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The recovery of tryptophan A auxotrophs at high frequency in a strain of *Salmonella typhimurium*

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1. INTRODUCTION

In a previous paper (Riyasaty & Dawson, 1967) we showed that the slow-growing reversions of the *tryA47* mutant of *Salmonella typhimurium* arise by a genetic change, *47S*, that is inseparable by recombination from the *47* site. These experiments were sufficiently extensive for it to be likely that this change was in the same codon as the *47* site or in an adjacent one. We searched for auxotrophs in cultures of these reversions and found that about half of all the auxotrophs recovered were mutant in the *tryA* gene. This paper is an account of these results and of the dependence of the recovery of this high proportion of *tryA* auxotrophs on the presence of the *47S* site.

2. MATERIALS AND METHODS

The wild-type LT-2 strain of *S. typhimurium* and the auxotroph *tryA47* derived from wild-type LT-7, were obtained from the collection of the late Dr Demerec at the Carnegie Department of Genetics, Cold Spring Harbor, Long Island, New York. Phage PLT-22 was used for all transductions except when phage-sensitive transductants were required, when its variant H4 was used.

All mutants were isolated by a penicillin-screening technique (Davies, 1948) using 50 Oxford units of penicillin G per ml.

Auxotrophs that are listed in Table 1 as having unidentified requirements have been shown not to grow on minimal media with the following supplements:

Amino acids: Alanine, arginine, aspartic acid, iso-leucine, lysine and valine.
Bases: Adenine, cytosine, guanine, thymine and uracil.
Vitamins: Ascorbic acid, inositol, nicotinic acid, d-pantothenate Ca, pyridoxine hydrochloride, riboflavin and thiamine.

The amino acids, the bases and indole were added to minimal medium to give concentrations of 0·002 %. Anthranilic acid was added to give a concentration of 0·0005 % and the vitamins to give concentrations of 0·0002 %.

The composition of media, preparation of phage stocks and the transduction procedures were as described by Smith-Keary (1960).

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3. RESULTS

(i) *The auxotrophs recovered from* tryA47S *and from wild-type*

We have previously designated the slow-growing reversions of *tryA47* as *tryA47S*. Twelve cultures were inoculated with small numbers of cells of one of these slow-growing reversions. Auxotrophs were recovered from each culture separately by

Table 1. *The growth requirements of auxotrophs arising by independent mutations in five related strains*

	(a)	(b)	(c)	(d)	(e)
Number of cultures screened	12	20	10	11	20
Try or Ind or Anth*	22		1		22
Try or Ind	5	1			5
Try			1		1
Phe + Tyr + Try + PABA + PHBA				5	
Phe + Tyr + Try	2	2	2	2	4
Phe + Tyr		1	1	1	
Phe		1			
Tyr		2	1		
Pro	1	4	8	2	5
Ser or Gly	1	1		2	3
Met		3		7	3
Leu		8	3		
His	2	1	1	2	
Glu			1		
Cys					2
Thr	1	3		2	3
Unidentified	5	9		3	5
Totals	39	36	19	26	55

(a) *tryA47S*.
(b) Wild-type LT-2.
(c) *tryA47S* in which the 47*S* site had been replaced by 47+ from wild-type LT-2 by transduction.
(d) fast-growing reversion of *tryA47*.
(e) wild-type LT-2 in which the 47+ site had been replaced by 47*S* by transduction from *tryA47S*.

* *Abbreviations:* Anth: anthranilic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ind: indole; Leu: leucine; Met: methionine; PABA: p-aminobenzoic acid; PHBA: p-hydroxybenzoic acid; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Try: tryptophan; Tyr: tyrosine.

the penicillin screening method. In this and all subsequent experiments only non-leaky auxotrophs were scored. The nutritional requirement of each auxotroph was sought and the results are set out in column (a) of Table 1. When auxotrophs with the same requirement are recovered from the same culture they could derive

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from either the same or separate mutations. Such auxotrophs were crossed by transduction and if prototrophic recombinants were obtained it was concluded that they had arisen by separate mutations at different sites. If no prototrophic recombinants arose the mutants were only scored as deriving from different mutations if their rates and characteristics of reversion were conspicuously different. These were the criteria of independent mutational origin that were used in this and all subsequent experiments. The data show that a very high proportion of the auxotrophs will grow when the minimal medium is supplemented with anthranilic acid. Some of these auxotrophs have been genetically analysed by transduction. Each has the *47S* site unchanged and has a further mutation elsewhere in the *tryA* gene. The data from these experiments are presented in Section ii.

Auxotrophs were similarly sought in twenty cultures of the wild-type LT-2 strain. Fifty-seven were found and at least thirty-six derive from separate mutations. Their growth requirements are summarized in column (b) of Table 1. None grew on minimal medium supplemented with anthranilic acid.

This result contrasts sharply with the high frequency of mutants in the *tryA* gene that was found when auxotrophs were sought in *tryA47S*. The only known genetic differences between the wild-type LT-2 strain and *tryA47S* is the presence of the *47S* site in the *tryA* gene of the latter. However, the LT-2 strain and the *tryA47* strain, from which *tryA47S* is derived, came from slightly different wild-type strains and have been maintained as separate stocks for many years. Unrecognized genetic differences could have accumulated. These, rather than the presence or absence of the *47S* site, might be responsible for the different proportions of *tryA* auxotrophs obtained from the two strains. We therefore scored auxotrophs in:

(1) A stock of *tryA47S* in which a small region of the genome, including the *47S* site, had been replaced by an equivalent region including 47^{+} from wild-type LT-2 by transduction with H4 phage. A fast-growing transductant that was sensitive to phage PLT-22 was used.

(2) A fast-growing reversion of *tryA47* in which the *47* site had reverted to 47^{+}. We have previously shown that such a reversion is due to a genetic change that is inseparable by recombination from the *47* site (Riyasaty & Dawson, 1967). This reversion therefore is identical to *tryA47S* except for the change of *47S* to 47^{+}.

(3) A stock of wild-type LT-2 in which a small region of the genome, including the 47^{+} site, had been replaced by *47S* by transduction from strain *tryA47S*. We obtained this stock by isolating a tryptophan-requiring auxotroph of LT-2 and using this as a recipient for phage grown on *tryA47S*. A phage-sensitive, phenotypically slow-growing transductant was selected.

The results of penicillin screening these strains and scoring non-leaky auxotrophs of independent origin are summarized in columns c, d and e of Table 1. The first two strains, which did not have the *47S* site yielded few anthranilic acid-requiring auxotrophs. A high proportion of these auxotrophs were recovered from strains with the *47S* site in their *tryA* gene.

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(ii) *The genetic analysis of the* tryA *auxotrophs recovered from* tryA47S

We first tested whether any of the *tryA* auxotrophs had arisen by the *47S* site mutating back to *47*. Of the thirty *tryA* mutants that were recovered, seven were unstable, and these have not been analysed. Of the remaining twenty-three, nineteen were reciprocally crossed by spot-transductions with *tryA47*. Twelve of these nineteen gave prototrophic recombinants, and so could not be mutant at the *47* site: none gave prototrophic recombinants in homologous transductions. The reversion frequencies of the remaining seven were then compared with that of *tryA47*. Two of the auxotrophs did not revert and five gave between two and three reversions *per* 3×10^8 cells plated, compared with nearly ten times this frequency for *tryA47*. We conclude that an insignificant proportion of the *tryA* auxotrophs arose by the *47S* site mutating to *47*.

The occurrence of many prototrophic recombinants in some of the spot-transductions between the *tryA* auxotrophs and *tryA47* and few, if any, in others suggests that the mutations in these auxotrophs are not all at the same site. When a number of *tryA* auxotrophs were recovered from the same culture they were crossed by transduction and if prototrophic recombinants were found the auxotrophs were scored as deriving from separate mutations. For example, of six *tryA* auxotrophs isolated from one culture all except two gave prototrophs when they were reciprocally crossed with one another. Similarly for six auxotrophs recovered from another culture. Five of the six auxotrophs in each of these cultures were therefore mutant at different sites of the *tryA* gene.

As most of the *tryA* auxotrophs arose by mutation at other than the *47S* site it was important to know whether their *47S* site was unchanged. We know from studies on *pro-401* (Smith-Keary & Dawson, 1964) that this strain behaves as if a controlling episome attached to a site in the proline operon were able to transpose to other sites in the same operon. These transpositions can be to sites in different complementation groups and the expression of the complementation group in which the controlling episome is located is suppressed. A possible interpretation of the present data is that *47S* is a site at which a controlling episome is attached and its transposition to other sites in the *tryA* gene is recognized by the recovery of auxotrophs. If this were so these auxotrophs should have 47^+ in place of the *47S* site; they cannot have *47* in place of *47S* or we would not have obtained prototrophic transductants when these auxotrophs were crossed with *tryA47*. Transductions between *tryA47* and any of the *tryA* auxotrophs would yield prototrophic recombinants which would grow as wild-type if the site were 47^+ but as slow-growing phenotypes if the site were still *47S*. The data from such transductions with eight *tryA* auxotrophs are set out in Table 2.

In all these transductions the number of prototrophic colonies is greater on the transduction plates than on the control plates thus confirming that the mutant sites in the auxotrophs are not at the *47* site. All the transductions yielded slow-growing colonies but only four out of the eight yielded wild-type and semi-fast colonies. The frequencies of these wild-type and semi-fast colonies were low and no greater

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Table 2. *Transduction between* tryA47 *and* tryA *auxotrophs recovered from* tryA47S. *Selection for* try^{+} *phenotypes. Samples of transductants scored as either fast, or semi-fast, or slow*

	Colonies on transduction plates			Colonies on control plates		
		Number characterized			Number characterized	
tryA auxotrophs used as recipients	Total number	Fast and semi-fast	Slow	Total number	Fast and semi-fast	Slow
1	61	14	22	46	26	0
5	40	0	6	1	0	1
12	15	4	9	8	5	3
16	40	2	4	6	3	0
17	30	1	12	1	0	1
29	23	0	15	1	1	0
30	17	0	11	6	0	6
45	12	0	9	2	0	2

than their frequencies on the control plates. We conclude that the *47S* site is unchanged in the *tryA* auxotrophs.

4. DISCUSSION

We have studied the relative frequencies of different auxotrophs in cultures of two strains of *S. typhimurium* and in cultures of strains derived from them. None of these strains are unstable in that variant phenotypes cannot be readily recognized by characterizing colonies that grow from a random sample of the cells of a culture. The proportion of auxotrophic cells in a culture has first to be selectively increased before they can be isolated and characterized. The selective increase is achieved by growing the culture in minimal medium with penicillin. Dividing cells are killed by the penicillin while cells that are not dividing are not killed. It is, however, unlikely that different auxotrophs will respond identically to this treatment. Leaky mutants will probably tend to be killed by the penicillin. Mutations in genes which produce those enzymes that exist in low concentrations in the cells will be recovered most readily; cessation of growth, and immunity to penicillin, will rapidly follow their mutation. Some auxotrophs may be fed by some cells while others are fed by none. It is therefore unlikely that the relative frequencies of the different types of auxotrophs recovered by this method accurately reflects their relative frequencies in the original culture. It is only when great care is taken to treat each culture in precisely the same way, when repeat experiments give similar results and when conspicuous differences are obtained from cultures of different strains that we can be sure that our results reflect differences in the pattern of mutation in these strains. Our experiments have these features in that all cultures were treated in as similar a way as possible and repeat experiments have given similar results. The most striking feature of the results is that about 50% of the

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auxotrophs recovered from strains that have the *47S* mutant site in their *tryA* gene are *tryA*$^-$ phenotypes, while an average of less than 5 % of such auxotrophs are recovered from strains that are wild-type at this site (Table 3).

Table 3. *The percentages of auxotrophs which will grow on Try or Ind or Anth* (tryA$^-$) *and those which will grow on Try or Ind* (tryB$^-$, tryC$^-$ and tryE$^-$) *among those arising by independent mutations in five related strains*

	Total number of auxotrophs recovered	Number deriving from independent mutations (n)	Percentages of (n) which will grow on:	
			Try or Ind or Anth	Try or Ind
(a)	50	39	56·4	12·8
(b)	57	36	0	2·8
(c)	32	19	5·2	0
(d)	64	26	0	0
(e)	91	55	40·0	9·0

(a), (b), (c), (d), (e): see footnote to Table 1.

The estimated percentages of *tryA* auxotrophs in strains with *47S* are minimum estimates. When a particular phenotype arises often there will be many cultures in which it occurs more than once. Only those that can be shown to have been independent mutations will be scored as such. There will be some that derived from independent mutations at close sites that will not be distinguished and so wrongly scored as deriving from the same mutation.

We have shown that the *tryA* auxotrophs are mutant in the *tryA* gene. We therefore have an example, in the language of classical cytogenetics, of a position effect phenomenon. The presence of *47S* in some way enables us to recover a large number of further mutations in the *tryA* gene. The data suggest that there are also more auxotrophs that will grow on tryptophan or indole, but not on anthranilic acid, in strains that have the *47S* site. Ten such auxotrophs were found among ninety-four auxotrophs deriving from independent mutations in strains with the *47S* site, while only one among eighty-one was found in strains without *47S*. The enhanced recovery of tryptophan-requiring auxotrophs seems not to be confined to those arising by mutation in *tryA*. It includes those due to mutation in adjacent genes.

We do not yet know whether *47S* is part of a mechanism that induces mutation at a relatively high rate in this region or causes mutations that arise even in the absence of 47*S* to be expressed as auxotrophs and so recognized. If this second explanation is to account for the frequencies of the indole-requiring mutants we need to postulate that the polypeptide coded by the *tryA* gene can be associated with the polypeptides coded by other genes in the same operon and affect their catalytic activity. Ito & Yanofsky (1966) have shown an association between the

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polypeptides coded by those genes in *Escherichia coli* that are equivalent to the *tryA* and *tryB* genes of *Salmonella*. Distinguishing between these alternative explanations will be facilitated by both a biochemical study of the enzymes and the isolation and characterization of derivatives of the anthranilic acid-requiring and indole-requiring auxotrophs from which the *47S* site has been removed. Some of this work is in progress.

Strains without *47S* may differ in the relative frequencies of auxotrophs with requirements other than tryptophan. Omitting those that would grow on minimal medium supplemented with tryptophan, 23% of the auxotrophs deriving from independent mutations that were recovered from *tryA47S* in which the *47S* site had been replaced by 47^{+} transduced from wild-type required proline. From the fast-growing reversion of *tryA47*, 19% required phe + tyr + try + PABA + PHBA and 27% required methionine. These percentages are based on small numbers of auxotrophs and we regard the data as merely suggestive of further differences in the patterns of mutation in these strains. None of these possible differences is correlated with the presence or absence of the *47S* site.

SUMMARY

1. Auxotrophs were sought in a slow-growing reversion of the *tryA47* strain of *S. typhimurium*. This reversion differs from *tryA47* by a genetic change that is inseparable from the *47* site and has been designated *47S*. Out of thirty-nine auxotrophs that derived from independent mutations, twenty-two grew on minimal medium supplemented with anthranilic acid. Eight of these auxotrophs were examined and each was shown to have the *47S* site unchanged and to carry a further mutation in the *tryA* gene. These further mutations were shown to be at different sites in different auxotrophs.

2. Auxotrophs were sought in the wild-type LT-2 strain. None out of thirty-six were mutant in the *tryA* gene.

3. The *47S* site in *tryA47S* was replaced by 47^{+} transduced from wild-type LT-2. Auxotrophs were sought in this strain and only one out of nineteen was mutant in the *tryA* gene.

4. Auxotrophs were sought in a wild-type reversion of *tryA47*. Out of twenty-six none were mutant in the *tryA* gene.

5. The 47^{+} site in wild-type LT-2 was replaced by *47S* transduced from *tryA47S*. Auxotrophs were sought in this strain and twenty-two out of fifty-five were mutant in the *tryA* gene.

6. We conclude that *tryA* auxotrophs are only recovered at a high frequency when the *47S* site is present in the *tryA* gene.

7. In strains with the *47S* site in the *tryA* gene the frequency of auxotrophs that will grow on minimal medium supplemented with indole but not on minimal medium supplemented with anthranilic acid is appreciably higher than in those strains without the *47S* site in the *tryA* gene. These auxotrophs are mutant in genes that are in the same operon as *tryA*.

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