"Mutants of Escherichia coli with Abnormal Patterns of Repression of Arginine Biosynthetic Enzymes."

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Abstract:

Experimental work was done to attempt to isolate further mutants of the arginine regulatory gene in Escherichia coli W of the same type as a strain known as W2-250. This mutant carries the argR* regulatory gene which confers upon it the diminished ability to repress or derepress its arginine biosynthetic enzymes.

Experimental work involved establishing a suitable method of exaggerating the slow growth rate of this strain on the arginine intermediate N-acetyl-L-ornithine, such that this could be the basis of protecting similar mutants when selecting them from a parent strain (W2-40) in a penicillin selection procedure. This was achieved by growing both strains in diphasic a medium containing 15ug/cm^3 of L-arginine and an excess (30ug/cm^3) of acetylornithine, in which it was found that the argR strain entered a premature stationary phase when the arginine was exhausted but the argR strain continued growth at a slower rate than normal in the acetylornithine. It was also found that a culture of W2-250 which had entered this stationary phase could be diluted 1:1 in fresh minimal medium + acetylornithine and still remain in a stationary condition for up to 24 hours.

Mutants of strain W2-40 produced using ultraviolet light were cycled twice through a system involving this stationary phase in diphasic medium and dilution 1:1 in fresh medium + 500 units of benzyl-penicillin/cm^3 in order to select against the parent-type. Further selection was carried out on minimal agar plates supplemented with acetylornithine and canavanine on which the parent strain will grow, but the argR* type is inhibited until arginine was added to the agar. The colonies which appeared at this stage were screened on variously supplemented solid media to select those most like W2-250.

Those selected on these criteria were then screened using the acetylornithinase assay and the diphasic medium, with W2-250 included for comparison.

A total of nine possible mutants of the desired type were isolated, but problems with the preparation of enzyme samples precluded a definite decision on their identity.

In the course of this work it was also discovered that an arg_8 bradytroph (W2-25/8) could behave similarly to W2-250 in diphasic media. It was also found that there was some transferable "factor" in the medium of cells which had reached the premature stationary phase which could prevent further growth of either W2-250 or W2-25/8 in non-repressive minimal medium. This is discussed in detail.

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Introduction:

The arginine biosynthetic pathway of Escherichia coli has eight enzyme catalysed steps in which L-glutamic acid is converted to L-arginine (56,65,67,68). There are eight corresponding structural genes, one for each of the eight enzymes (55,68) which are partially scattered and partially scattered at five separate loci on the genome (73). In addition there are genes involved in control (24) coding for permease[a] enzymes (59,73) and for five arginine transfer RNA's (12) and their synthetase (15). There is also an additional gene (arg I) for enzyme four (ornithine transcarbamylase) which has been mapped in E. coli strains B (22) and K-12 (53). (See tables AI & A-II in the appendix.)

The arginine pathway is controlled by a repression (58) mechanism in which the protein product (33,54) of the regulatory gene argR (the apo-repressor) interacts with the corepressor arginine (24), or even its precursors (6,43), and this then interacts with the protein synthesising machinery making the arginine biosynthetic enzymes, causing their cellular levels to decrease (45,58,61). The reverse of this (derepression) occurs in the absence of an external supply of arginine, although the system is sensitive to the internal levels of arginine (48). This form of control occurs in E. coli strains W, K and C (36), and is "negative" insofar as the regulatory gene product switches the structural genes off under appropriate conditions (1,2). However there is some recent evidence for the existence of a second regulatory gene for the arginine pathway in E. coli (8), this being the gene argM or arg 4' which was believed to code for an inducible form of ornithine transcarbamylase identical with that produced by arg 4 (5,19,37,63). It appears that a mutant version of this regulatory gene was responsible for causing the arg 4 gene to induce while it did not prevent repression of all the other structural genes (8). The gene for arginyl-t-RNA synthetase also the control of the arginine regulatory genes (15) as are the synthetases for leucyl, prolyl and methionyl t-RNA's which are under the

[a]Maas, W.K.(1965) Federation Proc. 24 - 1240

control of their respective regulatory genes (4). The arginine genes are seen to increase their cellular levels together at the onset of derepressive conditions (57,58) but in a co-ordinated but non-parallel fashion (61), unlike histidine genes which increase both co-ordinately and in parallel (3).

In strains where the internal supply of arginine from its intermediates is normal, the enzymes will reach a partially repressed "poised" level (48), but in strains where this supply is inadequate for normal growth (restrictive) the enzymes reach fully derepressed levels (45).

This behaviour applies to the similar arginine regulatory patterns of E. coli strains, W, K and C which have the $argR^+$ gene (36) but not to strains with other allelic forms of the $argR^+$. For instance the $argR^+_B$ allele found in E. coli strain B confers upon that strain the property of slight inducibility of all the arginine structural genes in the presence of arginine (24). An $argR^+_B$ allele can be converted by a single point mutation to an $argR^+$ repressible type (36), which suggests that induction and repression are closely related phenomena, a conclusion already arrived at from studies of the "inducible" ornithine-transcarbamylase (63). A single point mutation can also convert the $argR^+$ (repressible) to the $argR^-$, (50) which confers constitutiveness of the arginine structural genes: i.e. the genes are fully derepressed irrespective of arginine. Such $argR^-$ strains are recognised by their resistance to the arginine analogue canavanine (50). A derivative of an E. coli-W $argR^+$ strain is the strain W2-250 which has the unusual regulatory gene $argR^*$ and exhibits diminished repressibility and derepressibility of the arginine structural genes (Bacon: personal communication, 62,68); this feature resembles the control patterns of a tryptophan mutant except that this mutant has a lesion in the tryptophanyl-t-RNA-synthetase gene and not in the "tryp R" gene. Gorini and his coworkers (36) have produced some unusual $argR^+$(K-12)/$argR^+_B$ hybrid alleles which cause hybrid control patterns such as high level inducibility.

The arginine functional group of genes (a regulon[a]) because of their separateness on the genome are a marked contrast to most other known functional groups of genes which fall into single clusters termed operons. This latter term comes from the theory of Jacob & Monod (34) although the concept of operational cluster of contiguous genes came from Hartman (29). Jacob & Monod envisaged a regulatory gene coding for a soluble substance which had the function of switching off a group of contiguous genes when the correct conditions prevail by interacting with a control site at one end of the operon, termed the operator site. Such a model suits the histidine system with its single functional group of genes (3,39) but the arginine regulon is scattered, but is under the control of one regulatory gene, such that one operator or its equivalent must be present for each group of genes at least (61). In fact one operator mutant, the only one so far in the arginine system, has been isolated for the argI gene, and this shows diminished repressibility of the argI gene only (38,53). The single cluster of four arginine genes (21) is actually two regulatory groups (16), one of argE only, and the other of argC, B & H (10), the latter incidentally having its operator sited at the opposite end to that of other known multigene operons in E. coli (3,11,9,64). However it is possible that the arginine genes come together into a spatially close group in the cell, which would facilitate easier control (63,64).

In the original conception of Jacob & Monod's model, it was proposed that the regulatory gene product exerted its effect at the site of the operator-gene DNA, a situation called transcriptional control, which is true for the lactose operon. However there has since accumulated a certain amount of evidence about the arginine pathway which suggests that its regulatory gene product acts at the level of translation of m-RNA into protein. It is envisaged that the gene, the ribosome, the messenger and other factors form the protein-synthesising complex upon which the repressor and arginine act in concert during repression (Vogel-68, See Appendix, also 13, 28,52). This complex for the arginine system has even been seen (47) in the electron-microscope. The nature of the

[a]Maas, W.K. & Clark, A.J. (1964) J.Mol.Biol. 8 - 365

complex appears to vary with the job it is doing: its sensitivity to canavanine is different when it is synthesising acetylornithinase to when it is synthesising acetylornithine transaminase (19), which might give a clue to the pacesetting phenomenon (60) which is exaggerated in the argR$^+$ strain W2-250 indicating the argR$^+$ product may have a greater than normal affinity for the complex.

Studies of N-acetyl-glutamate-semialdehyde-dehydrogenase mutants show that some have diminished repressibility of the whole arginine pathway (8,51), which suggests involvement of this enzyme in the repression complex, whereas the first enzyme of the pathway is involved in feedback inhibition (26). Only some of the revertants of these mutants have regained normal repressibility patterns. The authors were unable to show that this enzyme binds arginine (8) although it has been shown that anthranilate synthetase in the tryptophan pathway will bind to the complex involved in repression which includes tryptophan[a], and histidine enzyme 1 binds histidine (11).

Studies of the possible involvement of arginyl-t-RNA or its synthetase in repression have yielded a negative result for all five t-RNA species (12,43), and the synthetase (31,32,70) unlike the reports of involvement of leucyl-t-RNA (30) histidyl-t-RNA (11) and other t-RNA species (39) in the control of synthesis of their respective amino-acids. The possibility of a small undetectable fraction of arginyl-t-RNA being involved in the repressive complex has not been ignored (31) and there has been one report of changes in the rates of arginyl-t-RNA charging under repressive and derepressive conditions (14).

Some very convincing evidence for translational control of the arginine pathway has come from Vogel and his co-workers (46,68,69) and others (71) as a result of studies using antibiotics to uncouple the various steps such that Vogel's workers managed to uncouple translation and transcription in the arginine system and to show repression of the latter by arginine. However the possibility of even indirect

[a]Somerville & Yanofsky (1965) Studies on the regulation of tryptophan biosynthesis in Escherichia coli, J.Mol.Biol.,11 p.747.

control of transcription would be difficult to ignore completely (19,71).

The actual mechanism of repression is unelucidated at present and probably will not be so until the working of the ribosome are a lot more fully understood, and in the case of arginine at least could be quite complex with so many "possibles" involved. For instance the argCBH messenger RNA is rapidly degraded in the presence of arginine, and may be even read more slowly as well (40), and another report suggests that the repressor substance itself may be more labile in the presence of repressive arginine concentrations (49). There is also some evidence to suggest the synthesis of some important factor during derepression growth, which combines with arginine at the onset of repression causing the levels of the enzymes to drop well below the expected repressed levels before eventually increasing to those levels after a few generations when the "factor" is diluted out (41,42). This suggests that derepression is not just a passive state of "non-control" but is in some way involved in pre-setting the cells for repressive conditions. Wozny et.al (71) have even suggested that translational repression in fact occurs after the initiation of translation which seems to be in keeping with the idea that all genes in a pathway were evolved from a single ancestor gene (Evolving Genes and Proteins. eds V. Bryson and H.J. Vogel (Academic Press: New York) 1965). This could mean that each protein of a given pathway has retained an amino-acid sequence in common with all the others in the pathway, and it is this common sequence which is the site for post-translation-initiation control by some influences involving arginine and the $argR^+$ product at least.

This thesis will deal only with one small part of this very wide field of the control of the arginine biosynthetic pathway in E. coli: this is the mutant strain W2-250 which has the abnormal regulatory gene argR , which means that the arginine structural genes can neither repress or derepress properly under appropriate conditions, (Bacon, personal communication, Vogel 62,68). I will show how I have attempted to isolate further argR mutants from the $argR^+$ strain, W2-40, by utilizing the pacesetting phenomenon in

diphasic medium (60. appendix figure A1) which is exaggerated in the strain W2-250. Then I have screened those mutants further on canavanine supplemented medium after they had grown in the presence of arginine, which differentiates between the $argR^{+}$ strain which is inhibited up to 50% of normal growth rate on such medium (45) and the $argR^{*}$ which is completely inhibited until arginine is added (Bacon: personal communication). Further studies of the nature of the argR were not planned but its growth patterns on diphasic media proved to be both useful and very interesting.

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