N123	E. MOUSTACCHI
N123UVSrho5	**a** *his rho5*	$\omega-C^{S}E^{S}O^{S}P^{S}$	rho5	E. MOUSTACCHI
6–2/7	**a** *ade-1*	$\omega+C^{R}{}_{321}E^{S}O^{R}{}_{4}P^{S}$	6–2/7	P. S. PERLMAN (spore from D243 × IL458)
1–2/3	**a** *ade-1*	$\omega-C^{R}{}_{321}E^{R}O^{R}{}_{4}P^{S}$	1–2/3	P. S. PERLMAN (spore from D243 × IL126)
1–1/1	*α ade-1*	$\omega-C^{R}{}_{321}E^{S}O^{R}{}_{4}P^{S}$	1–1/1	P. S. PERLMAN (spore from D243 × IL126)

each of arginine, histidine, isoleucine, leucine, lysine, methionine, tryptophan, adenine, and uracil. RD, etc., are similar complete media described by JAYARAMAN *et al.* (1966). YEPD, etc. contain 1% Difco yeast extract, 2% Difco proteose peptone, and carbon sources as above except that glucose is present at 2%. Antibiotics were added to agar media (containing 2% agar) at about 50°, as follows: chloramphenicol, 3 mg/ml; erythromycin, 1 mg/ml; oligomycin, 2 μg/ml. All experiments were done at 30°.

Physiological states: Logarithmic phase cultures were grown on complete medium with shaking for at least six generations at an O.D. (A_{600}) of 1.0 or less, and usually mated at $A_{600} = 0.5$ (approximately 10^{7} cells/ml). When glucose is the carbon source, such cells are substantially repressed; when glycerol, ethanol, or galactose is the carbon source, they are derepressed (stationary phase glucose cultures are actually using ethanol as their carbon source) (PERLMAN and MAHLER 1974). Stationary phase cultures are derepressed on all media; lag phase cells are repressing (PERLMAN and MAHLER 1974), and were obtained by transferring stationary phase cells to fresh medium and shaking for two hours. In most experiments the same carbon source was used for growing both parent cultures and in the mating and prototroph selection media. In some experiments, one parent was grown on glucose to log phase (repressed) and the other on glycerol or galactose to log phase (derepressed). Two mating mixtures were prepared; one was mated in glucose and plated on glucose (repressed); the other was mated in glycerol or galactose and plated on medium with the same carbon source (derepressed).

Mating and zygote growth: Mating mixtures contained about 10^{7} cells of each auxotrophic haploid parent per ml of complete medium. In some early experiments, the mating mixture was pelleted and permitted to mate for ½–1 hr, then resuspended and shaken for 1½ hr; in most experiments the mixture was simply shaken for 2 or 4 hours. Cells were then washed in distilled water or minimal medium and several dilutions were plated on minimal medium, with appropriate carbon sources, to select prototrophic zygotes .About 10^{5} cells of each parent culture were plated on MMD as controls to detect prototroph revertants; if enough were present to affect the results, the experiment was discontinued. Several hundred cells from each parent culture were also plated on YEPD or RD and the resulting colonies covered with soft agar containing triphenyl

tetrazolium chloride to determine the percent ρ^- cells in the culture. Such cells often lack one or more mitochondrial genes and variations in their frequencies could affect the results of crosses.

Output ratios: For *total progeny* analyses, all cells were washed off of prototroph selection plates containing several hundred or more zygotes; the cell suspensions, in 0.01 or 0.1 M EDTA, were diluted and about 100 cells were plated on MMD to make replica master plates. After incubation, these plates were replica-plated onto YEPD or RG plus appropriate antibiotics to determine the mitochondrial genotypes of the colonies, and hence of the diploid cells taken from the prototroph selection plates. For *zygote colony* analyses, 30 to 100 individual zygote colonies were picked off prototroph selection plates, suspended in EDTA, and the output ratios determined by replica-plating as above.

RESULTS

Effects on bias in homopolar crosses

Homopolar crosses (called homosexual by BOLOTIN *et al.* 1971) are those in which both parents are ω^+ or both are ω^-; the ω locus plays no role in determining the outcome of such crosses. Table 2 summarizes the results of one-factor and two-factor homopolar crosses in which both parents were either repressed or derepressed. Table 3 gives a more detailed analysis of one such cross.

The inequality of the reciprocal recombinant classes in Table 3 is typical of the D22 × DP1 cross and can probably be ascribed to selection favoring the C^SE^S

TABLE 2

Effects of glucose repression on output ratios in homopolar crosses

Exp't. No.	Strains (a × α)	Mating genotype	Carbon* source	Output ratios (%)			
				C^SE^R	C^RE^S	C^SE^S	C^RE^R
P8a	D22 × DP1	$\omega^+C^SE^R \times \omega^+C^RE^S$	D	46.6	44.0	7.6	1.8
			Gal	33.9	55.6	6.5	4.1†
			G	27.6	66.8	3.2	2.4†
P11b	55R5 × IL16	$\omega^-C^SE^R \times \omega^-C^RE^S$	D	39.0	50.7	6.7	3.7
			Gal	54.6	36.0	6.7	2.8†
			G	52.5	33.4	13.0	1.1†
P13b	6-2/7 × D6	$\omega^+C^RE^S \times \omega^+C^SE^R$	D	66.7	22.5	3.4	7.5
			G	35.0	45.7	3.5	15.7†
P12	D243 × 2-36	$\omega^+C^SE^S \times \omega^+C^RE^R$	D	4.0	5.0	31.0	60.2
			G	5.2	5.5	59.8	29.4†
				C^SO^R	C^RO^S	C^SO^S	C^RO^R
P23	N123 × 1-1/1	$\omega^-C^SO^S \times \omega^-C^RO^R$	D	5.1	5.9	29.3	59.8
			G	5.1	9.7	38.1	47.1†
			E	5.1	7.3	41.9	45.8†
P23	rho5 × 1-1/1	$\omega^-C^SO^S \times \omega^-C^RO^R$	D	2.8	5.0	54.6	37.6
			G	5.5	3.5	74.7	16.3†
			E	3.6	5.0	76.0	15.4†

* D = glucose; Gal = galactose; G = glycerol; E = ethanol. All cultures were in logarithmic growth phase as defined in MATERIALS AND METHODS.

† Significantly different from repressed cells at 5% level.

TABLE 3

Details of effects of glucose repression on output ratios in the homopolar cross $\omega^+C^SE^R \times \omega^+C^RE^S$ *(D22 × DP1, Expt. P8a)*

	Output ratios (%+S.E.)							
Carbon source*	C^SE^R	C^RE^S	C^SE^S	C^RE^R	C^S	E^R	$\frac{C^SE^R}{C^RE^S}$	Percent recomb.
Glucose	46.6±2.2	44.0±2.2	7.6±1.2	1.8±0.6	54.2	48.4	1.05	9.4
Galactose	33.9±1.7	55.6±1.8	6.5±0.9	4.1±0.7	40.4	38.0	0.61	10.6
Glycerol	27.6±1.8	66.8±1.0	3.2±0.7	2.4±0.6	30.8	30.0	0.41	5.6

* The log-phase cultures of D22 and DP1 contained, respectively, 1.0% and 14% ρ^- cells in glucose, 0.5% and 5.6% ρ^- in galactose, and no ρ^- in glycerol. These differences are insufficient to account for the differences in output ratios, even if one assumes that all ρ^- cells lack *C* and *E* mitochondrial genes.

phenotype over the C^RE^R phenotype. Reciprocal recombinants are more nearly equal in some other homopolar crosses (D243 × 2–36), and the degree of inequality shows no clear relationship to glucose repression; the latter is somewhat surprising, since one would expect selection for mitochondrial phenotypes to be less effective under conditions where mitochondrial gene action appears to be dispensable and at least partly repressed. The data for D22 × DP1 also suggest an effect of the carbon source used in the experiment on the total frequency of recombination: recombination is higher on glucose or galactose than on glycerol (or glucose in lag phase). This effect is seen only in the D22 × DP1 cross; averaging the recombination frequencies of all homopolar crosses gives 10.5% or 10.6% recombination when the carbon source is glucose or glycerol, respectively.

The most striking effect of glucose repression is on bias. Bias is seen as a deviation of the ratio of parental genotypes from 1, and as a deviation of the transmission of individual alleles from 50%. The cross shown in Table 3 was essentially unbiased when glucose was used as the carbon source, but was biased in favor of the transmission of markers from the DP1 parent when the carbon source was galactose or glycerol. In all cases the transmissional bias is in the same direction for the *C* and *E* genes and the effect of glucose repression is similar, as expected for these closely-linked loci. Each of these crosses has been repeated one or more times; the bias varies considerably between repetitions, but in every case the effect of changes in the carbon source is in the same direction (BIRKY, unpublished data; CHOU 1973). Moreover, although galactose and glycerol do not always give the same output ratios, whenever galactose shifts bias relative to glucose it is in the same direction as glycerol. Although galactose and glucose are both fermentable carbon sources, galactose does not repress mitochondrial functions to nearly the extent that glucose does. This indicates that a major effect of using different carbon sources depends upon differences in repression. For example (Table 2) in the D22 × DP1 cross, glucose favors the transmission of mitochondrial genes from D22, while galactose and glycerol favor DP1 genes. Ethanol acts like glycerol (experiment P23). This is also seen in experiment P1

(Table 4) which includes stationary phase glucose cultures in which the chief carbon source is ethanol produced by fermentation during the preceding log phase; these derepressed cultures are biased in the same direction as those grown in glycerol (experiment P1, etc., Table 4).

Glucose repression in different crosses favors different mitochondrial genotypes (C^SE^R in D22 × DP1, C^RE^S in 55R5 × IL16, C^RE^R in D243 × D6); it also favors mitochondrial genes from parents of different mating types (e.g., **a** in D22 × DP1, α in 55R5 × IL16). Repression effects are seen in both $\omega^+ \times \omega^+$ and $\omega^- \times \omega^-$ crosses involving stocks from several different sources.

Effects on transmission in individual zygotes

The effects of glucose repression on bias were seen above in total progeny analyses, in which the progeny of many zygotes are pooled, and the results represent the behavior of the "average" zygote. Figure 1 shows the transmission of alleles at the *C*, *E*, and *O* loci for sets of 50 individual zygotes grown, mated, and plated in glucose or glycerol; the total progeny analysis of this same cross is

TABLE 4

Effects of glucose repression on output ratios in heteropolar crosses

Exp't.	Strains $\omega^+ \times \omega^-$	Mating genotype	Carbon* source	Growth‡ phase	Output ratios (%)					
					C^SE^R	C^RE^S	C^SE^S	C^RE^R	C^S	E^R
P1	D22 × IL16	$\mathbf{a}C^SE^R \times \alpha C^RE^S$	D	log	62.5	3.6	33.9	0	66.1	62.5
			D	lag	50.5	4.0	45.5	0	54.5	50.5
			D	stat	50.9	12.1	37.1	0	63.0	50.9†
P9	D22 × IL16	$\mathbf{a}C^SE^R \times \alpha C^RE^S$	D	log	77.3	1.7	21.0	0	98.3	77.3
			Gal	log	48.0	11.8	40.2	0	88.2	48.0†
			G	log	51.9	4.8	43.3	0	95.2	51.9†
P13	D6 × 1-2/3	$\alpha C^SE^R \times \mathbf{a}C^RE^S$	D	log	84.1	0	16.0	0	100	84.0
			G	log	67.2	3.4	27.6	0	94.8	69.0†
					C^RE^S	C^SE^R	C^RE^R	C^SE^S	C^R	E^S
P11	DP1 × 55R5	$\alpha C^RE^S \times \mathbf{a}C^SE^R$	D	log	72.4	9.8	16.5	1.2	88.9	73.6
			Gal	log	75.3	7.6	14.5	2.6	89.8	77.9
					C^SE^S	C^RE^R	C^SE^R	C^RE^S	C^S	E^S
P12	D243 × IL126	$\mathbf{a}C^SE^S \times \alpha C^RE^R$	D	log	47.0	21.6	30.8	0.6	77.8	47.6
			Gal	log	60.7	1.8	37.5	0	98.2	60.7†
					C^RE^R	C^SE^S	C^RE^S	C^SE^R	C^R	E^R
P12	2-36 × N123	$\alpha C^RE^R \times \mathbf{a}C^SE^S$	D	log	82.1	1.5	16.0	0.4	98.1	82.5
			Gal	log	79.7	7.3	12.9	0.1	92.6	79.8
			G	log	81.9	2.3	15.6	0.3	97.5	82.2

* D = glucose; Gal = galactose; G = glycerol.
† Significantly different from repressed cells at 5% level.
‡ Log, lag and stat refer to logarithmic, lag and stationary phase cells as defined in MATERIALS AND METHODS.

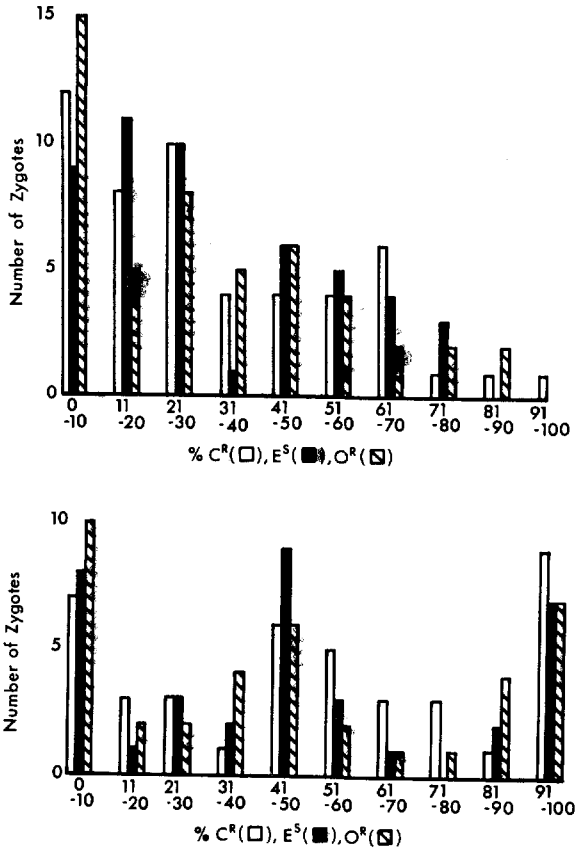

FIGURE 1.—Transmission frequencies of mitochondrial genes in the progeny of 50 individual zygotes from the cross $\omega^+C^SE^RO^S \times \omega^+C^RE^SO^R$ (D6 × 6-2/7). Bars represent the number of zygotes transmitting the C^R (open bar), E^S (solid bar), or O^R (hatched bar) alleles from 6-2/7 to various percentages of their diploid progeny, in intervals of 10%. Upper graph: experiment done with glucose as carbon source (cells repressed). Lower graph: glycerol carbon source (cells derepressed).

shown in Table 2 (experiment P13b) where it is treated as a two-factor cross for purposes of comparison with other matings. It is evident that three classes of zygotes are formed in this cross under derepressed conditions: one class transmits mitochondrial genes from the D6 parent preferentially; one class preferentially transmits genes from the 6-2/7 parent; and the third class transmits genes from both parents about equally well. When, however, the same cross was done with repressed cells, the class transmitting genes preferentially from 6-2/7 is no longer evident, and has been replaced by two classes showing moderate degrees of bias in favor of D6.

The effect of glucose repression on individual zygote classes is seen even more strikingly in Figure 2, with data from experiment P23 involving the mutant N123 *rho5*. Under repression, there are no distinct classes of zygotes with respect

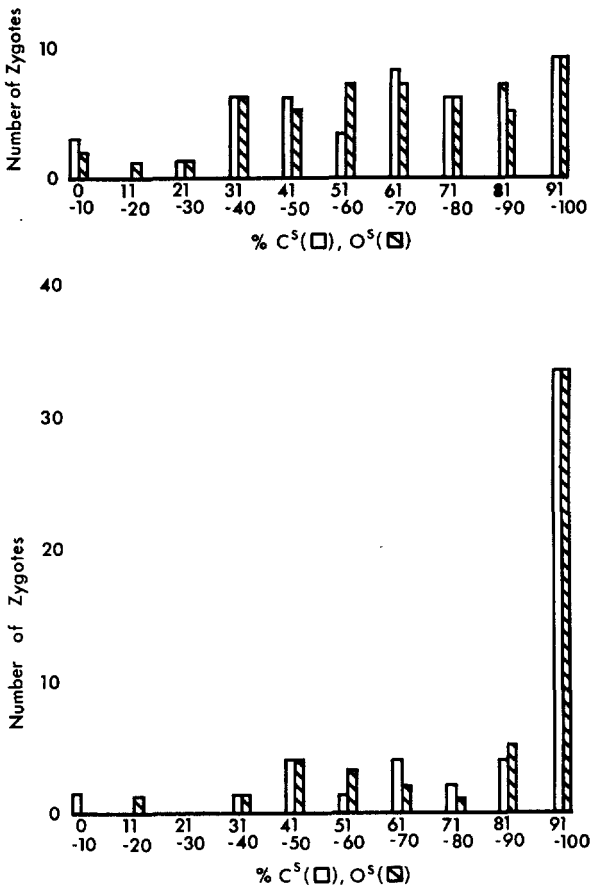

FIGURE 2.—Transmission frequencies of mitochondrial genes in the progeny of 50 individual zygotes from the cross $\omega^{-}C^{S}O^{S} \times \omega^{-}C^{R}O^{R}$ (*rho5* × 1-1/1). Bars represent the number of zygotes transmitting the C^{S} (open bar) or O^{S} (hatched bar) alleles from the N123*rho5* parent to various percentages of their diploid progeny, in intervals of 10%. Upper graph: experiment done with glucose as carbon source (cells repressed). Lower graph: glycerol carbon source (cells derepressed).

to mitochondrial gene transmission: there appears to be a single population of zygotes with high variance and moderate bias in favor of the 1-1/1 parent. But when the cells are derepressed, there is a striking shift toward transmission of genes from *rho5*; moreover, about half of the zygotes (22 out of 50) are *uniparental*, transmitting *only* mitochondrial genes from the *rho5* parent. Eight of these uniparental zygotes were tested further to determine whether they were strictly uniparental or merely very strongly biased. About 10^5 cells from each zygote colony were pipetted onto medium containing chloramphenicol or erythromycin; no cells grew, showing that these zygotes transmitted mitochondrial genes from 1-1/1 to less than 0.001% of their progeny. These phenomena will be described in more detail elsewhere (BIRKY, manuscript in preparation).

Effects on polarity and bias in heteropolar crosses

Heteropolar crosses are those in which the parental genophores differ at the *omega* mitochondrial locus: $\omega^+ \times \omega^-$. Among the progeny of such zygotes there is a strong preferential recovery of the ω^+ allele and of the *C* allele linked to it (C^+); a weaker preferential recovery of the *E* allele (E^+) linked to ω^+; but no effect on "unlinked" loci such as *O*. Consequently the reciprocal recombinants are always highly unequal, with $C^+E^- > C^-E^+$; also the parentals are generally unequal, with $C^+E^+ > C^-E^-$ (BOLOTIN *et al.* 1971). The effects of glucose repression on such crosses are shown in Tables 4 and 5.

Glucose repression modifies bias in heteropolar as well as in homopolar crosses. This can be seen in the three-factor cross in Table 5, where the non-polar *O* locus shows essentially no transmissional bias in derepressed cells but a strong bias toward the D6 parent in glucose-repressed cells. This is paralleled by an increased transmission of the E^R, and to a lesser extent of the C^S, alleles from D6. When the cross is considered as a two-factor cross by ignoring the *O* locus and looking only at the polar *C* and *E* loci, glucose repression appears to have increased the polarity of the cross; the ratio of parental genotypes is more strongly biased in favor of (C^SE^R). At the same time the total recombination frequency appears to have decreased.

Each cross shows a different response to glucose repression; in some (e.g., D22 × IL16) repression favors the ω^+ genome, in others (e.g., D243 × IL126) the ω^- genome, while one (DP1 × 55R5) shows no effect. As in the homopolar crosses, derepression favors mitochondria of different genotypes and from parents of both mating types in different crosses. The D22 × IL16 cross has been performed five times; the effects of glucose repression were in the same direction each time, although the precise output ratios varied.

Effects of glucose repression of only one parent or of zygotes

In the preceding experiments both parents and the zygotes were all kept either repressed or derepressed throughout. Four experiments were also done in which repressed cells were mated with derepressed cells; the results were complicated

TABLE 5

Details of the effects of glucose repression in the heteropolar cross $\omega^+C^SE^RO^S \times \omega^-C^RE^SO^R$ *(D6 × 1-2/3, Exp't. P13)*

	Output ratios (% ± S.E.)							
Carbon source	$C^SE^RO^S$	$C^RE^SO^R$	$C^SE^RO^R$	$C^RE^SO^S$	$C^SE^SO^R$	$C^RE^RO^S$	$C^SE^SO^S$	$C^RE^RO^R$
Glucose	68.1 ±3.7	0	16.0 ±2.9	0	13.5 ±2.7	0	2.5 ±1.2	0
Glycerol	48.6 ±3.5	2.4 ±1.1	18.6 ±2.7	1.0 ±0.7	23.3 ±2.9	0.5 ±0.5	4.3 ±1.4	1.4 ±0.8

	C^S	E^R	O^S	C^SE^R	C^RE^S	C^SE^S	C^RE^R
Glucose	100	84.0	70.6	84.0	0	16.0	0
Glycerol	94.8	69.0	54.3	67.1	3.3	27.6	1.9

TABLE 6

*Effect on output ratios of glucose repression in zygotes**

Exp't.	Strains a × α	Mating genotype	Carbon source a	Carbon source α	Carbon source Zygotes	Output ratios (%) C^S	Output ratios (%) E^R
P9	D22 × DP1	$\omega^+C^SE^R \times \omega^+C^RE^S$	D	D	D	73.2	71.5
			D	D	G	58.1	53.0†
			G	G	D	59.1	54.0
			G	G	G	50.3	46.2
	D22 × IL16	$\omega^+C^SE^R \times \omega^-C^RE^S$	D	D	D	98.3	77.3
			D	D	G	96.4	77.4
			D	D	Gal	79.3	36.9†
			Gal	Gal	D	95.9	70.2
			Gal	Gal	Gal	88.2	48.0†
			G	G	D	94.7	61.7
			G	G	G	95.2	51.9
P11	55R5 × IL16	$\omega^-C^SE^R \times \omega^-C^RE^S$	G	G	D	61.3	56.9
			G	G	G	65.5	53.6
						C^S	E^S
P12	N123 × 2–36	$\omega^-C^SE^S \times \omega^+C^RE^R$	D	D	D	1.9	17.5
			D	D	G	0.9	9.2
			G	G	D	9.1	27.1
			G	G	G	2.6	17.9†

* Both parents were grown to log phase on glucose (D), galactose (Gal), or glycerol (G), then mated in medium with the same or a different carbon source. Zygotes were grown on plates with the carbon source used for mating.

† Output ratio is significantly different at the 5% level from that obtained when zygotes were grown in glucose.

and show no clear pattern which might be useful in interpreting the effects of repression on mitochondrial genetics.

To determine whether repression acts on parents or zygotes, three experiments were performed in which both parents were either repressed or derepressed, but the mating was done, and the zygotes reared, under the opposite conditions. The results of these experiments are summarized in Table 6 as a series of pairwise comparisons. In four cases the repression of the zygotes alone significantly modified the output ratios. It thus appears that glucose repression can act upon events taking place in the zygote and its progeny to modify output ratios without necessarily affecting parameters in the parents. (An important *caveat* must be added to this interpretation: it is possible that glucose repression in these experiments is acting very rapidly—in less than two hours—on the parent cells *after* the mating mixture is formed but before the cells fuse to form zygotes, *or* that a significant amount of mating is taking place on the prototroph selection plates).

DISCUSSION

The results of these experiments indicate that both bias and polarity can be modified by glucose repression. The phenomena of polarity and bias can both be

viewed as the preferential recovery of mitochondrial genomes in the progeny of zygotes: preferential recovery of the mitochondrial allele ω^+ and linked genes in polarity; preferential recovery of genomes from a participular parental strain in bias. Possible mechanisms for such preferential recovery include selection and input effects. These are discussed below.

Selection

The existence of selection is suggested by the unequal recovery of reciprocal recombinants in crosses of strain D22 by DP1. This selection might be for cellular phenotypes (intercellular selection), or for mitochondrial phenotypes within cells (intracellular selection) as has been demonstrated in yeast by BIRKY (1973 and unpublished) and in paramecium by ADOUTTE and BEISSON (1972). Selection of either sort is unlikely to explain the repression effects because different genotypes are being selected in different experiments. This argument is not conclusive, since these crosses involve different C^R alleles and different nuclear genotypes, but it would be surprising if these features could cause a complete reversal in the direction of selection.

Input effects

The most parsimonious approach to understanding repression effects on polarity and bias is to seek a common mechanism for both; such a common mechanism might be an effect on input ratios. A useful paradigm for the rationalization of mitochondrial transmission genetics has been an analogy to the phage-infected bacterial cell (WOLF, DUJON and SLONIMSKI 1973; AVNER *et al.* 1973; DUJON, SLONIMSKI and WEILL 1974; LINNANE, HOWELL and LUKINS 1974; BIRKY 1975) as described by VISCONTI and DELBRUCK (1953). In a phage-analogy model it is postulated that the yeast zygote contains a pool of mitochondrial DNA molecules, *m* from one parent and *n* from the other. Due to the frequent fusions of mitochondria in the zygote and its heteroplasmic buds, the mitochondrial genophores are able to pair and recombine (mate) in both homologous and heterologous combinations; this mating may be random or nearly so with respect to genotype (WOLF, DUJON and SLONIMSKI 1973; DUJON, SLONIMSKI and WEILL 1974) and there may be multiple rounds of mating (DUJON, SLONIMSKI and WEILL 1974). The result (if recombination is formally, i.e. statistically, reciprocal) will be the production of equal numbers of recombinants, while parental genotypes and pairs of alleles will remain in the ratio of *m*:*n*. These same ratios will appear in the output percentages of homoplasmic diploid cells, providing all genotypes of cells and mitochondria replicate at equal rates.

Direct evidence for such effects of input on output has been obtained in several ways. PERLMAN and DEMKO (1974) and YOUNG (personal communication) have mated haploid cells with either haploid or diploid cells; the latter contain about twice as many mitochondrial DNA molecules. PERLMAN and DEMKO (1974) have also used cycloheximide to increase the mitochondrial DNA content of cells prior to mating. Finally, GOLDTHWAITE, CRYER and MARMUR (1974) have mated glucose-repressed with derepressed cells, using strains in which repression results in a drastic decrease in mitochondrial DNA content. In every case the change in

input ratios of mitochondrial DNA molecules has been accompanied by a change in output ratios of homoplasmic diploid cells; the changes are in the expected directions, but not always of the magnitude expected if input bias were the *sole* determinant of output bias.

In most of my experiments, *both* parents were either repressed or derepressed. If effects of repression on bias were to be explained by changes in mitochondrial DNA content, one would have to postulate that some stocks showed a larger decrease in mitochondrial DNA than others, upon repression. For example, in the mating D22 × DP1, repression favors the recovery of D22 markers. This would require that repression cause a more extreme decrease in the mitochondrial DNA content of D22 than of DP1, so that in matings of repressed cells the input ratios would be biased in favor of DP1, while in derepressed cells the input ratios would be unbiased or biased in favor of D22. Studies on the effects of repression on mitochondrial DNA content do, in fact, suggest that different strains differ in this respect; at the extremes, GOLDTHWAITE, CRYER and MARMUR (1974) found an approximately threefold decrease upon derepression in the one stock studied quantitatively, while GRIMES, MAHLER and PERLMAN (1974) found no increase in another haploid stock and its isogenic diploid.

On this hypothesis, if repression favors the transmission of markers from stock A in a mating of $A \times B$, and of stock B in a mating of $B \times C$, then it must also favor A in an $A \times C$ mating. This transitive relationship is satisfied by my data, in which repression favors D22 over IL16, IL16 over 55R5, 55R5 over DP1, and D22 over DP1 in separate experiments.

DUJON, SLONIMSKI and WEILL (1974) have developed a mathematical treatment of the phage-analogy model, in which output ratios are related to input ratios, number of rounds of mating, and recombination frequencies in homopolar crosses. They postulate that polarity is due to asymmetrical gene conversion and incorporate this additional factor into formulae for predicting the output ratios of heteropolar crosses. From their graphs, and from the corresponding data on UV-irradiated crosses in BOLOTIN *et al.* (1971), it appears that an increase in the input of the ω^- parent relative to the ω^+ parent results in (i) an increase in the output of the ω^- parental genotype; (ii) a decrease in the output of the ω^+ parental genotype; (iii) an increase in the major (C^+E^-) recombinant (C allele from ω^+, E allele from ω^-); (iv) a large increase in the output of the E^- allele; and (v) a smaller increase in the output of the C^- allele and a larger increase in the O^- output. Over a wide range of input ratios the rare C^-E^+ recombinant will remain nearly zero and effects on this genotype will be difficult to detect. All of these features are seen in comparing the glucose-repressed and derepressed crosses in my data.

Two lines of evidence, on the other hand, indicate that effects of repression on the mitochondrial DNA content of parent stocks are not sufficient to account for the changes in bias and polarity.

1) CHOU (1973) has measured mitochondrial DNA levels in repressed and derepressed cells of stock DP1 and found less than a twofold difference. But the genetic data would require differences of at least 2.5-fold (experiment P8) to 3.2-fold (experiments P7 and P9).

2) In the cross N123 rho5 × 1-1/1, derepression results in the appearance of many uniparental zygotes which completely fail to transmit mitochondrial genes from the 1-1/1 parent; this behavior cannot be explained by input ratios.

The input ratios as defined in the DUJON model are the ratios of *genetically functional* mitochondrial genophores in the early zygote. These ratios are not necessarily the same as the ratios of molecules in the parental haploid stocks, which ratios could be modified in the zygote in either of two ways: (i) by preferential replication of molecules from one parent or the other, as seen by ANDERSON (1968), BRÅTEN (1973) and SAGER (1972, but *cf.* CHIANG 1971); (ii) by preferential enzymatic destruction of molecules from one parent or the other.

Preferential replication of mitochondrial DNA has been postulated to explain zygote heterogeneity in yeast (BIRKY 1975). A possible mechanism for this is suggested by STRAUSBERG and PERLMAN (1974 and personal communication), who showed that yeast mitochondria from the two parents mix rather slowly in the zygote, as result of which first buds formed at one end of the zygote tend to include mitochondria primarily from one parent; such buds produce most of the resulting zygote colony, or all of it if only a few buds are produced before zygotes die. The only direct evidence relevant to selective destruction or replication of mitochondrial DNA in zygotes comes from the studies of SENA (personal communication; SENA *et al.* 1973); in the cross she studied, the first bud contains less mitochondrial DNA than the zygote, which indicates that not all of the DNA is replicated.

Another variable which may be important in glucose repression effects is the packaging of mitochondrial DNA into mitochondria. Serial electron micrographs by GRIMES, MAHLER and PERLMAN (1974 and personal communication) have shown that diploid repressed and derepressed cells contain the same amount of mitochondrial DNA packaged into about 4–5 organelles in repressed cells or about 22 organelles in derepressed cells. Similar results have been obtained by C. DAMSKY (personal communication) and STEVENS (1974), although HOFFMAN and AVERS (1973) have studied another strain in which there is only a single large, branched mitochondrion under repressing or derepressing conditions.

These experiments emphasize the importance of carefully controlling and clearly defining the physiological state of yeast cells used in studies of mitochondrial transmission genetics.

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