ONE-STEP MICRO-CONSTITUTIVE MUTATIONS IN THE PENICILLINASE REGION OF STAPHYLOCOCCUS AUREUS

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A number of micro-constitutive mutants have been obtained by treatment of the magno-inducible Staphylococcus aureus strain 8325-18 with ethyl methane sulphonate (Novick, 1963; Richmond, 1965a). The enzyme levels in the two strains examined here are 0.01% and 0.1% of the full magno-constitutive level, respectively, and the induction ratio is close to 1.0 in both cases (Table 1). These strains are particularly interesting since their genetic constitution does not seem to fit easily into the latest version of the 'operator' hypothesis for the control of enzyme synthesis (Jacob and Monod, 1961; Jacob, Ullman and Monod, 1964; Jacob and Monod, 1965).

Table 1. Specific enzyme activity (enzyme units/mg. dry wt. organisms) of the micro-constitutive mutants μ55 & μ56.

Strain No.	Phenotype	Specific enzyme activity (units/mg. dry wt. organisms)		Induction Ratio
		Uninduced	Induced	
8325($\alpha.i^+p^+$)	magno-inducible	10	300	30
8325($\alpha.i^-p^+$)	magno-constitutive	300	300	1
μ55	micro-constitutive	0.03	0.035	1.2
μ56	micro-constitutive	1.3	1.6	1.2

Three further points are important when considering the nature of these mutations:

(1) The mutants almost certainly do not synthesise 'muteins' (mutant proteins: Collins et al., 1965) since the enzymes synthesised by the 'micro' strains are indistinguishable from the wild-type enzyme by the criteria of relative rates of hydrolysis of various substrates, and reaction with specific anti-exo-penicillinase serum (Richmond, 1963; 1965b). The enzyme synthesised by strain μ56 has been purified and its specific activity is very close to that found for the purified wild-type enzyme (Richmond, 1963). There is, therefore, good prima facie evidence that the micro-constitutive mutations do not lie in the penicillinase structural (p) gene.

(2) The mutants are probably the result of point mutations and not deletions, since revertants to the magno-inducible phenotype of the parent have been obtained spontaneously from μ56 at a frequency of about $1{:}5 \times 10^{8}$.

(3) The mutations can be shown, by the criteria of co-transduction and co-elimination (Novick, 1963; Richmond, 1965a; Novick and Richmond, 1965) to lie on the same penicillinase plasmid as the genes responsible for the structure (p) and inducibility (i) of penicillinase. Mapping experiments show that the mutation in strain μ56 lies very close to another mutation known to lie in the p gene, and probably between the p- and i-regions.

The behaviour of these micro-constitutive mutants might be interpreted in terms of the original version of the operator'

hypothesis for the control of enzyme synthesis (Jacob and Monod, 1961) as due either to an altered repressor which now no longer recognises the inducer yet binds to the operator site more strongly, or to an altered operator which no longer recognises the *i*-gene product and is also very much less effective at initiating expression of the structural gene. In practice, it is possible to distinguish between these two possibilities by examining the behaviour of the genome bearing the micro-mutation when present with a magno-constitutive (i^-) genotype as part of a plasmid diploid (Richmond, 1965c; Novick and Richmond, 1965).

An appropriate diploid culture was constructed by using strain μ56 as recipient in a transduction experiment in which

Table 2. Physiological characters of parent strains, diploids and segregants in an experiment in which phage from strain 147(ß.$i^-_{223}p^+$) was used to construct a diploid with the micro-constitutive strain μ56.

	Specific enzyme activity (enzyme units/mg. dry wt. bacteria) Uninduced	Induced
Parent strains		
147(ß.$i^-_{223}p^+$)	161	298
micro-constitutive, μ56	1.2	1.6
Diploid		
147(ß.$i^-_{223}p^+$)/μ56	5.6	271
Segregants		
8325(ß.$i^-_{223}p^+$)	142	307
micro-strain (μ56)	1.4	1.4
Stock strains		
8325(ß.$i^-_{223}p^+$)	161	319
147(ß.i^+p^+)	7.1	258

phage was obtained by irradiating the magno-constitutive strain 147(ß.$\underline{i}^{-}_{223}\underline{p}^{+}$) (see Novick and Richmond, 1965, for this nomenclature). Table 2 gives the physiological characteristics of the parent, diploid and segregant strains obtained. The presence of the µ56 genome in the diploid repressed the expression of the magno-constitutive genotype, in the absence of inducer, close to that found in the uninduced wild-type strain 147(ß.$\underline{i}^{+}\underline{p}^{+}$). These results show that the micro-constitutive mutation in strain µ56 has not altered the properties of, and cannot therefore exist in, the penicillinase i-gene. Similar results have been obtained with strain µ55.

Another possibility is that some other enzyme - such as one converting penicillin to an effective intracellular form - could be involved in the system and that this enzyme is impaired by the micro-mutation. If this were so the ability to express the structural gene cis to a micro mutation should be restored by the wild-type allele acting trans in a diploid. Examination of induced diploids of the type 147(α.µ56/ß.$\underline{i}^{-}_{223}\underline{p}^{+}$) shows that less than 3% of the penicillinase synthesised is of the immnulogical A-type characteristic of the α-plasmid, whereas about 50% of the enzyme would have been of this type had the effect of the micro-constitutive mutation been relieved by a trans effect (see Richmond, 1965c, for methods).

Within the framework of the Jacob and Monod (1961) hypothesis, and in view of its probable map location between i and p, the point mutation in strain µ56 would be in the operator locus, and be responsible, at one and the same time,

for the abolition of repressor recognition and for a marked lowering of the expression of the penicillinase structural gene. Such a duality of effects was envisaged in the original definition of the operator (Jacob and Monod, 1961) but recent work has now led Jacob and Monod (1965) to suggest that these two operator functions are separate and to propose a new locus the promoter region - which is responsible for the expression of the operon. This new hypothesis implies that the o-locus, which recognises the repressor, is concerned neither with the initiation, nor with the rate of 'reading' of the operon. However, if the Monod/Jacob hypothesis is to be retained as an interpretation of the behaviour of the staphylococcal penicillinase system, the properties of these particular micro-constitutive mutants are not really compatible with the new postulate which appears to require the complete separation of 'promoter' and 'repressor-recognition' functions.

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