THE EFFECT OF ARABINOSE-SPECIFIC ENZYME SYNTHESIS ON RECOMBINATION IN THE ARABINOSE GENES OF *ESCHERICHIA COLI*

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Received June 14, 1967

FIVE genes are known to be specifically involved in the metabolism of the pentose sugar L-arabinose by *Escherichia coli* B/r. The *araA* and *araB* genes are the structural genes for the L-arabinose isomerase and L-ribulokinase (LEE and ENGLESBERG 1962). The *araD* and *araE* genes are probably structural genes for L-ribulose-5-phosphate 4-epimerase and L-arabinose permease respectively (ENGLESBERG *et al.* 1962; ISAACSON and ENGLESBERG 1964). *araC* is a regulatory gene whose product is required for synthesis of the enzymes and permease (HELLING and WEINBERG 1963; ENGLESBERG, IRR, POWER and LEE 1965).

It is now well established (at least in microorganisms) that all genes do not function to produce a gene product (enzyme) all of the time. Appropriate regulatory mechanisms ensure that many enzymes are produced only in response to a specific need (see JACOB and MONOD 1961). In *E. coli* synthesis of the arabinose enzymes and permease is induced by arabinose; when the arabinose is removed from the medium synthesis ceases. If regulation of enzyme synthesis occurred at the level of the gene (transcription level rather than or in addition to regulation at the level of translation of mRNA into protein), one would expect a gene in its "active" state to behave differently in recombination or any other process involving the gene, than if it were not actively engaged in mRNA and protein synthesis. This prediction is confirmed by the results reported here. In the experiments described in this paper, I looked to see what effect arabinose-specific enzyme synthesis had on recombination in the structural genes for these enzymes. The frequency of recombination was found to be higher when the enzymes were being synthesized than when they were not being synthesized. The frequency of recombination in the adjacent leucine genes was unaffected.

MATERIALS AND METHODS

Media: H: 14 g K_2HPO_4 , 6 g KH_2PO_4 , 0.5 g Na_3 -citrate- $2H_2O$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.2 g $(NH_4)_2SO_4$, per liter. Oxoid Ion-agar No. 2 was added to 0.9% for solid medium (H agar). L-agar and L-broth were described previously (BERTANI 1951). Soft agar is L-broth containing 0.35% Difco agar.

Bacteriophage: Lysates of phage P1bt (GROSS and ENGLESBERG 1959) were prepared by the soft-agar layer method (SWANSTROM and ADAMS 1951) in 30×18 cm Pyrex cake-pans using

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L-agar as a bottom layer. Twenty ml of soft L-agar containing 4 ml of an overnight culture of the recipient in L-broth and 10^7 plaque-forming phage particles were poured per pan as a top layer. After $4\frac{1}{2}$ hours incubation at 37°C these lysates were collected by adding 20 to 30 ml 2% ammonium acetate to each pan, scraping the top layer into a centrifuge bottle and emulsifying. After two cycles of low speed centrifugation (10 min, 7000 rpm, Sorvall GSA rotor), the supernatant solution was stored overnight at 4°C . Then these lysates were concentrated and purified by subjecting them to two cycles of alternate low speed and high speed (2 hr, 13,000 rpm, Sorvall GSA rotor, in KEL-F-lined stainless-steel bottles) centrifugation. Pellets from high speed centrifugation were allowed to resuspend themselves overnight in 2% ammonium acetate. The phage yield in the crude lysate from six pans was generally about 1×10^3 plaque-forming particles, but the yield is markedly dependent on the host strain. When necessary the desired genetic markers from a strain giving a low phage yield were transduced into other strains giving better yields. In each case the starter phage had been grown previously on the same recipient in order to eliminate any possibility of contamination of the final lysate with heterologous starter phage which, depending on its origin, might increase the frequency of rare transductants.

Bacterial strains: Genetic loci are designated by the following abbreviations: *ara*, arabinose; *leu*, leucine; *thr*, threonine; *lac*, lactose. All strains used were derivatives of *E. coli* B/r. Strains containing the *leu-1* (*leu* of GROSS and ENGLESBERG 1959) or *leu-3* (received from E. ENGLESBERG in the strain BC 28-12-1-B *leu*) mutations require leucine for growth. All *araA* mutants used lack a functional isomerase, the *araB* mutants lack the kinase, and *araD* mutants lack epimerase, *araC5* and *araC19* are uninducible, and *araC6* is constitutive for all arabinose enzymes and the arabinose permease. Multiple mutations were constructed as previously (HELLING and WEINBERG 1963). The order of the mutations is shown in Figure 1. All of the loci shown are jointly transducible, but the *araE* locus is not linked by transduction to the other *ara* genes (ISAACSON and ENGLESBERG 1964). For the origins and further properties of these mutations see GROSS and ENGLESBERG (1959) and ENGLESBERG *et al.* (1965).

In each transduction the recipient strain was lysogenic for phage P1bt in order to eliminate problems arising from phage-induced lysis. Sensitive strains lysogenized for use as recipients are listed in Table 1.

The lysogenic strains were obtained by incubating the sensitive recipients plus phage (added at a multiplicity of 5 to 10) in L-broth containing 2.5×10^{-3} M CaCl_2 for 2 hours at room temperature, followed by incubation at 37°C for 3 to 4 hours. Virtually all survivors were lysogenic. After single colony isolation, lysogeny was verified by demonstrating production of phage from the clone.

Procedure for transduction: Bacteria growing exponentially in H + 0.1% glycerol + 40 $\mu\text{g}/\text{ml}$ L-leucine were harvested by centrifugation and washed in 10^{-2} M MgSO_4 . The cells were

FIGURE 1.—Genetic linkage map of *ara* mutations, and the biochemical pathway of L-arabinose utilization.

TABLE 1
Recipient strains

Strain	Genetic loci			
	<i>araA</i>	<i>araB</i>	<i>araC</i>	<i>leu</i>
BH1	54	a22	+	1
BH2	54	a22	19	1
BH3	54	a22	c6	1
BH5	+	24	+	1
BH6	+	+	19	1

resuspended in 10^{-2} M MgSO_4 + 7.5×10^{-3} M CaCl_2 and phage was added to the multiplicity of about 0.5. After 5 minutes at 37°C for adsorption, the mixture was diluted tenfold with 10^{-2} M MgSO_4 and centrifuged. The pellet was resuspended in H + 0.01% glycerol + 0.012 M MgSO_4 (but lacking leucine), generally to a density of 1 to 3×10^8 cells/ml, and divided in two parts to one of which arabinose was added (to 0.2%). The two suspensions were shaken at 37°C .

Assay for transductants: *ara*⁺ *leu*⁺ transductants: 1.0 ml samples were pipetted from the suspension lacking arabinose into petri dishes containing 4 ml melted H agar. Samples from the arabinose-containing suspension were pipetted into melted H agar containing 0.2% arabinose. The agar was allowed to harden and the plates were incubated at 37°C . A top layer of H agar + 0.2% arabinose was added for selection several hours after infection. Only the *ara*⁺ *leu*⁺ transductants are able to grow in this medium. The number of colonies which appeared was independent of the time at which the selective layer was added in the interval 1½–24 hours after infection. Counts were made after 48 hours at 37°C . *leu*⁺ transductants: 0.1 ml samples were spread on H agar plates containing glycerol as carbon source.

RESULTS

Purified phage grown on bacteria mutant in the *araA* gene (containing the mutation *araA2*) were used to infect BH1 recipients (containing the *araA* gene mutation *araA54*) and arabinose was added to part of the mixture after adsorption. Periodically samples were plated selecting for *leu*⁺ or for *ara*⁺ *leu*⁺ recombinants. For *ara*⁺ *leu*⁺ recombinants to appear, recombination must have occurred in the *araA* gene between *araA2* and *araA54* (Figure 2).

The numbers of transductants remained constant for almost three hours after infection until cells began multiplying at an apparently normal rate. If *ara*-

FIGURE 2.—Partial, schematic representation of crossovers necessary for the formation of *ara*⁺ *leu*⁺ recombinants following infection of an *araA54 araBa22 leu* recipient by an *araA2* (and otherwise wild-type) donor phage. Numbers refer to the *ara* mutational sites.

FIGURE 3.—The effect of arabinose on the numbers of *ara*⁺ *leu*⁺ transductants recovered following infection of an *araA54 araBa22 leu* recipient lysogenic for P1bt, by an *araA2* donor phage. Solid symbols represent *ara*⁺ *leu*⁺ transductants; open symbols represent total *leu*⁺ transductants. Triangles (Δ , $\blacktriangle$) indicate samples from the culture with arabinose; circles ($\circ$, $\bullet$), from the culture lacking arabinose. Block figures ($\blacksquare$) indicate samples from the culture lacking arabinose pipetted into agar containing arabinose at the point indicated. See MATERIALS AND METHODS for procedure and conditions of assay.

binose was added to the cells immediately after resuspension, a higher number of *ara*⁺ *leu*⁺ transductants was recovered whereas the number of *leu*⁺ transductants remained unchanged (Figure 3). The ratio (*ara*⁺ *leu*⁺ transductants/all *leu*⁺ transductants) changed from 0.020 in the absence of arabinose to 0.030 in the presence of arabinose. The absolute frequencies varied somewhat from experiment to experiment but the effect of arabinose was relatively constant (Table 2, cross 1)

No effect of the presence of arabinose during growth of the phage on later transduction and recombination in the *ara* genes could be detected.

In order to estimate the time during which the presence of arabinose can produce an increase in the number of recombinants, samples were pipetted at various times from the flask lacking arabinose into melted agar containing arabinose (Figure 3, block symbols). It can be seen that the arabinose effect (i.e., the increased number of recombinants in the presence of arabinose) is limited to the first hour after infection. This may reflect the time of integration of donor genes into the recipient genome.

Arabinose also increased the frequency of *ara*⁺ *leu*⁺ transductants to a small extent when phage grown on an *ara*⁺ host were used (Table 2, cross 2). The size of the arabinose region of the chromosome must be small compared to the size of the DNA carried by the transducing phage. IKEDA and TOMIZAWA (1965) estimate the molecular weight of the bacterial DNA in a "normal" transducing particle to be 60 million daltons (the size of the lytic phage DNA). If the average molecular weight of a nucleotide is 330, and an average gene contains about 900 nucleotide pairs, then each transducing phage particle must carry about 100 bacterial genes. Since the arabinose segment of chromosome contains only four known genes (or roughly 4% of the inserted DNA if the full complement of

TABLE 2

The effect of arabinose on recombination in the arabinose genes

Cross No.	Phage	Recipient	<i>ara</i> ⁺ <i>leu</i> ⁺ / <i>leu</i> ⁺		+ARA/—ARA
			—ARA	+ARA	
1	<i>araA2</i>	BH1 (8)	.0203	.0307	1.51 ± .07
2	<i>ara</i> ⁺	BH1 (3)	.4540	.5150	1.13 ± .12
3	<i>araA2</i>	BH2 (4)	.0218	.0255	1.17 ± .10
4	<i>araA2</i>	BH3 (5)	.0391	.0393	1.01 ± .03
5	<i>araA2</i>	BH1* (3)	.0348	.0335	0.96 ± .09
6	<i>araBa22</i>	BR5 (6)	.0140	.0168	1.20 ± .07
7	<i>araC5</i>	BH6 (4)	.0077	.0077	1.00 ± .06

Samples were plated about 2 hours after infection. Number of independent experiments performed to obtain the average ratios shown is indicated in parentheses. In each experiment at least 700 *leu*⁺ transductants and 350 *ara*⁺ *leu*⁺ transductants were counted to obtain the individual frequencies. —ARA and +ARA indicate absence or presence of arabinose after infection as detailed in MATERIALS AND METHODS. Ratios are shown ± standard error of estimate. In every case the frequency of total *leu*⁺ transductants was the same whether or not arabinose was present after infection.

* Arabinose was added to the growth medium 1.5 generations before the cells were harvested and used as recipients in a

transducing DNA is injected), and since the locus *leu* is sufficiently close to *ara* that joint transduction occurs almost as frequently as either single transduction (GROSS and ENGLESBERG 1959), it is clear that in the case of a wild-type donor no recombination in *araA* or any other *ara* gene was necessary for the recovery of *ara*⁺ *leu*⁺ transductants. As expected, the ratio *ara*⁺ *leu*⁺/*leu*⁺ is some 20- to 25-fold higher when a wild-type donor is used rather than an *araA2* donor (Table 2, compare crosses 1 and 2). If we assume the distance between *araA2* and *araA54* to be about half the length of an average gene, then we are looking at recombination within a distance corresponding to roughly 0.5% of the introduced transducing DNA, when we select *ara*⁺ recombinants in the transduction *araA2* phage × BH1 recipient.

Is there a selection for ara⁺ cells? Because the number of *leu*⁺ transductants recovered was unaffected by the presence of arabinose, the increased number of *ara*⁺ *leu*⁺ transductants cannot be due to a shift from abortive to complete transduction caused by arabinose, but rather it must result from an increased number of recombination events ("switches") in the arabinose *A* gene, or from an immediate selective advantage of cells having an *ara*⁺ phenotype over those cells unable to metabolize arabinose. It seemed unlikely that arabinose produces a strong initial selective advantage for *leu*⁺ cells able to use arabinose as a carbon source, because glycerol was present throughout in the liquid medium and could be metabolized by both *ara* and *ara*⁺ cells. The cells were not adapted to use arabinose. Furthermore the recombination event leading to an *ara*⁺ phenotype is intracistronic (HELLING and WEINBERG 1963), so that recombination must occur before the cell can use arabinose. It is difficult to be sure that partial, intracistronic complementation does not occur between *araA54* and *araA2*, but it is very unlikely because *araA2* forms no mutant protein immunologically similar to the wild-type isomerase (LEE and ENGLESBERG 1962).

If intragenic complementation *did* occur, leading to some utilization of *ara*-

binose as a source of carbon and energy, in addition to the glycerol, and resulting in increased recovery of *ara*⁺ *leu*⁺ transductants, other carbohydrates might be expected to have the same effect. However, the addition of D-xylose or D-galactose (which are substrates for the L-arabinose permease, but do not induce formation of the arabinose-specific enzymes (NOVOTNY and ENGLESBERG 1966), or D-lactose to the infected cells in the absence of arabinose had no effect on the number of *ara*⁺ *leu*⁺ or *leu*⁺ transductants recovered, so it appears that the increased number of *ara*⁺ *leu*⁺ recombinants in the presence of arabinose was due to increased recombination and was not the result of selection.

The effect of mutations in the araC gene: If genes engaged in enzyme synthesis show a higher frequency of recombination, the addition of an *ara* constitutive mutation to the BH1 genome should lead to elevated rates of *ara* gene recombination in the absence of arabinose. Table 2, cross 4 shows this to be the case, where the recipient (BH3) was transduced with the same stock of *araA2* phage used in cross 1. The frequency of *ara*⁺ *leu*⁺ recombinants (which necessarily result from a genetic exchange in the region between *araA2* and *araA54*) was high even though the infected cells were not exposed to arabinose for several hours after infection. The addition of arabinose had little or no effect on the frequency of *ara*⁺ *leu*⁺ or *leu*⁺ transductants. This confirms our conclusion that the increased frequency of *ara*⁺ *leu*⁺ recombinants observed previously in the presence of arabinose was not the result of selection due to the presence of arabinose soon after infection.

If the BH1 cells were pre-loaded with arabinose by growing them in the usual way, but adding 0.1% L-arabinose to the medium a generation or more before harvesting for use as recipients, the ratio *ara*⁺ *leu*⁺/*leu*⁺ was high (almost as high as the frequency obtained with a constitutive recipient), and little affected by the presence of arabinose *after* infection (Table 2, cross 5). If a minimal medium containing one half the phosphate and five times as much ammonium was used throughout the experiment, and the cells were pre-loaded with arabinose or contained a constitutive *araC* mutation so that they had a functional arabinose permease (ENGLESBERG *et al.* 1965), both the transduction frequency (frequency of *leu*⁺ transductants) and the recombination frequency (*ara*⁺ *leu*⁺/*leu*⁺) were lower if arabinose was added *after* infection. The phosphate and ammonium concentrations seemed to have little effect on recombination frequency if arabinose was *not* present after infection. Most or all of the decreased recombination was not an effect specific for the *ara* genes; instead the decrease seemed to affect recombination between two *leu* mutations in the same way, and presumably depressed recombination frequencies in general. This effect is probably related to the general inhibition of growth and protein synthesis in cells which concentrate arabinose but can not metabolize it—an inhibition which is potentiated by low potassium phosphate and high ammonium sulfate medium containing (ISAACSON and ENGLESBERG 1964; ISAACSON 1964).

If the cells were grown in H + glycerol + arabinose + 0.2% D-fucose prior to infection, the recombination frequencies were similar to those obtained following growth in the usual H + glycerol medium. D-fucose is a substrate for the

L-arabinose permease (NOVOTNY and ENGLESBERG 1966), and inhibits the induction of arabinose-specific enzyme synthesis (ENGLESBERG *et al.* 1965; HELLING, unpublished data). Presumably in this case synthesis of the arabinose enzymes and *araE* gene-specific permease were not induced during growth prior to infection, so the cells were unable to accumulate arabinose via this permease.

In contrast to the results obtained with the BH1 recipient, the ratio $ara^+ leu^+ / leu^+$ is low and affected rather little by the presence of arabinose if the recipient contains the mutation *araC19* which prevents the induction of arabinose-specific enzyme synthesis by arabinose (BH2 recipient, Table 2, cross 3). (Because *araC* lies between *araA* and *leu*, the regions where crossing over is necessary for formation of an $ara^+ leu^+$ recombinant should be unaffected by the presence of an *araC* mutation (Figures 1, 2).) Lack of an arabinose effect might be expected because *araC* mutants are not inducible for any of the enzymes. I attribute the small arabinose effect observed to the formation by the introduced *araC*⁺ gene of a functional *araC* gene product which leads to arabinose enzyme and permease synthesis (HELLING and WEINBERG 1963). No effect of arabinose on the ratio $ara^+ leu^+ / leu^+$ was detectable when both the transducing phage and the recipient contained an *araC* mutation (*araC5* phage and BH6 recipient, Table 2, cross 7).

The specificity of the arabinose effect: All of the preceding experiments concerned recombination in the *araA* gene between *araA2* and *araA54*. Arabinose also increases the frequency of recombination in the *araB* gene as shown by the transduction, *araBa22* phage $\times$ BH5 recipient (Table 2, cross 6).

Arabinose did not detectably affect the frequency of transduction to *lac*⁺ or to *thr*⁺ when a wild-type donor transduced a *lac* or *thr* derivative of BH1. To show further that arabinose affected recombination only in the *ara* genes, the effect of arabinose on the frequency of transductants recombinant in the *leu* genes (*leu*⁺) was tested in a transduction using a *leu-3* donor and a *leu-1* recipient (BH1). No detectable effect of arabinose on the frequency of *leu*⁺ transductants can be seen (Table 3). Likewise the frequency of *leu*⁺ recombinants recovered was unaffected by arabinose when the recipient contained an *ara* constitutive mutation (*leu-3* $\times$ BH3 recipient, Table 3).

DISCUSSION

The results presented clearly demonstrate that *ara* genes undergo recombination more readily when actively involved in protein synthesis than when they

TABLE 3

Lack of effect of arabinose on recombination between leu-1 and leu-3

Phage	Recipient	$ara^+ (+ARA) / ara^+ (-ARA)$	$leu^+ (-ARA) / ara^+$	$leu^+ (+ARA) / ara^+$	$leu^+ (+ARA) / leu^+ (-ARA)$
<i>leu-3</i>	BH1 (4)	1.11	0.089	0.080	0.98 $\pm$ 0.08
<i>leu-3</i>	BH3 (2)	1.00	0.076	0.078	1.03 (1.01-1.05)

See Table 2.

are not so involved. Recombination is increased in the *araA* and *araB* genes by the inducer of *ara* gene-specific enzyme synthesis (arabinose), which does not affect recombination in the adjacent *leu* genes. Cells constitutively producing the arabinose enzymes show enhanced *ara* gene recombination even in the absence of the sugar arabinose. Recombination in the *ara* regulatory gene, *araC*, on the other hand, appears to be unaffected by the presence of arabinose, presumably because the *araC* gene product is produced constitutively. The *araC* mutant recipients also lack the arabinose permease whose structure is probably controlled by *araE* (see ENGLESBERG *et al.* 1965). Lack of an arabinose effect in the *araC* mutant recipient BH2 is probably not the result of a permease deficiency however, because even the *araC*⁺ recipients *lack* this permease until induction commences upon addition of arabinose. Furthermore low-level uptake of arabinose occurs even in the *araC* mutants by way of a second permease also inducible by arabinose (E. ENGLESBERG, private communication). These results indicate that some difference exists between the physical state of an induced gene and of a noninduced gene. It is difficult to imagine gene-specific effects on recombination which do *not* act at the level of the gene. Support for this conclusion comes from the results of KÖLSCH and STARLINGER (1965a,b) who found a difference in ultraviolet light sensitivity and photoreactivation between genes in a repressed and derepressed state.

LUZZATI, CLAVILIER and PERE (1963) found that histidine prevents some 95% of the gene conversion at the *ad3* locus in *Saccharomyces cerevisiae*. Mutation at this locus leads to a simultaneous requirement for histidine and adenine. Adenine had no effect on gene conversion. This result is strictly analogous to the results reported here if we assume that histidine (but not adenine) is capable of repressing enzyme synthesis from the *ad3* locus. The results were explained by postulating that a cytoplasmic repressor formed in the presence of histidine interacted at the genic level to inhibit gene conversion (which results from nonreciprocal recombination).

One can imagine several consequences of gene-product formation, which might facilitate recombination: 1. If the repressor binds to the DNA of an operon it might sterically hinder recombination. This explanation is unlikely to be correct because the arabinose genes are subject to positive control, i.e., a cytoplasmic *araC* gene product ("activator") and arabinose are *required* for induction of enzyme synthesis. (Induction does *not* occur on removal of a repressor as it apparently does in the *lac* operon.) One might hypothesize that the *araC* gene product does normally bind to the *ara* operon DNA in an uninduced cell, and that induction involves removal of the *araC* product from DNA and participation of this product in transcription or in translation of *ara* gene mRNA into protein. Even if this were the case, steric hindrance of recombination by activator in an uninduced cell appears unlikely because a mutant which lacked a functional *araC* gene (BH2 recipient), and in which the DNA of the *ara* operon was presumably free of any specific inducer or repressor substance normally produced from a functional *araC* gene (as judged on the basis of lack of reversion, low residual or induced enzyme levels, and complementing properties of the *araC19*

mutant gene (HELLING and WEINBERG 1963; unpublished data), showed no significantly greater *ara* gene recombination than a cell with a functional *araC* gene (BH1 recipient). It can be argued that a functional *araC* gene product made from the exogenote soon after its insertion immediately binds to the *ara* operon DNA in the cell, so this is not really a valid test of the hypothesis. However if this were the case the frequency of *ara* gene recombination should be increased by the *presence* of arabinose after infection to the same extent when an *araC* mutant recipient (BH2) is used as when an *araC*⁺ recipient (BH1) is used (compare crosses 1 and 3, Table 2). Clearly it is not. Other explanations based on attachment of activator to DNA can be ruled out in the same manner.

2. The *ara* gene mRNA might bind together two homologous DNA single strands by annealing with each (in different regions) thus increasing the likelihood of homologous regions of DNA finding each other and exchanging parts (O. MAALOE, personal communication). This seems unlikely but cannot be ruled out by these results.

3. DNA making mRNA might be located at the periphery of the bacterial nuclear body, or at some other place where it is more accessible for recombination. This does not seem to explain the results reported here because little effect on recombination in the adjacent *leu* genes was noted.

4. Single-stranded regions might be much more frequent in the *ara*-gene DNA when mRNA is produced, and free ends (single-strand breaks) may appear. Free ends must appear at some point during recombination if recombination occurs by breakage and reunion, but this may be unrelated to any process involved in enzyme induction. According to current thinking single strands are required for the strand-specific pairing which leads to recombination (e.g., HOWARD-FLANDERS and BOYCE 1966). Thus the increased recombination on induction might result from separation of the complementary strands of DNA in the genes producing enzymes. The mRNA on the reading strand of DNA might cause some steric hindrance of recombination, but the other strand should be free of such hindrance. This model predicts that regulation of enzyme formation, in part or in whole, involves regulation of mRNA synthesis. This conclusion was also suggested on the basis of experiments measuring specific mRNA production from induced or noninduced operons. It was shown that mRNA specific for lactose (ATTARDI, NAONO, ROUVIERE, JACOB and GROS 1963; GUTTMAN and NOVICK 1963, HAYASHI, SPIEGELMAN, FRANKLIN and LURIA 1963), galactose (ATTARDI *et al.* 1963), histidine (MARTIN 1963), and tryptophan (IMAMOTO, MORIKAWA and SATO 1965) operons accumulated when the relevant enzymes were being synthesized, but not under conditions of repression. Although this did *not* necessarily mean that any difference in mRNA production exists between induced and noninduced genes, less direct methods indicate that the synthesis of mRNA specific for the *lac* operon of *E. coli* (KEPES 1963; NAKADA and MAGASANIK 1964; KEPES and BEGUIN 1966) and histidase operon of *Bacillus subtilis* (HARTWELL and MAGASANIK 1963) does proceed on induction and cease on removal of inducer.

Models have been presented in which regulation of enzyme synthesis involves control of mRNA translation into protein (e.g. STENT 1965; GRUBER and CAM-

PAGNE 1965). The results in this paper do not rule out control at that level, but they do show that control of enzyme synthesis extends to the level of the DNA.

I thank G. BERTANI for his hospitality and criticisms, O. MAALOE for suggesting the problem, and E. ENGBERG for providing some of the mutants. Support from a National Science Foundation Postdoctoral Fellowship is gratefully acknowledged. This investigation was supported in part by Institutional Research Grant No. IN-40F to The University of Michigan from the American Cancer Society, a Horace Rackham Faculty Research Grant, and NSF Grant GB-4542.

SUMMARY

The frequency of transductants resulting from intragenic recombination of two genes of the L-arabinose gene-complex was increased if arabinose, the inducer of enzyme synthesis from these genes, was present soon after infection. This frequency was high in the absence of arabinose if the recipient contained an additional mutation leading to constitutive production of arabinose-specific enzymes. Other sugars had no effect on *ara* gene recombination and arabinose did not affect recombination in the adjacent *leu* genes. These results indicate that control of arabinose-specific enzyme synthesis involves control of mRNA synthesis. Enhanced recombination in active genes is probably due to increased single-stranded areas of DNA and/or more single-strand breaks.

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