Strand-Specificity in the Transformation of Yeast With Synthetic Oligonucleotides

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ABSTRACT

Cyc1 mutants of the yeast *Saccharomyces cerevisiae* were directly transformed with both sense and antisense oligonucleotides to examine the involvement of the two genomic DNA strands in transformation. Sense oligonucleotides yielded approximately 20-fold more transformants than antisense oligonucleotides. This differential effect was observed with oligonucleotides designed to make alterations at six different sites along the gene and was independent of the oligonucleotide sequence and length, number of mismatches and the host strain. Competition studies showed that antisense oligonucleotides did not inhibit transformation. Although the mechanism for this strand specificity is unknown, this difference was maintained even when *CYC1* transcription was diminished to approximately 2% of the normal level.

TRANSFORMATION of yeast directly with synthetic oligonucleotides has proven to be a useful technique for making site specific mutations in the yeast *Saccharomyces cerevisiae*, especially of the *CYC1* gene encoding iso-1-cytochrome *c* (MOERSCHELL, TSUNASAWA and SHERMAN 1988; MOERSCHELL *et al.* 1990; MOERSCHELL and SHERMAN 1991; YAMAMOTO *et al.* 1992). Yeast strains used in this procedure should have a target allele bearing a single-site mutation that reverts at a low frequency. A host strain with such an allele can be transformed with oligonucleotides, and the transformants recovered by using a selection that requires at least partially functional iso-1-cytochrome *c*. For example, this technique has been used to alter the beginning of the *CYC1* gene to produce N-terminal variants of iso-1-cytochrome *c* (MOERSCHELL *et al.* 1990). Some of the parameters effecting the frequencies of oligonucleotide transformation has been reported by YAMAMOTO *et al.*(1992). During the course of these experiments, sense oligonucleotides (oligonucleotides having a sequence similar to the transcribed message) were observed to transform yeast more efficiently than antisense oligonucleotides (oligonucleotides having a sequence complementary to the transcribed message).

This paper describes a systematic analysis of transformation with two different types of oligonucleotides, either sense or antisense. We have examined oligonucleotides with different lengths and mismatches; and we have employed target alleles with different sites along the gene. We have also examined *cyc1* alleles with diminished levels of transcription. In each case, the sense oligonucleotides transformed yeast much more efficiently than the antisense oligonucleotides.

MATERIALS AND METHODS

Genetic nomenclature and yeast strains: The symbols *CYC1* and *CYC1*$^+$ denote, respectively, any functional allele and the wild-type allele encoding iso-1-cytochrome *c* in the yeast *S. cerevisiae*. The *cyc1-31*, *cyc1-812*, etc. alleles cause a complete deficiency of iso-1-cytochrome *c*. *CYC7*$^+$ denotes the wild-type allele encoding iso-2-cytochrome *c* and *cyc7-67* denotes a partial deletion of the *CYC7* locus that results in the complete deficiency of iso-2-cytochrome *c*.

CYC1$^+$ *CYC7*$^+$ strains can grow on both lactate and glycerol media; *cyc1*$^-$ *CYC7*$^+$ strains can grow on glycerol medium but not lactate medium, whereas *cyc1*$^-$ *cyc7-67* strains cannot grow on either lactate or glycerol media (DOWNIE, STEWART and SHERMAN 1977; DOWNIE *et al.* 1977).

Construction of *cyc1* strains: The *CYC1* gene on plasmid pAB812 (FETROW, CARDILLO and SHERMAN 1989) was modified with oligonucleotides by site-directed mutagenesis *in vitro* (KUNKEL, ROBERTS and ZAKOUR 1987), using *Escherichia coli* CJ236 as the *dut-1 ung-1* strain and *E. coli* phage R408 as the helper phage. In addition to the *CYC1* gene, this plasmid contains *E. coli* f1(IG), ori and bla segments, and the yeast *URA3* gene. The *cyc1-1008*, *cyc1-1009*, *cyc1-1010* and *cyc1-1011* alleles, having the sequences described below, were derived with, respectively, the plasmids pAB772, pAB770, pAB778 and pAB768.

The yeast strains used in this study include the isogenic series shown in Table 1. Strain B-8022 was directly derived from B-7528 by oligonucleotide transformation. This B-8022 strain was transformed separately with plasmids

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TABLE 1

Yeast strains

Strain no.	Genotype	Source
B-7528[a]	*MATa cyc1-31 cyc7-67 ura3-52 lys5-10*	MOERSCHELL, TSUNASAWA and SHERMAN (1988)
B-8022[a]	*MATa CYC1⁺ cyc7-67 ura3-52 lys5-10*	YAMAMOTO *et al.* (1992)
B-8079[a]	*MATa cyc1-812 cyc7-67 ura3-52 lys5-10*	This study
B-8120[a]	*MATa cyc1-1008 cyc7-67 ura3-52 lys5-10*	This study
B-8121[a]	*MATa cyc1-1009 cyc7-67 ura3-52 lys5-10*	This study
B-8122[a]	*MATa cyc1-1010 cyc7-67 ura3-52 lys5-10*	This study
B-8123[a]	*MATa cyc1-1011 cyc7-67 ura3-52 lys5-10*	This study
B-8277[a]	*MATa cyc1-1097 cyc7-67 ura3-52 lys5-10*	This study
B-7627	*MATa cyc1-812 cyc7::CYH2 ura3-52 his3-Δ1 leu2-3 leu2-112 trp1-289 can1-100 cyh2*	MOERSCHELL, DAS and SHERMAN (1991)

[a] Isogenic strains.

pAB772, pAB770, pAB778 and pAB768; subsequently stable transformants were selected. These transformants were plated on FOA medium (BOEKE, LACROUTE and FINK 1984) to select strains having the *CYC1* locus replaced with a single copy of the *cyc1⁻* allele from the plasmid. These strains derived with plasmids pAB772, pAB770, pAB778 and pAB768 were denoted as B-8120, B-8121, B-8122 and B-8123, respectively. B-8277 was similarly constructed except that the *cyc1-31* alteration was introduced into the *cyc1-947* sequence, that lacked the four TATA or potential TATA elements (LI and SHERMAN 1991).

Yeast transformation: Yeast transformation was performed as described previously by YAMAMOTO *et al.* (1992) or MOERSCHELL *et al.* (1990) using, respectively, 50 or 200 μg of oligonucleotides. Oligonucleotides were prepared as described previously by MOERSCHELL, TSUNASAWA and SHERMAN (1988). Standard YPD medium was used for selecting the transformants.

RESULTS

Transformation of yeast with synthetic oligonucleotides was investigated with host *cyc1* strains having nonsense/frameshift mutations at various positions along the gene. Such nonsense/frameshift mutants generally revert only at low frequencies and cause a complete deficiency of iso-1-cytochrome *c*. Because the strain cannot utilize nonfermentable carbon sources, whereas strains having as low as 1% of the normal amount of iso-1-cytochrome *c* can grow on such media, it is possible to select for *cyc1* transformants having altered iso-1-cytochromes *c* with even extremely low specific activities.

Oligonucleotides used for transformation of either the *cyc1-31*, *cyc1-812*, *cyc1-1008*, *cyc1-1009*, *cyc1-1010* or *cyc1-1011* strains, that could correct the nonsense/frameshift mutation, were denoted as selectable oligonucleotides. Oligonucleotides denoted as heterologous oligonucleotides were not complementary to the region of the host *CYC1* gene bearing the nonsense/frameshift mutation and therefore could not correct the lesion to produce selectable transformants.

The host yeast strains were treated with oligonucleotides using standard transformation protocols of either YAMAMOTO *et al.* (1992) or MOERSCHELL *et al.* (1990). The treated cells were plated on YPD medium that supports the growth of both the transformed *CYC1* strains and the original *cyc1* strain. However, after several days growth, the *CYC1* transformants appeared as large colonies over the lawn of the original *cyc1* strain because of the continued growth of the transformants on nonfermentable carbon sources after depletion of glucose from the medium.

Different types and combinations of oligonucleotides were tested to explore the parameters affecting the differential behavior of sense and antisense oligonucleotides.

Yeast was transformed with a matched series of sense and antisense oligonucleotides to test their relative transformation efficiencies. Each oligonucleotide in the sense series had a different mismatch compared to the target allele, and had a counterpart oligonucleotide in the antisense series with the complementary mismatch (Table 2). Although different types of mismatches produced slightly different frequencies of transformation, every sense oligonucleotide produced about 50–100 times more transformants than the corresponding antisense oligonucleotide.

Two nonisogenic yeast strains, each having the *cyc1-812* mutant allele, were compared. Both strains were transformed with sense and antisense oligonucleotides (Table 3). Strain B-8079 was transformed considerably more efficiently than B-7627. However, antisense oligonucleotides transformed both strains much less efficiently than sense oligonucleotides.

A series of isogenic *cyc1* strains were tested with oligonucleotides that were unrelated in sequence because each allele had the nonsense/frameshift mutation at a different site in the gene (Table 4). In each case, antisense oligonucleotides transformed the yeast much less efficiently than the sense oligonucleotides.

Yeast strain B-7528 was transformed with oligonucleotides of different lengths and containing different

TABLE 2

Frequency of transformation of *cyc1-31* with various sense and antisense oligonucleotides having different types of two base-pair mismatches

	Allele or oligonucleotide no.		Transformants[a]
1 2 3 4 5 6 7 8 9 10			
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	*CYC1*$^+$	Sense	
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T		Antisense	
5′ AAATTAATA ATG ACT GAA TA- AAG GCC GGT TCT GCT AAG A	*cyc1-31*	Sense	
3′ TTTAATTAT TAC TGA CTT AT- TTC CGG CCA AGA CGA TTC T		Antisense	
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	OL89.69	Sense	2100
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL89.70a	Antisense[b]	13
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL89.70b	Antisense[b]	17
Met-Thr-Glu-Leu-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTA AAG GCC GGT TCT GCT AAG A	OL89.134	Sense	305
3′ TTTAATTAT TAC TGA CTT AAT TTC CGG CCA AGA CGA TTC T	OL89.139	Antisense	6
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTT AAG GCC GGT TCT GCT AAG A	OL89.135	Sense	600
3′ TTTAATTAT TAC TGA CTT AAA TTC CGG CCA AGA CGA TTC T	OL89.137	Antisense	4
Met-Thr-Glu-Leu-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTG AAG GCC GGT TCT GCT AAG A	OL89.136	Sense	419
3′ TTTAATTAT TAC TGA CTT AAC TTC CGG CCA AGA CGA TTC T	OL89.138	Antisense	3
Met-Thr-Glu-Lys-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA AAG AAG GCC GGT TCT GCT AAG A	OL89.132	Sense	668
3′ TTTAATTAT TAC TGA CTT TTC TTC CGG CCA AGA CGA TTC T	OL89.131	Antisense	1

Amino acid replacements and nucleotide mismatches between *cyc1-31* and the oligonucleotides are underlined.
[a] Tranformation frequency of B-7528 with 200 μg of oligonucleotide, using the procedure of MOERSCHELL, DAS and SHERMAN (1991).
[b] OL89.70a and OL89.70b are two independent preparations of the same oligonucleotide.

numbers of mismatches (Table 5). Previously, these factors were shown to affect the efficiency of transformation (YAMAMOTO *et al.* 1992). However, the relative difference in transformation efficiency of sense and antisense oligonucleotides was maintained with oligonucleotides having different lengths and mismatches (Table 5).

Because antisense oligonucleotides potentially could hybridize to the *CYC1* mRNA, the inefficiency of transformation with antisense oligonucleotides may be attributed to an inhibitory effect. This possibility was addressed with mixtures of sense and antisense oligonucleotides as shown in Figure 1. Two strains were tested, each with a different *cyc1* allele. There was no evidence that antisense oligonucleotides inhibited transformation by the sense oligonucleotides. In fact, the presence of the antisense oligonucleotides synergistically increased the numbers of transformants obtained. With one exception, transformants were not obtained with heterologous oligonucleotides alone. The two colonies obtained using B-8079 with oligonucleotide C were a rare event.

The *cyc1-1097* allele was used to test if the differential effect of sense and antisense oligonucleotides on transformation frequency could be due to differences in transcription rates. In addition to the nonsense/frameshift mutation corresponding to *cyc1-31*, the *cyc1-1097* allele lacks TATA and potential TATA elements as shown in Table 6. The lack of TATA elements reduces *CYC1* transcription to approximately 2% of the normal level (LI and SHERMAN 1991). As presented in Table 6, the *cyc1-31* and *cyc1-1097* strains responded remarkably similar to the differential effect of sense and antisense oligonucleotides. As expected, spectroscopic examinations revealed that the *cyc1-31* transformants contained normal levels of iso-1-cytochrome *c*, whereas the *cyc1-1097* transformants contained low levels corresponding to approximately 2%. The results suggest that the difference in transformation frequencies with sense and antisense oligonucleotides may not be simply related to the differences of transcription of the two DNA strands.

DISCUSSION

Sense oligonucleotides typically transformed yeast about 50–100 times more efficiently than antisense oligonucleotides. This difference was independent of the base sequence, length, and numbers of mismatches.

Antisense oligonucleotides have been used *in vivo*

TABLE 3

Transformation of two *cyc1-812* strains with sense and antisense oligonucleotides

	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	Allele or oligonucleotide no.		Transformants[a] B-7627	Transformants[a] B-8079
5′		TCA	GAG	TAC	TTG	ACT	AAC	CCA	AAG	AAA	TAT	ATT	CCT	GGT	ACC	AAG	ATG	GCC	T	*CYC1*$^+$	Sense		
3′		AGT	CTC	ATG	AAC	TGA	TTG	GGT	TTC	TTT	ATA	TAA	GGA	CCA	TGG	TTC	TAC	CGG	A		Antisense		
5′		TCA	GAG	TAC	TTG	ACT	AAC	CCA	AAG	AAA	TA-	ATT	CCT	GGT	ACC	AAG	ATG	GCC	T	*cyc1-812*	Sense		
3′		AGT	CTC	ATG	AAC	TGA	TTG	GGT	TTC	TTT	AT-	TAA	GGA	CCA	TGG	TTC	TAC	CGG	A		Antisense		
						Thr	-Asn	-Pro	-Lys	-Lys	-Tyr	-Ile	-Pro	-Gly	-Thr	-Lys	-Met	-Ala	-				
5′						ACT	AAC	CCA	AAG	AAA	TAT	ATT	CCT	GGT	ACC	AAG	ATG	GCC	T	OL89.174	Sense	24	468
3′						TGA	TTG	GGT	TTC	TTT	ATA	TAA	GGA	CCA	TGG	TTC	TAC	CGG	A	OL89.175	Antisense	2	22
		Ser	-Glu	-Tyr	-Leu	-Thr	-Thr	-Pro	-Lys	-Lys	-Tyr	-Ile	-Pro	-Gly	-Thr	-Lys	-						
5′		TCA	GAG	TAC	TTG	ACT	ACC	CCA	AAG	AAA	TAT	ATT	CCT	GGT	ACC	AAG				OL87.204	Sense	13	619
3′		AGT	CTC	ATG	AAC	TGA	TGG	GGT	TTC	TTT	ATA	TAA	GGA	CCA	TGG	TTC				OL89.176	Antisense	1	14

Amino acid replacements and nucleotide mismatches between *cyc1-812* and the oligonucleotides are underlined.

[a] Transformation frequency with 200 μg of oligonucleotide, using the procedure of MOERSCHELL, DAS and SHERMAN (1991).

to inhibit translation of transcripts having a complementary sequence (SIMONS 1988). In principle, the antisense oligonucleotides could produce the necessary site-specific mutation, but prevent growth of the transformant by inhibiting translation of the *CYC1* mRNA. This possibility was ruled out by the data shown in Figure 1. Transformation of either strain was not inhibited by simultaneous transformation with an antisense oligonucleotide.

Because of the comparison of the *cyc1-31* and *cyc1-1097* strains (Table 6), the differential action of sense and antisense oligonucleotides cannot be simply related to the difference in the rates of transcription. However, because *cyc1-1097* is not completely deficient in transcription, one could argue that the low transcription of *cyc1-1097* is sufficient to promote transformation and that only a complete deficiency, such as with a nontranscribed strand, may be required for diminution of transformation.

In this regard, we wish to point out other genetic phenomena related to transcription or to differences of transcribed and nontranscribed strands. A number of genes exhibited preferential repair of pyrimidine dimers in the transcribed strands, including the hamster DHFR gene (MELLON, SPIVAK and HANAWALT 1987); the yeast *URA3* gene (SMERDON and TOMA 1990); and the *E. coli lacI* gene (MELLON and HANAWALT 1989). Although this strand selectivity is prominent for the repair of pyrimidine dimers, it is limited to the repair of only a few other types of lesions. Only a slight strand bias was observed in the repair of cisplatin intrastrand adducts (BOHR *et al.* 1991); whereas little or no strand bias was observed for the repair of 6–4 photoproducts (BOHR *et al.* 1991), 4-nitroquinoline-1-oxide induced damage (SNYDERWINE and BOHR 1991), and dimethylsulfate-induced damage (BOHR *et al.* 1991; SCICCHITANO and HANAWALT 1989).

BOHR (1991) suggested that the degree of strand bias in the repair process may be related to the degree by which the lesion inhibits transcription. Although it is known that UV lesions block transcription *in vivo*, no information is available for other lesions. These biases in the repair of specific strands led HANAWALT (1989) to propose that a "transcription coupling factor" may be associated with repair enzymes.

Furthermore, strand bias in the production of mutation has been observed for several genes after various treatments, and these effects have been attributed to the preferential repair of strands. More mutations are formed in the nontranscribed strand of the human HPRT gene treated with UV and benzo[**a**]pyrene (VRIELING *et al.* 1989; MCGREGOR *et al.* 1991; CHEN, MAHER and MCCORMICK 1990) and of the hamster DHFR gene treated with UV (VRIELING *et al.* 1991). However, in contrast to the genes which are tran-

TABLE 4

Frequency of transformation of various *cyc1* alleles with sense and antisense oligonucleotides corresponding to the wild-type sequence

	Allele or oligonucleotide no.		Transformants[a]
1 2 3 4 5 6 7 8 9 10			
5′ AAATTAATA ATG ACT GAA TA- AAG GCC GGT TCT GCT AAG A	*cyc1-31*	Sense	
3′ TTTAATTAT TAC TGA CTT AT- TTC CGG CCA AGA CGA TTC T		Antisense	
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	OL86.69	Sense	1641
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL91.18	Antisense	7
14 15 16 17 18 19 20 21 22 23 24 25 26			
5′ CTT TTC AAG ACT AGA TGT -TA -AA TGC CAC ACC GTG GAA A	*cyc1-1008*	Sense	
3′ GAA AAG TTC TGA TCT ACA -AT -TT ACG GTG TGG CAC CTT T		Antisense	
Leu-Phe-Lys-Thr-Arg-Cys-Leu-Gln-Cys-His-Thr-Val-Glu-			
5′ CTT TTC AAG ACT AGA TGT CTA CAA TGC CAC ACC GTG GAA A	OL90.263	Sense	133
3′ GAA AAG TTC TGA TCT ACA GAT GTT ACG GTG TGG CAC CTT T	OL90.277	Antisense	3
31 32 33 34 35 36 37 38 39 40 41 42 43			
5′ CAT AAG GTT GGT CCA AAC TA- -AT GGT ATC TTT GGC AGA C	*cyc1-1009*	Sense	
3′ GTA TTC CAA CCA GGT TTG AT- -TA CCA TAG AAA CCG TCT G		Antisense	
His-Lys-Val-Gly-Pro-Asn-Leu-His-Gly-Ile-Phe-Gly-Arg-			
5′ CAT AAG GTT GGT CCA AAC TTG CAT GGT ATC TTT GGC AGA C	OL90.257	Sense	535
3′ GTA TTC CAA CCA GGT TTG AAC GTA CCA TAG AAA CCG TCT G	OL90.272	Antisense	51
37 38 39 40 41 42 43 44 45 46 47 48 49			
5′ TTG CAT GGT ATC TTT GGC TAA -AC TCT GGT CAA GCT GAA G	*cyc1-1010*	Sense	
3′ AAC GTA CCA TAG AAA CCG ATT -TG AGA CCA GTT CGA CTT C		Antisense	
Leu-His-Gly-Ile-Phe-Gly-Arg-His-Ser-Gly-Gln-Ala-Glu-			
5′ TTG CAT GGT ATC TTT GGC AGA CAC TCT GGT CAA GCT GAA G	OL90.260	Sense	938
3′ AAC GTA CCA TAG AAA CCG TCT GTG AGA CCA GTT CGA CTT C	OL90.276	Antisense	91
74 75 76 77 78 79 80 81 82 83 84 85 86			
5′ ACT AAC CCA AAG AAA TA- ATT CCT GGT ACC AAG ATG GCC T	*cyc1-812*	Sense	
3′ TGA TTG GGT TTC TTT AT- TAA GGA CCA TGG TTC TAC CGG A		Antisense	
Thr-Asn-Pro-Lys-Lys-Tyr-Ile-Pro-Gly-Thr-Lys-Met-Ala-			
5′ ACT AAC CCA AAG AAA TAT ATT CCT GGT ACC AAG ATG GCC T	OL89.174	Sense	468
3′ TGA TTG GGT TTC TTT ATA TAA GGA CCA TGG TTC TAC CGG A	OL89.175	Antisense	22
78 79 80 81 82 83 84 85 86 87 88 89 90			
5′ G AAA TAT ATT CCT GGT ACC TA- ATG GCC TTT GGT GGG TTG	*cyc1-1011*	Sense	
3′ C TTT ATA TAA GGA CCA TGG AT- TAC CGG AAA CCA CCC AAC		Antisense	
Lys-Tyr-Ile-Pro-Gly-Thr-Lys-Met-Ala-Phe-Gly-Gly-Leu-			
5′ G AAA TAT ATT CCT GGT ACC AAG ATG GCC TTT GGT GGG TTG	OL90.259	Sense	150
3′ C TTT ATA TAA GGA CCA TGG TTC TAC CGG AAA CCA CCC AAC	OL90.275	Antisense	1

Nucleotide mismatches between *cyc1* mutations and the oligonucleotides are underlined.

[a] Transformation frequency with 50 μg DNA, using the procedure of YAMAMOTO et al. (1992).

scribed by RNA polymerase II, the yeast *SUP4-o* gene, which is transcribed by RNA polymerase III, contained more UV-induced mutations on the transcribed strand (ARMSTRONG and KUNZ 1990).

More direct evidence for the role of transcription in genetic phenomena come from experimental studies with varied transcription rates. Transcription activity was shown to correlate the repair of pyrimidine dimers in hamster and human metallothionein genes (OKUMOTO and BOHR 1987; LEADON and SNOWDEN 1988) and overall in mammalian DNA (CHRISTIANS and HANAWALT 1990); as well as in the repair of alkylation damage in the rat insulin gene (LEDOUX *et al.* 1990). Also, transcription was shown to stimulate recombination of yeast genes controlled by RNA polymerase II (THOMAS and ROTHSTEIN, 1989) and RNA polymerase I (KEIL and ROEDER 1984; STEWART and ROEDER 1989; VOELKEL-MEIMAN and ROEDER 1990; LIN and KEIL 1991). KOROGODIN *et al.* (1991) have presented evidence consistent with the view that spontaneous mutation rates are higher when genes are derepressed, most likely as a result of enhanced transcription.

The preferential action on the transcribed strand and the increased frequency of a variety of genetic phenomena with transcription could be due to strand separation causing an open conformation that allows accessibility to the action of various enzymes. Al-

TABLE 5

Frequency of transformation of *cyc1-31* with 40 or 50 nt long sense and antisense oligonucleotides having various base-pair mismatches

	Allele or oligonucleotide no.		Mismatches	Transformants[a]
1 2 3 4 5 6 7 8 9 10 11 12				
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-Gly-				
5′ ACACTAAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG AAA GGT	*CYC1*$^+$	Sense		
3′ TGTGATTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC TTT CCA		Antisense		
5′ ACACTAAATTAATA ATG ACT GAA TA- AAG GCC GGT TCT GCT AAG AAA GGT	*cyc1-31*	Sense		
3′ TGTGATTTAATTAT TAC TGA CTT AT- TTC CGG CCA AGA CGA TTC TTT CCA		Antisense		
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-				
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	OL89.69	Sense	2	1287
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL91.18	Antisense		11
Met-Cys-Pro-Pro-Leu-Ala-Gly-Ser-Ala-Lys-				
5′ AAATTAATA ATG TGC CCC CCC CTT GCC GGT TCT GCT AAG A	OL90.18	Sense	11	457
3′ TTTAATTAT TAC ACG GGG GGG GAA CGG CCA AGA CGA TTC T	OL91.20	Antisense		2
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-Gly-				
5′ ACACTAAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG AAA GGT	OL87.73	Sense	2	2109
3′ TGTGATTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC TTT CCA	OL91.19	Antisense		19
Met-Ser-Glu-Phe-Leu-Ala-Gly-Ser-Ala-Lys-Lys-Gly-				
5′ ACACTAAATTAATA ATG TCT GAA TTC TTG GCC GGT TCT GCT AAG AAA GGT	OL86.124	Sense	6	801
3′ TGTGATTTAATTAT TAC AGA CTT AAG AAC CGG CCA AGA CGA TTC TTT CCA	OL91.21	Antisense		33

Amino acid replacements and nucleotide mismatches between *cyc1-31* and the oligonucleotides are underlined.

[a] Transformation frequency with 50 μg DNA, using the procedure of YAMAMOTO *et al.* (1992).

TABLE 6

Frequency of transformation of *cyc1-31* and *cyc1-1097* mutants having respectively, 100% and ~2% levels of transcription

	Allele or oligonucleotide no.		Transformants[a]
5′ ACACTAAATTAATA ATG ACT GAA TA- AAG GCC GGT TCT GCT AAG A	*cyc1-31*	Sense	
3′ TGTGATTTAATTAT TAC TGA CTT AT- TTC CGG CCA AGA CGA TTC T		Antisense	
1 2 3 4 5 6 7 8 9 10			
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	OL89.69	Sense	694
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL91.18	Antisense	4
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-Gly-			
5′ ACACTAAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG AAA GGT	OL87.73	Sense	2267
3′ TGTGATTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC TTT CCA	OL91.19	Antisense	19
5′ ACACTAAATTAATA ATG ACT GAA TA- AAG GCC GGT TCT GCT AAG A	*cyc1-1097*	Sense	
3′ TGTGATTTAATTAT TAC TGA CTT AT- TTC CGG CCA AGA CGA TTC T		Antisense	
1 2 3 4 5 6 7 8 9 10			
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-			
5′ AAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG A	OL89.69	Sense	799
3′ TTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC T	OL91.18	Antisense	31
Met-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-Gly-			
5′ ACACTAAATTAATA ATG ACT GAA TTC AAG GCC GGT TCT GCT AAG AAA GGT	OL87.73	Sense	3009
3′ TGTGATTTAATTAT TAC TGA CTT AAG TTC CGG CCA AGA CGA TTC TTT CCA	OL91.19	Antisense	9

-180 -170 -160 -150 -140 -130 -120

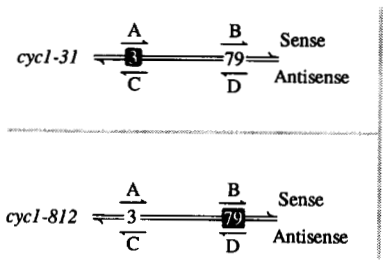
cyc1-31 GCATATATATATGTGTGCGACGACACATGATCATATGGCATGCATGTGCTCTGTATGTATATAAAACT
cyc1-1097 GCATCGCG-TATGTGTGCGACGACACATGATCATATGGCATGCATGTGCTCTGTATGTAGCGCAAACT

-110 -100 -90 -80 -70 -60 -50

cyc1-31 CTTGTTTTCTTCTTTTCTCTAAATATTCTTTCCTTATAAATTAGGTCCTTTGTAGCATAAATTACTA
cyc1-1097 CTTGTTTTCTTCTTTTCTCTAGGCCTTCTTTCCTTGCGCATTAGGTCCTTTGTAGCATAAATTACTA

Nucleotide mismatches between *cyc1-31* or *cyc1-1097* and the oligonucleotides are underlined. The *cyc1-1097* allele lacks TATA and potential TATA elements as indicated by double underlines at the bottom.

[a] Transformation frequency with 50 μg DNA, using the procedure of YAMAMOTO *et al.* (1992).



Oligo.	Transformation frequencies *cyc1-31*	*cyc1-812*
A	986	0
B	0	362
C	9	2
D	0	23
A+B	1127	404
A+D	1203	38
C+D	25	32
C+B	15	413

FIGURE 1.—Transformation frequencies with mixtures of sense and antisense oligonucleotides. The *cyc1-31* strain (B-7528) and the *cyc1-812* strain (B-8079) were transformed with 50 μg of various combinations of oligonucleotide as shown in the figure by using the procedure of YAMAMOTO *et al.* (1992). Oligonucleotides A, B, C and D denote OL89.232, OL89.174, OL89.70b and OL89.175, respectively. The sequences of the oligonucleotides are presented in the tables as follows: OL89.70b, Table 2; OL89.69, which has the same sequence as OL89.232, Table 2; OL89.174, Table 3; and OL89.175, Table 3. Oligonucleotides A and C give rise to functional transformants with the *cyc1-31* strain; whereas oligonucleotides B and D give rise to functional transformants with the *cyc1-812* stain. Oligonucleotides A and B are sense oligonucleotides; whereas oligonucleotides C and D are antisense oligonucleotides.

though it is inviting to speculate that the strand selectivity for enhanced oligonucleotide transformation is one of these genetic phenomena related to transcription, the *cyc1-31* and *cyc1-1097* results (Table 6) forces us to consider alternative mechanisms.

Oligonucleotide transformation has some similarities with the very short patch (VSP) repair system in *E. coli*. The VSP repair system in *E. coli* is both strand and sequence specific (LIEB 1983, 1985), with a repair tract of about 10 to 20 bases (LIEB 1983). This repair tract length is similar to the number of contiguous mismatched residues tolerated by the oligonucleotide transformation system in yeast (YAMAMOTO *et al.* 1992). An enzyme has been found in *E. coli* that cuts a specific mismatched T residue in one strand (HENNECKE *et al.* 1991). HENNECKE *et al.* (1991) suggested that exonucleolytic removal of a portion of the nicked strand followed by template directed repair was the mechanism for VSP repair. However, no sequence specificity appears to be required for oligonucleotide transformation of the *cyc1* mutants (see Table 4).

Strand selectivity of genetic phenomena also can be attributed to the differences in leading and lagging strands during DNA replication, as was suggested to occur for mutations of the HPRT gene in an excision repair-deficient Chinese hamster cell line (VAN ZEELAND *et al.* 1990). While no difference was observed for the formation of a class of deletions in a *E. coli* plasmid (WESTON-HAFER and BERG 1991), the differential response of mutation of the leading and lagging strands was reported by TRINH and SINDEN (1991).

Since oligonucleotide transformation does not depend on *RAD52* function (YAMAMOTO *et al.* 1992), the recombinational process appears distinct from transformation of linear duplex DNA, and does not appear to involve double-strand breaks. To account for strand selectivity in oligonucleotide transformation, independent of transcription, we suggest that oligonucleotides are preferentially incorporated into either the leading or lagging strand during DNA replication.

Although we can only speculate on the mechanism by which strands are preferentially selected for oligonucleotide transformation, the results presented in this paper has firmly established the phenomena.

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