TEMPERATURE-SENSITIVE REVERTANTS*

Shiau-Ta Chung and G. Robert Greenberg

Department of Biological Chemistry
The University of Michigan
Ann Arbor, Michigan 48104

Received September 27, 1971

SUMMARY: About 50% of a series of non-temperature-sensitive, spontaneously revertable auxotrophs of *Escherichia coli* showed temperature-sensitive revertants when the selection was at 25° rather than at 37°. This procedure provides a simple device to select temperature-sensitive mutants.

Many non-temperature-sensitive revertable mutants of *Escherichia coli* show temperature-sensitive (*ts*) revertants if the selection is carried out at lower (permissive) temperatures. In such cases many more revertants are selected at the lower temperature, and these additional colonies normally are *ts*. This simple observation makes possible the selection of *ts* markers almost at will. The present note reports a study of the reversion of 22 mutants of *E. coli* chosen at random. Sixty-eight percent showed spontaneous revertants at 25° under the conditions of assay, and about 1/2 of these were *ts*. It is likely that some mutations in all structural genes would, on reversion, give rise to a *ts* system.

By examining mutations in all segments of a given gene it should be possible to construct maps indicating the relative rate of reversion to the *ts* state, and such a map might be expected to be related to the structure and stability of the protein.

---

* This study was supported by Grant AI-08133 from the National Institutes of Allergy and Infectious Diseases, United States Public Health Service.

EXPERIMENTAL

Bacteria. Bacterial stocks were grown at 37° on Bactonutrient agar slants and maintained at 4°. For thymine-requiring strains 20 to 50 µg/ml thymine were added. All organisms were purified at least once on L-agar plates at 37°, with additional thymine included for Thy⁻ organisms. *Thy-104* is a high thymine-requiring (Alikhanian *et al.*, 1966, and Lomax and Greenberg, 1968) mutant of *E. coli* B and was isolated by Dr. Beverly Dale (Dale, 1968). Strain 605 is a Thy⁻ organism which has different properties than *thyA* cultures. It was isolated in a K-12 strain and transduced into an *E. coli* B background by P1bt transducing phage. The other strains employed are described in Table I.

Reversion studies. Revertants were selected by spreading 0.1 ml of an overnight culture grown in L-broth in bubbler tubes at 37° on agar plates containing minimal salt solution (Vogel and Bonner, 1956) with appropriate metabolites added at 20 µg/ml each to allow for selection of the revertant. In each case the plates were incubated at 25° ± 0.5° and at 37° ± 1°. The plates were observed for 3 to 4 days. Sometimes it is advisable to look for revertants for a week or more. In most instances the revertant colonies were tested for temperature sensitivity and metabolite requirement without further purification. In this paper the term 'revertant' means phenotypic revertant and includes apparent full revertants and partial revertants, and it could also include suppressor mutations (Allen and Yanofsky, 1963). The effect of mutagens has not yet been tested in the selection of *ts* revertants.

RESULTS

Table I lists 22 mutations in 18 strains whose reversion rates were examined. Of these, 7 showed no revertants when plated at 25°. Of the 15 mutations which reverted at 25°, 9 showed more revertants when plated at 25° than at 37°. In 8 of the 9 cases the majority of the revertants growing at 25° was shown to be *ts*, a fact reflected by the

TABLE I

SELECTION OF REVERTANTS AT PERMISSIVE AND NON-PERMISSIVE TEMPERATURES

Escherichia coli Strains						
Lab. No.	Origin (h) and Designation	Relevant Genotype (i)	Marker Studied (i)	No. of Revertants (j) 37°	25°	*ts* (k)
85	B 302 (a)	*met arg thy*	*arg*	0	0	
603	B (o,b)	*argA*	*arg*	6	260 (n)	5/5
714	K-12 CP154 (c)	*argA his lysA thi*	*arg*	0	0	
444	K-12 KY895 (d)	*ilv tdk*	*ilv*	8	>2000 (m)	5/5
715	K-12 JC5029(c)	*thr ilv-318*	*ilv*	53	78 (l)	
638	B/r (e)	*leuB arg*	*leu*	0	0	
714	K-12 CP154 (c)	*argA his lysA thi*	*lys*	0	1 (l)	
607	B (o,b)	*lysA*	*lys*	6	3 (l)	
85	B 302 (a)	*met arg thy*	*met*	0	0	
614	K-12 (p,b)	*met*	*met*	2	36 (n)	10/10
86	B 335 (a)	*arg met pro thy*	*pro*	0	0	
20	B 96	*purH*	*pur*	1	2	1/2
322	B/r	*pyrA*	*pyr*	55	56	
38	B (f)	*pyr*	*pyr*	0	0	
77	15	*thy pyr*	*pyr*	46	135 (n)	1/5
82	K-12 JE1064(g)	*thy ser*	*ser*	1	1 (r)	
91	B (e)	*serA*	*ser*	0	0	
297	B (b)	*thr*	*thr*	5	290 (m)	3/5
77	15	*thy pyr*	*thy*	3	2 (l)	
85	B 302 (a)	*met arg thy*	*thy*	0	>1000 (m)	5/5
605	B (o,b)	*arg thy*	*thy*	1	12 (n)	22/52 (q)
104	B (b)	*thy*	*thy*	1	20 (n)	40/50 (q)

(a) C. Pauling, B302 and B335 are derivatives of *E. coli* B3 (Thy^-); (b) this laboratory; (c) D. Mount; (d) T. Yura; (e) R. Helling; (f) A. Pardee; (g) M. Ishibashi; (h) *E. coli* B, B/r, K-12 or 15; (i) these abbreviations are according to Taylor (1970); (j) 0.1 ml of an overnight culture was spread on the plates; (k) fraction represents number of colonies of those tested which grew at 25° but grew at 37° only in the presence of the required metabolite; (l) not temperature-sensitive; (m) mainly small colonies; (n) colonies of various sizes; (o) transductant from K-12 strain; see *Methods*; (p) transductant from strain B; (q) from several plates; (r) not temperature-sensitive; between 4 and 8 days small colonies appeared at 37°, some of which were cold-sensitive, i.e., grew at 25° only with serine present.

ratio of the number of revertants at 25° *versus* those at 37°. In strain 605, of 52 Thy^+ revertants, 22 were *ts*, i.e., grew at 25° but not at 37°. Of the remaining 30 colonies, 28 grew at 37° but were unable to grow at 42°. In the same way in *thy-104*, of 50 Thy^+ revertants, 40 were *ts*, and of the remaining 10 colonies, 8 grew at 37° but not at 42°. Many *ts* revertants were slow-growing.

It is perhaps significant that the ratio of revertants growing at

25° to those growing at 37° ranged from about 2 to 1 to more than 1000 to 1, 7 of the 9 being more than 10 to 1. The *thy* mutation in strain 85 needs to be studied further to determine whether any revertants are detectable at 37°.

DISCUSSION

While the present study includes a relatively few examples, several interesting ideas emerge. Firstly, the technique of plating mutant cultures on minimal agar at 25° and 37° selects *ts* mutants in about 1/2 the instances in which reversion is detected. Secondly, those mutations which show a proclivity to revert to temperature sensitivity frequently show far more temperature-sensitive than non-temperature-sensitive reversions.

These observations provide an easy method of obtaining *ts* mutants. Since most reversions, excluding suppressors of chain-terminating mutations, are within the same gene (Benzer, 1955 and Yanofsky, 1960), i.e., second site reversions or, less frequently, at the primary site (Allen and Yanofsky, 1963), and since an externally suppressed mutation can yield a *ts* protein (Dirksen, Hutson and Buchanan, 1963), this procedure provides a relatively specific method to select for a *ts* marker in a given gene for conducting physiological studies by temperature shifts or studying *ts* proteins or for use as a genetic marker. This approach appears to be limited to auxotrophs, and such mutations as revertants of transport-negative systems or of fermentation or of drug-resistant mutants where a metabolite is not required for growth. In the last case the penicillin selection technique could be applied. There is *a priori* no obvious reason that *ts* mutations cannot be obtained by reversion in repressor genes and possibly in the operator site. Mutations in structural genes whose enzymes form indispensable products not available from the medium must, of course, be of the conditional lethal group, such as those pioneered by Edgar and coworkers in T4 bacteriophage (Edgar and Lielausis, 1964).

It is perhaps not surprising that *ts* reversions tend to greatly predominate over other reversions when they occur. This finding suggests that most of the reversions of such mutations are at sites which yield a *ts* protein. Two reasonable models present themselves: In one, each site which is prone to revert to temperature-sensitivity is peculiarly situated in the gene such that a large number of revertant sites can be selected which form an active, but *ts* protein. From another point of view, the number of conformations the revertant protein can assume or different amino acid substitutions it can tolerate and show activity is considerably greater at 25° than at 37°. In two, the mutant site is so situated that revertants which are at hot spot sites (or regions) are selected and the result is a *ts* protein. The *thyA* gene of *E. coli* has such a *ts* hot spot. About 40% of the *thy* mutants selected by the aminopterin (Alikhanian *et al.*, 1966) or trimethoprim (Dale, 1968) method are *ts*. It is reasonable to suggest that in this case model two may hold, and the Thy^+ reversions which are *ts* are at the *ts* hot spot. This high frequency of mutations at the hot spot could obscure other *ts* reversions. These questions will be resolved by a careful genetic analysis.

This method of selecting for *ts* revertants has the potential of selecting new mutations with interesting and useful properties. For example, when the *ts* partial revertants of *thy-104* were analyzed, several classes were found[1]. In one of these classes the revertants are Thy^+ at 25°, Thy^- at 37° but resistant to trimethoprim (a folic acid analog) in the presence of thymine at both 25° and 37°. Thus selection for temperature sensitivity uncovered a class of trimethoprim/thymine-resistant Thy^+ organisms, normally a property of Thy^- cells.

It may be worthwhile to reassess certain studies of reversion rates since *ts* revertants would be missed at 37°. As a corollary, it

---

[1] F. Rodriguez and G. R. Greenberg, in preparation.

seems reasonable to suggest that because of the possible predominance of *ts* mutants at 25°, fewer auxotrophic mutations might be selected at 25° than at 37°, employing a method such as penicillin selection. It is generally accepted that the mutation rate per generation per cell is independent of temperature (Witkin, 1953 and Ryan and Kiritani, 1959).

It is not unreasonable, depending on the protein, that the selection process might select revertants which would be cold-labile, i.e. stable at 37° and unstable at 25° (see strain 82 in the Table). In this context it should be clear that the assay of the reversions can be at a much lower temperature. Thus if the non-permissive temperature were 18° and the permissive temperature 25°, possibly cold-sensitive (O'Donovan, Kearney and Ingraham, 1965) rather than thermosensitive reversions would be obtained, but again it is quite conceivable that a revertant system could be stable at 18° and unstable at 25°.

By using the penicillin method at non-permissive temperatures, *ts* revertants can be concentrated, or the method of Kaplan and Anderson (1968) can be employed for enrichment, capitalizing on the fact that at non-permissive temperatures Thy^- organisms with a second *ts* mutation will not undergo thymineless death.

A misconception which may arise from a reversion to temperature sensitivity is that the original mutation was temperature-sensitive, whereas in fact the revertant predominates at the permissive temperature.

REFERENCES

Alikhanian, S. I., Ilijina, T. S., Kaliaeva, E. S., Kameneva, S. V. and Sukhodolec, V. V., Genet. Res. 8, 83 (1966).
Allen, M. K. and Yanofsky, C., Genetics 48, 1065 (1963).
Benzer, S., Proc. Nat. Acad. Sci. 41, 344 (1955).
Dale, B., Doctoral Dissertation, The University of Michigan (1968).
Dirksen, M. L., Hutson, J. C. and Buchanan, J. M., Proc. Nat. Acad. Sci. 50, 507 (1963).
Edgar, R. S. and Lielausis, L., Genetics 49, 649 (1964).
Kaplan, S. and Anderson, D., J. Bacteriol. 95, 991 (1968).
Lomax, M. S. and Greenberg, G. R., J. Bacteriol. 96, 501 (1968).
O'Donovan, G. A., Kearney, C. L. and Ingraham, J. L., J. Bacteriol. 90, 611 (1965).
Ryan, F. J. and Kiritani, K., J. Gen. Microbiol. 20, 644 (1959).

Stacey, K. A. and Simson, E., J. Bacteriol. 90, 554 (1965).
Taylor, A. L., Bacteriol. Rev. 34, 155 (1970).
Vogel, H. J. and Bonner, D. M., J. Biol. Chem. 218, 97 (1956).
Witkin, E. M., Proc. Nat. Acad. Sci. 39, 427 (1953).
Yanofsky, C., Bacteriol. Rev. 24, 221 (1960).