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Biotin-requiring Mutants of *Escherichia coli* K-12

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Mutants of *Escherichia coli* requiring biotin have been studied by several authors. C. H. Gray and E. L. Tatum (Proc. Natl. Acad. Sci. U.S., **30**:404, 1944) isolated a mutant that was closely linked to *gal* and to the λ prophage attachment site *attL* (J. Rothman, J. Mol. Biol. **12**:892, 1965). M. Schwartz (J. Bacteriol. **92**:1083, 1966) found that some λ-resistant mutants have a biotin requirement, presumably because of a deletion extending into a biotin gene. As the deletion also included the *malA* locus, this biotin gene would be near *malA* and *str*. A mutant of the Crookes strain of *E. coli* has been the object of detailed physiological (but not genetic) study (E. H. Pai and H. C. Lichstein, Biochim. Biophys. Acta **100**:43, 1966).

We have isolated nine biotin-requiring mutants from a prototrophic strain (R881) of *E. coli* K-12 treated with nitrosoguanidine, by the method of E. A. Adelberg, M. Mandel, and G. C. C. Chen (Biochem. Biophys. Res. Commun. **18**:788, 1965). These mutants grow on minimal medium supplemented with biotin but fail to grow (or grow very poorly) on minimal medium alone. We have studied these together with the Tatum mutant (strain W602, kindly given to us by J. Rothman). The W602 strain requires leucine and thiamine; these supplements were routinely added in all tests.

All 10 of these mutants are transducible by phage λ. A collection of high-frequency-transducing (HFT) variants of λ were isolated on W602 (G. Kayajanian, *in preparation*). Table 1 shows the ability of various HFT isolates to transduce the *bio* mutants of our collection. The transduction results are compatible with the following gene order: *attL*, (*bio-0*, *bio-4*, *bio-24*), (*bio-2*, *bio-17*), *bio-12*, *bio-3*, *bio-23*, *bio-18*, *bio-19*.

The mutants were grouped into nutritional classes by feeding tests. Washed cells of different cultures were spread onto adjacent sectors of a minimal agar plate. If growth of culture I was markedly stimulated along the margin of culture II, we say that II feeds I. We presume that feeding results from excretion of biotin or related compounds by culture II.

Wild-type *E. coli* is known to secrete various biotin-related compounds, some of which, like biotin, combine with avidin (C. H. Pai and H. C. Lichstein, Biochim. Biophys. Acta **100**:28, 1965). To test whether the mutants could be fed by avidin-uncombinable compounds, feeding plates were prepared with avidin incorporated into an agar overlay.

The results of feeding tests, with and without avidin, are shown in Table 2. The mutants fall into four nutritional classes: A (*bio-0*); B (*bio-2*, *bio-17*); C (*bio-3*, *bio-12*, *bio-18*, *bio-23*), and D (*bio-19*). Each class comprises a connected group on the genetic map. The mutants *bio-4* and *bio-24* are not included. The *bio-4* mutant is very leaky, and *bio-24* was isolated recently and has not yet been studied thoroughly. They seem to be similar to *bio-0* in most respects. The simplest scheme compatible with all the data is

TABLE 1. *Transduction of biotin-requiring mutants by high-frequency-transducing lysates of λ*[a]

No. of independently isolated transducing phages	Allele no. of *bio* mutation in recipient									
	0	4	24	2	17	12	3	23	18	19
4	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	−	−	−	−	−
1	+	+	+	+	+	+	−	−	−	−
2	+	+	+	+	+	+	+	−	−	−
3	+	+	+	+	+	+	+	+	−	−
20	+	+	+	+	+	+	+	+	+	−
8	+	+	+	+	+	+	+	+	+	+

[a] Lysates of the transducing phages (containing helper λ phage when the transducing phage was defective) were spotted onto lawns of mutants on minimal dextrose-tetrazolium plates, supplemented with leucine and thiamine, and observed over a period of 1 week or more for the appearance of transductants within the area of lysis. Symbols: + = transductants; − = no transductants. Each transducing phage listed was a separate isolate from low-frequency transduction. The *bio* mutation in strain W602, on which all the transducing phages were isolated, is designated as *bio-0*.

TABLE 2. *Results of feeding tests*

Allele in strain being fed	Ability of strains carrying indicated *bio* alleles to act as feeders									
	With avidin	Without avidin								
	bio$^+$	*bio*$^+$	*bio-2*	*bio-17*	*bio-0*	*bio-19*	*bio-3*	*bio-12*	*bio-18*	*bio-23*
bio-2	−	+	−	−	−	−	−	−	−	−
bio-17	−	+	−	−	−	−	−	−	−	−
bio-0	−	+	+	+	−	−	−	−	−	−
bio-19	−	+	+	+	+	−	−	−	−	−
bio-3	+	+	+	+	+	+	−	−	−	−
bio-12	+	+	+	+	+	+	−	−	−	−
bio-18	+	+	+	+	+	+	−	−	−	−
bio-23	+	+	+	+	+	+	−	−	−	−

$$W \xrightarrow{C} X \xrightarrow{D} Y \xrightarrow{A} Z \xrightarrow{B} \text{biotin}$$

where W, X, Y, and Z are biotin precursors, and X is uncombinable with avidin. The order of the two middle steps (A and D) is less certain than the others, being based on rather weak feeding between one mutant pair.

As all the mutants can grow with added biotin, those compounds excreted in its absence are presumably precursors (or derivatives thereof) rather than products of biotin metabolism.

Biotin biosynthesis is thus controlled by a closely linked cluster of at least four genes mediating different steps in the process. Physical data on the densities of the transducing phages (G. Kayajanian, *in preparation*) indicate that the size of this cluster is at least 14% of the molecular weight of λ phage, which would come to 4.2×10^6 daltons. This should be enough to code for at least 10 polypeptide chains of 200 amino acids each. This suggests that perhaps more genes in this region remain to be discovered.

We have recently found that mutants of groups A, C, and D can use desthiobiotin for growth in place of biotin, whereas group B mutants cannot. This corroborates the other evidence that the genetic block of group B mutants is beyond those of the other groups in the metabolic sequence and indicates that group B mutants are blocked between desthiobiotin and biotin.