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Studies on Repression of Arginine Biosynthesis in *Escherichia coli*

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INTRODUCTION

For the biochemical geneticist who is interested in the physiological action of the gene, the discovery of repression introduced an unwelcome complication. Prior to this, it was generally accepted that the primary action of a gene is to control the production of a single enzyme. Since then, as a result of studies on the genetic control of repression and induction of enzymes, we have come to recognize two kinds of genes: structural genes, which conform to the above picture of the gene; and regulating genes whose action is pleiotropic, affecting a series of metabolically related enzymes. Both types of genes function together in the production of an enzyme. In order to understand gene action it becomes necessary to analyze separately the contribution of each type of gene to the formation of an enzyme.

I first became aware of the complication introduced by repression in 1956, during a search for mutants affecting the nature of the enzyme-forming mechanism. Previously I had found in the case of a temperature-sensitive pantothenate auxotroph that a mutation may result in the production of an altered enzyme protein with increased heat-lability (Maas and Davis, 1952). Subsequently, I looked for mutations affecting the nature of the enzyme-forming mechanism, rather than the structure of the enzyme. I found such mutants, which were "cold-sensitive," being able to produce ornithine transcarbamylase (OTC), the enzyme that converts ornithine to citrulline, at 37°C, but not at 25°C. The enzyme produced at 37°C did not differ from that of the wild type. During the study of these mutants I found that OTC was produced in significant amounts only when arginine was absent from the growth medium, whereas in the presence of arginine only traces of the enzyme were formed. This was true not only for the mutants but also for the wild type. In order to understand how the mutation affected the nature of enzyme formation, it became necessary to find out how arginine affected enzyme formation. I am still engaged in trying to understand this controlling action of arginine. The present paper will describe what I have learned about this mechanism since the original observation in 1956.

I. EFFECT OF THE CONCENTRATION OF INTRACELLULAR ARGININE ON THE SYNTHESIS OF OTC

When arginine is added to a culture of *E. coli* growing in a mineral salt-glucose (minimal) medium, the rate of formation of OTC is greatly reduced (Maas, 1956). This effect is specific for arginine; neither citrulline nor ornithine inhibits enzyme synthesis. Furthermore, arginine inhibits enzyme formation in a mutant blocked between citrulline and arginine. If, after growth in the presence of arginine, the bacteria are washed and reinoculated into minimal medium without arginine, the formation of OTC is resumed at a high rate. It was the unusual kinetics of enzyme formation observed during this resumption of growth which first suggested that not only added but also endogenously produced arginine is able to repress the formation of OTC (Gorini and Maas, 1957).

To test the hypothesis that endogenously formed arginine is able to repress enzyme synthesis, conditions had to be found under which the intracellular level of arginine could be reduced below that of bacteria growing in minimal medium. This was achieved by growing an arginine auxotroph, blocked in a step before the OTC reaction, in a chemostat (Novick and Szilard, 1950) with arginine limiting the growth rate (Gorini and Maas, 1957). It was found that under these conditions the rate of OTC formation was about 25 times greater than in bacteria growing in minimal medium. In control experiments it was shown that this rise in the rate of OTC formation was not a consequence of the reduced growth rate imposed by the conditions of growth in a chemostat.

Subsequently, two more direct methods were found to demonstrate repression by endogenously produced arginine. The first involved the use of a mutant with a partial block (bradytroph) before the OTC reaction. Such a mutant will grow slowly in minimal medium, and its growth rate can be raised to that of the wild type by supplying arginine. Thus, in minimal medium, the endogenous production of arginine limits the growth rate. Under these conditions it was found that, as in the chemostat experiment, the rate of formation of OTC was increased 25 times over that

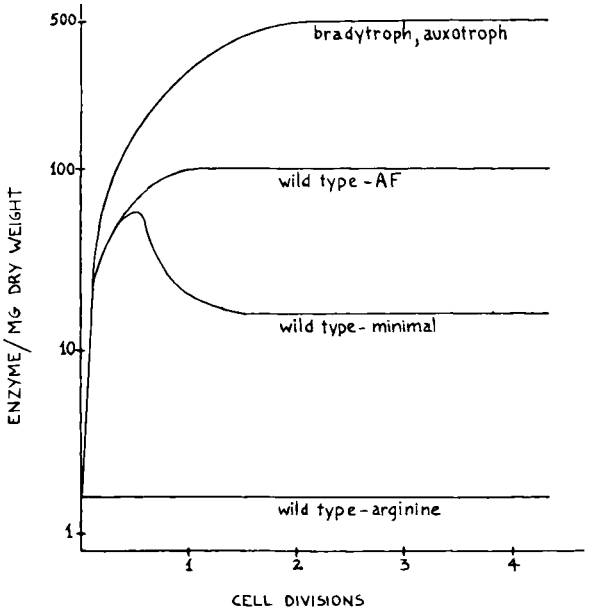

FIGURE 1. The effect of different media and conditions of growth on OTC production. For explanation, see text. Methods used were the same as those described in the legend of Table I.

rate, due to a limitation in the supply of carbamyl phosphate, a common precursor of arginine and pyrimidines (Novick and Maas, 1961).

From these experiments it was concluded that repression can be exerted by endogenously formed as well as exogenously supplied arginine and that the degree of repression depends on the intracellular concentration of arginine. The reason for not being able to demonstrate this graded response with added arginine is the presence in the bacteria of a specific concentrating mechanism (permease) for arginine and other basic amino acids which establishes a higher intracellular level of arginine even at a very low (1 μg/ml) concentration outside (Schwartz, Maas, and Simon, 1959). Figure 1 summarizes the various levels of enzyme reached in the media described above, after prior growth in the presence of arginine.

II. THE EXTENT OF REPRESSION IN THE ARGININE PATHWAY

There are seven steps in the pathway of arginine biosynthesis, starting from glutamate, the first specific precursor of arginine (Fig. 2). We have measured in cell-free extracts three of the enzymes (5, 6, 7)

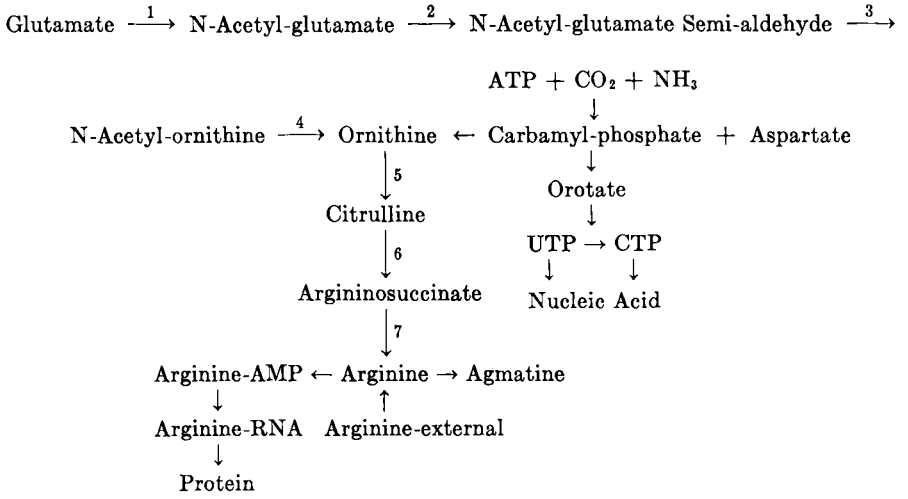

FIGURE 2. The pathway of arginine biosynthesis and neighboring reactions.

of the wild type growing in minimal medium (Novick and Maas, 1958, 1961). In the second method, a limitation of the endogenous formation of arginine was imposed in the wild-type strain by growing the bacteria in an enriched, but arginine-free (AF) medium (Novick and Maas, 1958, 1961). In this medium the bacteria grow about twice as fast as in minimal medium. Addition of arginine does not further accelerate growth. Yet, the rate of OTC formation is about 6 times faster than in minimal medium. It was shown that under these conditions the rate of arginine synthesis is decreased relative to the growth

catalyzing these reactions and have found the formation of these enzymes to be repressible by arginine (Table 1). Wiame and Bourgeois have found similar results for enzymes 2, 3, and 4. The details of the experiments will be published jointly.

The extraction of the remaining enzyme (1) which acetylates glutamate has been described (Maas, Novelli, and Lipmann, 1953), but so far the activities found in extracts have been too weak to permit determinations of the amounts of enzyme formed under various conditions of growth. Since we have found repression for six enzymes, we conclude tenta-

tively that arginine is able to repress the formation of all the enzymes that are specifically concerned with its biosynthesis.

We have also tested the repressibility by arginine of some of the reactions peripheral to arginine biosynthesis. These include the arginine-activating enzyme, which catalyzes the formation of arginine-sRNA via arginine-AMP (Boman, Boman, and Maas, 1961), the permease for arginine (Schwartz, Maas, and Simon, 1959), and carbamyl phosphate synthetase (Schwartz and Maas, 1959). The formation of none of the enzymes involved in these reactions was found to be affected by the presence of arginine in the growth medium. The formations of carbamyl phosphate synthetase and of the arginine permease were measured also in an arginine auxotroph growing with limiting arginine in a chemostat and were found not to be affected by deprivation of arginine. We conclude from these findings that arginine represses the formation of only those enzymes involved specifically in its own biosynthesis.

It may be seen in Table 1 that, although conditions of lowered intracellular concentration of arginine result in a release of repression of each enzyme, the magnitude of these releases is not the same for all enzymes. Particularly, the level of OTC increases much more than those of the other enzymes. Ames and Garry (1959) have shown that in the histidine pathway under conditions of release of repression, the levels of all the enzymes involved in histidine biosynthesis rise to the same extent. They have named this phenomenon coordinate repression. The genes which control the formation of the enzymes involved in histidine biosynthesis have been shown to be next to each other on the linkage map (Hartman, Loper, and Šerman, 1960). It has been suggested that this remarkable type of arrangement of genes forms the basis for a coordinate control of enzyme formation. The genes controlling the production of several enzymes of arginine biosynthesis have been found not to be linked. The results of the mapping experiments will be described in the next section.

III. MAPPING OF ARGININE AUXOTROPHS

In order to map the genes controlling the formation of the enzymes in the arginine pathway, it is necessary to have mutants blocked in these reactions. In *E. coli* K-12 we have obtained mutants for all of the reaction steps except 3 and 5. The positions of the blocks in the pathway have been determined by the nutritional requirements of the mutants, by syntrophism tests, and, for all except those blocked in step one, by absence of enzyme activity in cell-free extracts (Wiame, Bourgeois, and Maas, unpublished).

So far no mutants blocked in step 3 have been isolated from any wild-type strain of *E. coli*. This step involves a transamination reaction between N^{α}-acetylglutamic-semialdehyde and glutamate to form N^{α}-acetylornithine and α-ketoglutarate. Possibly the transaminase catalyzing this reaction is also involved in other transaminations, so that a block in this enzyme would result in a requirement for several growth factors. This may explain the failure to isolate mutants for this step. Mutants blocked in step 5 (OTC) have been isolated from *E. coli* W and *E. coli* B. Although we have searched intensively we have not found any in *E. coli* K-12. It seems unlikely that OTC is involved in other metabolic reactions; we have no explanations for the failure to isolate such mutants in K-12.

The positions of the genes for enzymes 2, 4, 6, and 7 on the linkage map of K-12 are shown in Fig. 3. For the purpose of orientation a number of other loci are indicated. It can be seen that three of the arginine loci are located near a gene for methionine synthesis and one near the locus for streptomycin resistance. The genes for some of the other enzymes have been

TABLE 1. REPRESSION BY ARGININE OF ENZYME FORMATION IN *E. coli* K-12

The numbers represent units of enzyme per mg of protein. One unit is the amount of enzyme which produces one μmole of product in one hour at 37°C. The enzyme preparations were sonic extracts. The bacteria were grown for at least four divisions in the medium indicated and harvested during the exponential growth phase.

Strain	Enzyme No.	Medium	
		AF	AF + arginine, 200 μg/ml
AB 313	5	382	1.7
	6	1.0	0.3
	7	2.7	0.3

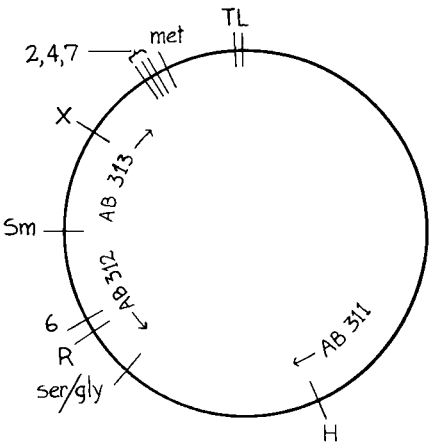

FIGURE 3. Map of genes controlling arginine biosynthesis. Explanation of symbols: T = threonine; L = leucine; met = methionine; X = xylose; Sm = streptomycin; ser/gly = serine or glycine; H = histidine; R = canavanine resistance. For further explanations, see text.

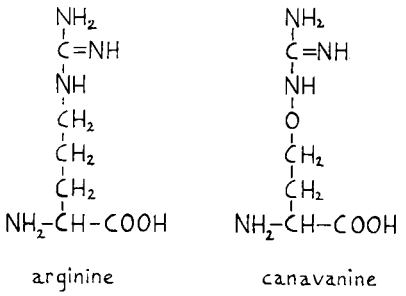

FIGURE 4. The structures of arginine and canavanine.

mapped by Gorini (1961) in *E. coli* B and have also been found to be in several regions of the map. It is thus clear that the genes controlling arginine biosynthesis are not all together in one block although some of them are clustered in one region. The mapping described here was carried out for step 6 by measuring in Hfr × F^- crosses time of entrance of the gene and linkage to other markers, and for the other three steps linkage to methionine in Hfr × F^- crosses and in transductions (Lavalle, Jacob, and Maas, unpublished).

IV. MUTATIONS AFFECTING REPRESSIBILITY

A. Method of Isolation of R Mutants

We have studied a number of mutants with a defective repression mechanism (R mutants) all of which were isolated by selection for resistance to the inhibition of growth by canavanine. Our reason for thinking that canavanine resistance may involve an alteration in repressibility was based on what we have learned about the effects of canavanine on growth. We shall therefore summarize our information on the mode of action of this compound before describing the isolation of R mutants.

Canavanine, a naturally occurring amino acid, is closely related in its structure to arginine (Fig. 4). It inhibits growth by competitively interfering with the utilization of arginine in protein synthesis (Schwartz and Maas, 1960). However, it does not inhibit the formation of all proteins. As a result, cell mass increases linearly rather than exponentially with time. Like arginine it prevents the formation of enzymes in the arginine pathway. It also inhibits the formation of at least one other enzyme, β-galactosidase.

At first we thought that canavanine did not repress enzyme formation in the same way as arginine, since it also affects the production of other enzymes. However, on the basis of recent findings with R mutants to be reported below, we now feel that canavanine does repress enzyme formation in the same manner as arginine and that its inhibition of the formation of other enzymes involves actions at different sites. Furthermore, we have come to the conclusion that the inhibition of growth, at least under some conditions, is due to its action as a repressor.

Originally, our reason for using canavanine resistance in the screening for R mutants was the following: since added arginine prevents canavanine inhibition, a mutation leading to increased endogenous production of arginine should have the same effect. Such a mutational change may result from lowered repressibility giving rise to higher levels of arginine-forming enzymes. We therefore looked among canavanine-resistant mutants for increased arginine production, using the feeding of an arginine auxotroph as a test. We found that about ⅓ of the mutants isolated in K-12 fed the indicator strain heavily. When these were tested for repressibility of OTC it was found that indeed the formation of this enzyme was no longer repressible. We refer to the arginine excreters as R_1 mutants.

To our surprise, we found that in some of the non-excreters, to which we refer as R_2 mutants, the repressibility by arginine was also decreased. Moreover, mapping experiments, to be described below, showed that all canavanine-resistant mutations were located in the same region of the chromosome. These included R_1 mutants and R_2 mutants with decreased repressibility as well as other R_2 mutants with unchanged repressibility. From these findings we conclude tentatively that all of these mutations affect the same mechanism. Since in R_1 mutants repressibility by arginine is clearly altered, the mutation in R_2 strains must also affect the arginine repressor mechanism. Since in R_2 strains with unchanged repressibility by arginine the only recognizable effect is the absence of repression by canavanine, this must mean that canavanine acts via the same mechanism of repression as arginine. To summarize, all canavanine resistant mutants of K-12 described are altered in the arginine repression mechanism in such a way that canavanine no longer represses enzyme formation. In some of them (R_1 and some R_2) repressibility by arginine is also decreased, whereas in others (the remaining R_2) it is unaltered.

B. Properties of R Mutants of K-12

1. *Repressibility*

In R_1 mutants of K-12, grown in the absence of arginine, we have found the level of enzymes 5, 6, and 7 to be as high or higher than the completely derepressed level found during arginine-limited growth in the chemostat (Table 2). The presence of arginine during growth slightly represses the formation of enzymes 6 and 7 but not of 5. Wiame and Bourgeois have found that the formation of enzymes 2 and 4 is similarly raised and only slightly affected by arginine. We think, on the basis of these results, that the mutation in these strains affects the repressibility of

all 7 enzymes. We have tested the arginine permease in these strains and found it to be the same as that of the wild type. The loss of repressibility is therefore not due to the inability of the bacteria to concentrate arginine.

It should be noted in Table 2 that the level of OTC in R_1 mutants is twice as high during growth in minimal medium as during growth in AF medium. The bacteria grow twice as fast in AF medium as in minimal medium. We shall return to the significance of this finding in the Discussion.

Our studies on R_2 mutants have begun recently, and we have measured so far only enzyme 5 in all of them and enzymes 6 and 7 in a few. As mentioned before, in some of them repression by arginine is decreased, in others it is not. In Table 2 one of each type is shown, with the enzyme levels of the parent wild type indicated in parenthesis. For those R_2 mutants with lowered repressibility by arginine (R_2[b] in Table 2), the lack of repression is more pronounced during growth in minimal medium than in AF medium. This difference seems to be related to the growth rate of the bacteria, since we have found that bacteria that were growing more slowly than usual in minimal medium (due to a change in the pregrowth conditions) were not repressible at all by arginine. It appears as though the slower the growth rate the more difficult it becomes for arginine to repress enzyme formation.

The arginine permease activity in R_2 mutants, in contrast to R_1 mutants, is slightly lower (10–20 per cent) than in the wild type. It is unlikely, however, that this decrease in the concentrating mechanism is responsible for canavanine resistance. Since we do not have radioactive canavanine available, we cannot measure the uptake of canavanine directly but only indirectly via its inhibitory effect on the uptake of arginine. The per cent of inhibition was found to be the same for R_2 mutants as for the wild type, thus indicating that canavanine is taken up by the R_2 cells. Furthermore, we have isolated in the W strain of *E. coli* canavanine-resistant mutants with a truly deficient permease for basic amino acids (Schwartz, Maas, and Simon, 1959). Here, the uptake of arginine was reduced about 5-fold over that of the wild type, and canavanine in the mutant no longer prevented the uptake of arginine.

2. *Mapping of R mutants*

For the mapping of the R mutations we isolated canavanine-resistant mutants from the 3 Hfr strains described by Taylor and Adelberg (1960). The head end of the entering chromosome and the direction of injection for the 3 strains is indicated in Fig. 3. The marker genes used in the crosses were threonine (T), leucine (L), histidine (H), xylose (X), and canavanine resistance (Can). The crosses were carried out as follows:

Hfr T^-, L^-, X^+, H^+, Can^r × F^-T^+, L^+, X^-, H^-, Can^s.

The mating was interrupted at 10 minute intervals, using T_s, as described by Hayes (1957). The time of entrance of X^+, H^+ and Can^r were scored. We found that in both R_1 and R_2 mutants ([a] and [b]) isolated from the same Hfr, Can^r entered at the same time, near the time of entrance of X. Thus, in AB312 crosses, Can^r entered 5 minutes after mixing of the strains, X^+ after 20 minutes. In crosses with AB313, X^+ entered after 10 minutes, Can^r after 25 minutes. In our crosses the time of entrance of H^+ and X^+ was in excellent agreement with the time reported by Taylor and Adelberg. We conclude from our findings that the R mutations we have mapped are located in the same genetic region, possibly in the same cistron. If this turns out to be true, it is likely, as we have mentioned before, that in all of these mutants the same biochemical mechanism is affected. Since in R_1 mutants we have found that the defect affects repressibility of most, and very likely, of all the arginine forming enzymes, we may assume that the R_2 mutants are similarly pleiotropic.

TABLE 2. THE LEVELS OF ARGININE-FORMING ENZYMES IN R MUTANTS OF *E. coli* K-12

The methods used here were the same as those described in the legend of Table 1.

Type of Mutant	Enzyme No.	Medium		
		AF	AF + arginine, 200 μg/ml	Minimal
R_1 (from AB 313)	5	1090	1045	2000
	6	2.4	3.0	3.5
	7	8.0	7.0	10.0
R_2 (a) (from AB 313)	5	300 (382)	2.0 (1.7)	
	6	0.9 (1.0)	0.2 (0.3)	
	7	2.6 (2.7)	0.2 (0.3)	
R_2 (b) (from AB 312)	5	500 (292)	61 (6.6)	

C. R MUTANTS IN STRAIN B

The wild-type B strain has a repressibility pattern which is very different from that of K-12. As shown by Gorini and Gundersen (1961), added arginine does not repress enzyme formation. However, that a repression mechanism for the arginine-forming enzymes exists is indicated by the occurrence of mutants in which the level of these enzymes is raised. In this connection it may be pointed out that the level of OTC in bacteria grown in the absence of arginine is considerably lower in B than in K-12; however, in the

TABLE 3. THE LEVELS OF ARGININE-FORMING ENZYMES IN *E. Coli* B AND R MUTANTS OF B

The methods used were the same as those described in the legend of Table 1.

Type of Strain	Enzyme No.	Medium	
		AF	AF + arginine, 200 μg/ml
B Wild Type	5	19	16
	7	0.78	0.99
Type 1 R	5	870	720
	7	8.4	5.4
Type 2 R	5	276	36
	7	1.35	0.75

mutants of Gorini, the level is about the same as in R_1 mutants of K-12.

We have isolated canavanine-resistant mutants in B and have found again two types in regard to the pattern of repressibility, similar to the R_1 and R_2 mutants in K-12. So far we have examined only enzymes 5 and 7 in these strains. In the first type, the enzyme level is as high as in R_1 mutants of K-12 and is not affected by growth in the presence of arginine. In the second type, the level of enzyme is not as high as in the first type, but considerably higher than in the wild type; here enzyme formation is repressed by arginine, as in the R_2 mutants (Table 3). These results confirm Gorini's finding of repression in strain B and show that this mechanism can be affected by mutation in a similar fashion as that of K-12. The reason for the difference in repressibility pattern between the two wild-type strains remains obscure. Gorini (1961) has provided evidence for the existence of 2 independent genes to account for the differences in both level and repressibility. Our results, on the other hand, suggest that the differences arise from different mutational states of one gene.

V. DISCUSSION

A. MECHANISM OF REPRESSION

We have shown that in *E. coli* K-12 repressibility is controlled by a gene R which is located on the linkage map quite far from most of the other genes of arginine biosynthesis. The action of this gene is pleiotropic, affecting the formation of most, if not all, the enzymes of arginine biosynthesis. Its action must therefore be mediated via the cytoplasm. A similar conclusion has been reached for a gene controlling repressibility of tryptophan biosynthesis (Cohen and Jacob, 1959).

Several types of mutants affecting repressibility have been described, and from our mapping data we have concluded that all of these are due to mutation in the R gene. These strains were isolated as canavanine-resistant mutants. We have presented evidence that canavanine, like arginine, represses enzyme formation in the wild type. In all of the mutants the ability of canavanine to repress enzyme formation is lost. In some of them (R_1), the ability of arginine to repress enzyme formation is also lost; in other (R_2), it is either impaired or not affected at all. Thus there are a number of alleles of this gene which produce different states of repression. So far, we know very little about the biochemical mechanism underlying repression. We hope that a study of the characteristics of these mutants will help us to characterize the repressor system in biochemical terms. A beginning has been made in this direction by our finding that in R_1 mutants there is more transfer RNA for arginine than in the wild type (Boman, Boman, and Maas, 1961).

A model has been proposed for the mechanism of repression, called the operator model (Jacob, Perrin, Sanchez, and Monod, 1960), based largely on experiments with the β-galactosidase system; this model assumes that the structural genes governed by the same repressor are located next to each other on the chromosome. Repression is then exerted on the activity of these genes, via another gene, the operator, which is located adjacent to them. The activity of the operator, in turn, is controlled by a repressor substance formed through the action of yet another gene analogous to the R gene described above.

We see from our mapping of the structural genes that the operator model, as proposed above, does not hold for arginine biosynthesis. We may adapt the model by assuming that each of the structural genes has a separate operator and that the repressor substance acts on all of these. This type of mechanism is depicted in Fig. 5. Our finding of the absence of coordinate repression lends some support to the idea of several operators. In the histidine pathway, in which coordinate repression has been found, the genes are next to each other and are presumably controlled by a single operator. More definitive evidence could be obtained if one isolated a mutant in which a single operator was affected. In such a mutant, the rate of enzyme synthesis rather than the structure of the enzyme should be affected. One may look for such mutants among bradytrophs, isolated as slow-growing revertants from complete auxotrophs.

The evidence for the production of a specific repressor substance comes from the finding that in zygotes repressibility is dominant over non-repressibility. This has been demonstrated for the tryptophan pathway (Cohen and Jacob, 1959) and for the β-galactosidase system (Pardee, Jacob, and Monod, 1959). In these systems the structural genes are together on the linkage map. For the arginine system

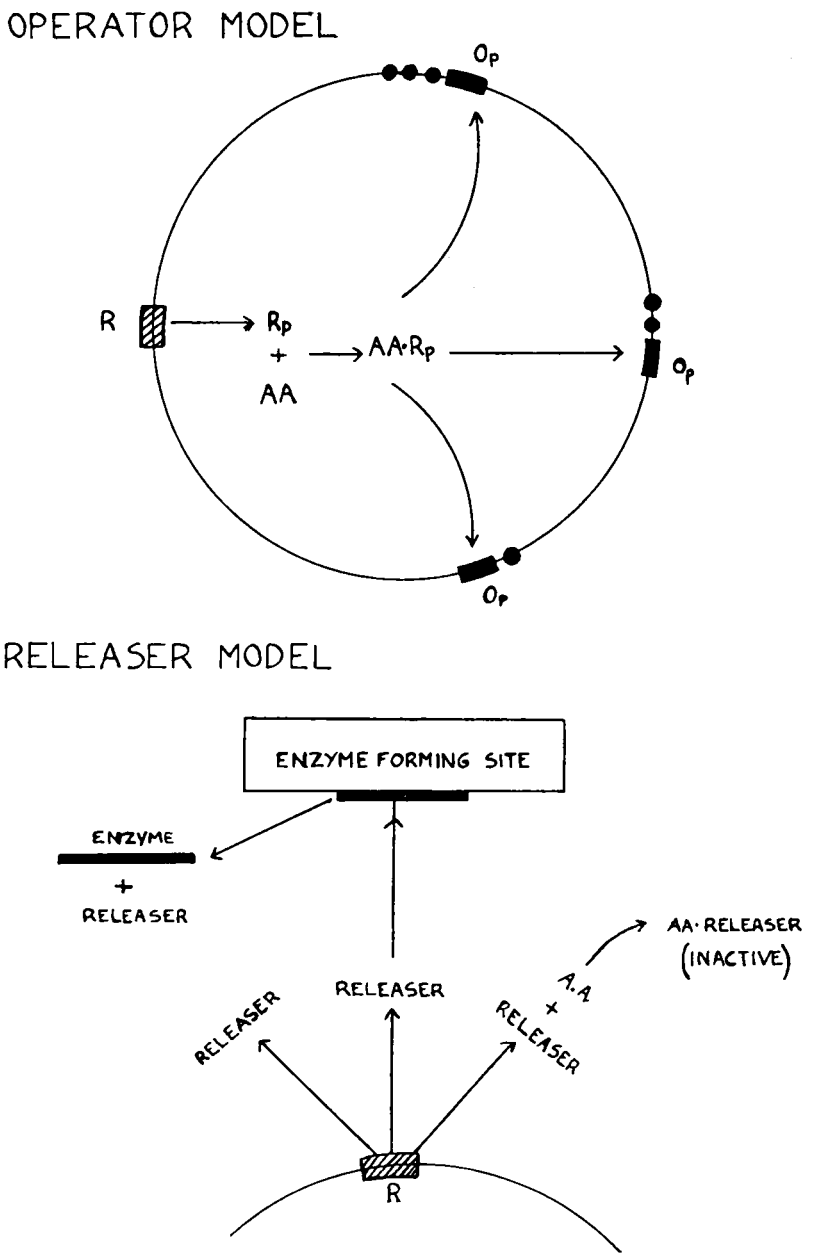

FIGURE 5. Postulated schemes for the mechanism of repression.

we do not know yet whether repressibility or non-repressibility is dominant. Because the structural genes are apart, it will be of interest to see whether or not the arginine system behaves differently.

Since we do not yet know the dominance relations for the R alleles, I would like to propose an alternative model for the mechanism of repression, in which we do not have to assume the production of a specific repressor substance. Although this scheme is pure speculation, it may be useful in our present state of ignorance in regard to the arginine system to consider other possibilities besides the operator model. This scheme is called the Releaser Model and is depicted in Fig. 5. Here repression occurs not on the level of the gene, as in the operator model, but on the level of the enzyme-forming site, presumably on the ribosome. The model postulates that the action of a specific releaser substance is necessary to liberate the enzyme protein from the ribosome. This releaser substance would be the primary product of the R gene. Besides being able to combine with the enzyme and release it from the ribosome, it can also combine with arginine. Once combined with arginine, it is no longer able to release enzyme from the ribosome. As a consequence, enzyme formation is repressed. In mutants with decreased repressibility, the R gene produces an altered releaser substance which, although still able to release the enzyme, has partially or completely lost the ability

to combine with arginine and thus to repress enzyme formation. According to this model, repressibility should not be a dominant trait. Zygote experiments in the arginine system should therefore provide a test for the validity of the releaser hypothesis. Superficially, the releaser model resembles that proposed for induction before it was shown that inducers act by preventing repression. Actually, we postulate here the production of a substance by the R gene which is neither an inducer nor a repressor. Repression results when this releaser substance combines with arginine. Induction results when another metabolite, for instance ornithine (Gorini, 1960), prevents the combination of releaser with arginine.

B. Control of Enzyme Formation in the Absence of Repression

In the introduction we have mentioned that the phenomenon of repression presents a complication if one wishes to study how a gene controls the production of a specific enzyme. This has been borne out by our experiences with the arginine system. However, since we are now able to obtain mutants no longer repressible by arginine, we can return to the original question.

For this purpose, we are beginning to analyze what factor or factors in R_i mutants limit the rate of enzyme formation. A possible clue was provided by the finding that the level of OTC, measured per mg of protein, is higher in slowly growing cells than in fast growing ones (Table 2). This may mean that, per cell, faster growing cells produce less OTC or, alternatively, more protein, than slowly growing ones. Actually the latter interpretation seems to be correct. It has been shown by Schaechter, Maaløe, and Kjeldgaard (1958) that, per bacterial nucleus, a doubling in the growth rate results in about a twofold increase in the protein content. Our results thus reinterpreted show that, whereas the general protein content of the cell rises with increasing growth rate, the OTC content remains constant.

In the above cited work of Schaechter *et al.*, it was shown that of the 3 major cell constituents, DNA, RNA, and protein, only the DNA content did not increase with increasing growth rates. Thus, the rate of OTC synthesis under conditions of different growth rates remains in step with the rate of DNA synthesis. This parallelism suggests that the formation of OTC, in R_i mutants, is under the control of DNA to a greater extent than the formation of the other proteins, most of which are presumably controlled by repressor mechanisms. Possibly the rate limiting factor for OTC formation in the absence of repressor is the supply of a direct gene product, such as messenger RNA.

SUMMARY

1. The rate of formation of the enzymes involved in arginine biosynthesis is controlled by the intracellular concentration of arginine. Direct evidence for this statement has been presented for six of the seven enzymes, and it is likely that the formation of the seventh enzyme is controlled in the same way. As the intracellular concentration of arginine decreases, the rate of enzyme formation increases. Quantitatively, this change is different for different enzymes, the rates of formation of some of them increasing more than those of others. Repression in this system is therefore not coordinate.

2. The genes controlling the formation of four enzymes have been mapped. Three of these are close together, the fourth is separated from the others. The genes for this pathway are thus not next to each other, as in some other biosynthetic pathways.

3. In a search for mutations affecting repressibility, mutants resistant to growth inhibition by canavanine (a structural analogue of arginine) were isolated. *Some* of these were altered in their repressibility by arginine. In *all* of them, the mutation was located in the same region of the linkage map, suggesting a change in the same gene. From this finding it is assumed that in *all* of the mutants the same biochemical reaction is affected.

4. We have studied three types of canavanine-resistant mutants. In all of them canavanine no longer inhibits formation of the enzymes of arginine biosynthesis. In one type repressibility by arginine is completely lost (demonstrated for five enzymes); in the second type, it is impaired; in the third type, it is as effective as in the normal strain. On the basis of these results we conclude that in the wild type canavanine represses enzyme formation via the same mechanism as does arginine.

5. The mechanism of repression is discussed in the light of the findings presented. It is concluded that the pleiotropic action of the gene controlling repressibility is exerted via a cytoplasmic substance. The operator model as proposed for the β-galactosidase system must be modified to be applicable to arginine biosynthesis. An alternative hypothesis, the releaser model, is described.

6. The question is raised: what factor limits the rate of enzyme formation in the absence of repression. Preliminary experiments are described suggesting that DNA or a direct product of DNA is this limiting factor.

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