# THE NATURE OF GENETIC INSTABILITY IN AUXOTROPHS OF *SALMONELLA TYPHIMURIUM* REQUIRING CYSTEINE OR METHIONINE AND RESISTANT TO INHIBITION BY 1,2,4-TRIAZOLE

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ABSTRACT

We tested the hypothesis that unstable suppression of auxotrophy in triazole-resistant derivatives of Cym⁻ mutants of *Salmonella typhimurium* is due to reversible insertion at the Cym⁻ site of genetic material originating in the *cysALKptsHI* region. We have shown that the unstable phenotype was co-transducible with markers in the *cysCDHIJ* region. The suppression of the Cym phenotype was *recA* dependent and frequencies of segregation were affected by UV irradiation. Restored enzyme activity in suppressed strains was determined by wild-type enzyme, suggesting that the unstable regions are located in *cys* gene regulatory regions. These results support the hypothesis. In contradiction, we found no evidence for a deletion in the *cysALKptsHI* region.

THE preceding paper (KINGSMAN, SMITH and HULANICKA 1978) reports the isolation of triazole-resistant (Trz$^r$) derivatives of six cysteine or methionine requiring (Cym⁻) mutants of *Salmonella typhimurium* (QURESHI, SMITH and KINGSMAN 1975). Some of the derivatives (CTS) of each Cym⁻ mutant were prototrophic, *i.e.*, the Cym⁻ phenotype was suppressed. This suppression was initially unstable (KINGSMAN 1976), resulting in the segregation of Cym⁻ auxotrophs at high frequency. Upon subculture, some of these derivatives remained highly unstable, segregating Cym⁻ auxotrophs at 50–70%, whereas others stabilized, segregating at frequencies less than 1%. All CTS strains possessed a Trz$^r$ marker co-transduced with *cysA* (HULANICKA and KLOPOTOWSKI 1972). The stabilized strains were Cym$^+$, whereas the unstable strains were apparently Cym⁻. To account for these and other observations (KINGSMAN 1976; KINGSMAN, SMITH and HULANICKA 1978) and in accord with the work of CORDARO and ROSEMAN (1972), it was suggested that when some Trz$^r$ mutants were isolated, a low molecular weight plasmid was formed by excision of part of the *cysALKptsHI* region of the *S. typhimurium* chromosome. Suppression of Cym⁻ then occurred by the integration of this plasmid (CSP) at

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the Cym⁻ site, resulting in correction of the Cym⁻ lesion. Instability was thought to result from reversibility of this integration.

This hypothesis led to a number of predictions: (1) If the instability of suppression of Cym⁻ results from the reversible integration of CSP, then it should be possible to select the Cym+ genotype and to transfer it in appropriate transduction crosses. Cym+ transductants should show the instability of the donor. The inserted CSP should also be detectable genetically due to its disruption of linkage relationships in the region of the suppressed Cym⁻ mutation, *i.e.*, the *cysCDHIJ* gene cluster (QURESHI, SMITH and KINGSMAN 1975). (2) If integration of CSP is dependent on similar base sequences at the Cym⁻ site and on CSP (KINGSMAN, SMITH and HULANICKA 1978), then the integrative recombination event should be *recA*-mediated (CLARK 1973). (3) If the Cym⁻ mutations and the correcting integrative events occur within regulatory regions of the relevant *cys* genes (QURESHI, SMITH and KINGSMAN 1975), then restored enzyme levels in CTS strains may be lower than in wild type, but the protein produced should be identical. (4) If CSP is formed by excision of part of the *cysALKptsHI* region, there should be a deletion in CTS strains corresponding to the excised piece.

We describe here the testing of these predictions with the exception of the demonstration of an insertion in the *cysCDHIJ* region, which was the subject of another publication (KINGSMAN 1977).

## MATERIALS AND METHODS

*Organisms, media, genetic crosses and segregation assays*: All strains were derived from *Salmonella typhimurium* LT2; they are listed in Table 1 of KINGSMAN, SMITH and HULANICKA (1978) with the exception of *cysA20ptsI31*, which was obtained from D. HULANICKA (Polish Academy of Sciences, Warsaw). The media used were also those of the same authors, and their procedures for genetic crosses and for the measurement of segregation frequencies were used.

*Ultra-violet irradiation*: In experiments in which UV irradiation was used to stimulate recombination, doses that gave 70% kill were chosen to provide opimal stimulation (ROMAN and JACOB 1958).

*Preparation of cell-free extracts*: Four hundred ml quantities of the appropriate media were inoculated with 10 ml stationary phase cultures of bacteria in the same medium, supplemented with 1% NB, and incubated on an orbital shaker (220 rpm) at 37° until the subcultures had reached an O.D. 650 of about 0.6*. The bacteria were then harvested by centrifugation at 1800 × **g** for 15 min, washed twice with distilled water and resuspended in 3 ml of buffer appropriate to the enzyme assay involved. Suspensions were subjected to two 15 sec bursts of ultrasonic disintegration using an M.S.E. 100 watt sonicator at 20 kc/sec. (8 microns peak to peak, ⅜″ probe). Cell debris was removed by centrifugation at 27,000 × *g* for 20 min. The method of LOWRY *et al.* (1957) was used for protein determinations.

*Assay of PAPS synthetase*: The assay was that of HULANICKA and KLOPOTOWSKI (1972).

*Assay of $SO_3$ reductase*: The assay was based on that of *SIEGEL* and KAMIN (1971), but using hydroxylamine as substrate to increase sensitivity (LEINWEBER, MONTY and SIEGEL 1965).

## RESULTS

*The genetics of instability in CTS strains*: Although KINGSMAN, SMITH and HULANICKA (1978) could not show transduction of unstable Cym+ when trans-

* Bacteria derepressed for cysteine biosynthesis were obtained by growing in the sulfur-free (SF) medium of QURESHI, SMITH and KINGSMAN (1975) containing 40 μg/ml of djenkolic acid.

ducing lysates were prepared on nonselective media (SMITH 1961), it seemed likely that if there is an unstable insertion at the Cym⁻ site in CTS21 and 151 (KINGSMAN, SMITH and HULANICKA 1978), then that insertion would be transduced, provided that selection was made for the insertion during preparation of the transducing lysate. To test this, three clones of each of the unstable strains CTS21 and 151 were grown overnight in NB, 10% NB in MM and 1% NB in MM, and P22L4 lysates were prepared in the same medium. This ensured three levels of increasing selection during preparation of the donor lysates. The transducing ability of each lysate was assessed in spot transduction crosses with *aroB57* as recipient. The ability of each lysate to transduce Cym⁻ was assessed by using as recipients a deletion *cysCD519*, which spans the original Cym⁻ site and the original Cym⁻ site and the original Cym⁻ mutation. Wild type, CymAa2Trz1 and CymBc15Trz551 were used as donor controls (Table 1). The lysates of all the clones prepared under the various conditions had about

TABLE 1

*The selection of Cym genotype in CTS21 and 151*

| Donor strain | Clone | Phage preparation conditions* | Recombinants per spot with these recipients: *aroB57* | *cysCD519* | CymA2 | *cysH1536* | CymBc15 |
|---|---|---|---|---|---|---|---|
| Wild type | 1 | A | ++ | ++ | ++ | ++ | ++ |
| | | B | ++ | ++ | ++ | ++ | ++ |
| | | C | ++ | ++ | ++ | ++ | ++ |
| CymA2Trz1 | 1 | A | ++ | — | — | nt | nt |
| | | B† | + | — | — | nt | nt |
| | | C† | — | — | — | nt | nt |
| CymBc15Trz5 | 1 | A | ++ | nt | nt | — | — |
| | | B† | — | nt | nt | — | — |
| | | C† | — | nt | nt | — | — |
| CTS21 | 1 | A | ++ | 4 | 12 | nt | nt |
| | | B | ++ | + | 21 | nt | nt |
| | | C | + | + | + | nt | nt |
| | 2 | A | ++ | 12 | + | nt | nt |
| | | B | ++ | + | + | nt | nt |
| | | C | ++ | ++ | + | nt | nt |
| | 3 | A | ++ | — | — | nt | nt |
| | | B | ++ | — | — | nt | nt |
| | | C | ++ | + | + | nt | nt |
| CTS151 | 1 | A | ++ | nt | nt | — | — |
| | | B | + | nt | nt | — | — |
| | | C | ++ | nt | nt | + | + |
| | 2 | A | ++ | nt | nt | — | — |
| | | B | ++ | nt | nt | — | 19 |
| | | C | ++ | nt | nt | + | 17 |
| | 3 | A | ++ | nt | nt | — | — |
| | | B | ++ | nt | nt | — | — |
| | | C | + | nt | nt | + | 15 |

* A = NB, B = 10% NB in MM, C = 1% NB in MM.
† Cym Trz$^r$ mutants grew very poorly on these media.
— = 0, + = 25–50, ++ = > 50, nt = not tested.

the same ability to transduce *aroB57* to prototrophy, showing that there were about the same number of transducing particles in each of the lysates. In the tests of the Cym status of the various clones, it was generally observed that an increased number of prototrophic transductants were obtained with increased selection of Cym$^+$ in the lysed cultures of CTS21 and 151, whereas the number was unaffected in the case of wild type. No transductants were formed with CymAaTrz1 and CymBc15Trz51 as donors. These facts confirmed that the Cym$^+$ genotype is selectable in unstable strains and are compatible with the detection of the Cym marker in these strains resulting from the lysed cultures containing a large proportion of segregants in which CSP was excised (KINGSMAN, SMITH and HULANICKA 1978).

According to the hypothesis, the prototrophs produced in the crosses between the *cys* deletions the original Cym mutants and CTS21 and 151 will have acquired the integrated CSP and should therefore be unstable. Five transductants from each cross were picked and tested for the segregation of auxotrophs at high frequency. Transductants from crosses with the CTS strains as donors were all highly unstable and retained this property through five single-colony subcultures. In contrast, the ability to grow on MA (37°) of transductants from crosses between the same recipients and wild type was completely stable, showing that apparent instability was not an effect of transduction. Next, ten segregants from each of the ten unstable transductants tested were streaked onto MA (37°) to determine whether, like CTS21 and 151, their segregation was reversible (KINGSMAN, SMITH and HULANICKA 1978). Segregants from all produced prototrophic clones on MA (37°) that maintained a high level of instability over ten subcultures, showing that the transductants behaved exactly like CTS21 and 151.

We conclude that the phenotypic instability of CTS21 and 151 is transferable by transduction; this provides strong evidence that it is a genetic phenomenon.

*Involvement of the* recA *system*: The role of the generalized bacterial recombination system in suppression of Cym$^-$ in CTS strains was assessed by (a) the isolation of Trz$^r$ derivatives of a Cym$^-$ *recA* mutant and their subsequent screening for the formation of Cym$^-$-suppressed clones (KINGSMAN, SMITH and HULANICKA 1977) and (b) testing the effect of UV irradiation, which stimulates recombination (ROMAN and JACOB 1958; JACOB and WOLLMAN 1961; HOLLIDAY 1971), on segregation in CTS strains.

(a) In a *recA* strain, CSP should be unable to integrate at the Cym$^-$ site and so not suppress the Cym$^-$ phenotype. Trz$^r$ derivatives of CymAa7 and CymAa7-*recA8* were isolated (KINGSMAN, SMITH and HULANICKA, 1978) and tested for their ability to grow on MA (37°). Eight out of ten Trz$^r$ isolates of CymAa7 gave "unstable" growth on MA (37°), which stablized after five subcultures. However, none of 50 Trz$^r$ derivatives of CymAa7*recA8* gave any growth on MA (37°), even after four days incubation to allow for the slightly slower growth rate of the *recA8* strain (KINGSMAN, unpublished). These results suggest that a functional *recA* system is required for the suppression of Cym$^-$ in CTS strains.

(b) The effects of UV irradiation on segregation in the two classes of CTS

strains will be discussed separately. In the unstable strains, KINGSMAN, SMITH and HULANICKA (1978) proposed that CSP rapidly alternated between the integrated (Cym$^-$-suppressing) and excised (Cym$^-$-nonsuppressing) states and that both integration and excision were *recA* mediated. UV stimulation of recombination should affect both integration and excision. This makes prediction of the effect of UV irradiation on the frequency of segregation in the unstable strains complex. If it does stimulate both integration and excision, a reduction in the between-clones variation of segregation frequencies, after irradiation, compared with spontaneous frequencies is anticipated. Thus, there should be a negative correlation between spontaneous segregation frequency and the UV-induced change in that frequency. Thirty clones of CTS21 and 20 clones of CTS151 were grown overnight in NB, and each culture was diluted $10^{-3}$ in NB. These dilutions were split into two. A 3 ml sample of one-half of each was irradiated, and 1 ml was used to inoculate 5 ml of fresh NB. The other half of each was further diluted $10^{-2}$ to stimulate the killing effect of UV, and 1 ml of this dilution was used to inoculate an identical volume of the same medium. Both sets of cultures were incubated at 37° for 45 min in the dark, diluted appropriately and the segregation frequencies measured by the standard segregation assay. Figure 1 shows that with both CTS21 and 151 there was a significant negative correlation between the UV-induced change in segregation frequency and the spontaneous segregation frequency ($p = < 0.001$ in each case). The regression lines for the

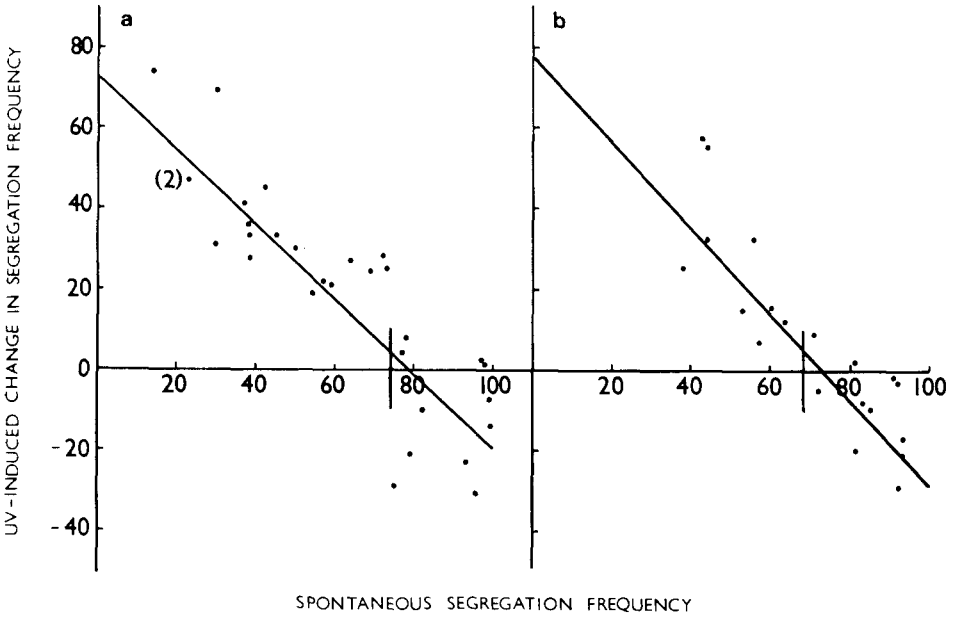


FIGURE 1.—The relationships between spontaneous segregation frequency and the UV-induced change in segregation frequency. The equations of the straight lines were calculated by the method of least squares. In both (a) and (b), from regression analysis of variance, $P = < 0.001$.

two strains are very similar, supporting the idea that events involved in each strain were the same. The straight lines intercept the $x$-axis at a spontaneous segregation frequency of about 75%. This shows that excision, rather than integration, of CSP is the more probable event. Figure 1 also suggests that UV irradiation reduces the segregation frequency in populations with a high spontaneous frequency. To test this unequivocally, we subjected the data to the right of the vertical lines in Figures 1A and B to Student's $t$-test; the probabilities that the means of those points differed from zero as a result only of error variance were 0.02–0.05 and 0.01–0.02, respectively. Therefore, these results are consistent with UV irradiation stimulating both integration and excision of CSP *via* the generalized recombination system.

In the stabilized strains, segregation frequencies are less than 1%, so that CSP integration is relatively stable. Thus the effect of UV irradiation should be only to increase segregation frequency. From Table 2, it is seen that for each of the stablized strains tested there was a marked increase in segregation after UV treatment. In the absence of UV irradiation, segregation was barely detectable, but its levels were increased to 1.6–11.2% for CTS71, 194 and 43 and to 9.3–93.7% for CTS84. The large increase in segregation frequency for CTS84 may relate to its segregation properties, which are intermediate between those of the unstable and the stabilized strains (KINGSMAN, SMITH and HULANICKA 1978). We conclude that the nature of the unstable and stabilized strains is essentially the same, but that in the stabilized strains CSP has a greater affinity for its integration site.

*The nature of the restored enzyme activities in CTS strains*: If the integration of CSP at the Cym$^-$ site leads to the restoration of a functional *cys* gene with CSP integrated alongside (KINGSMAN, SMITH and HULANICKA 1978), the bacteria should be prototrophic since active *cys* enzyme will be produced. This would

TABLE 2

*Ultra-violet induction of segregation in stabilized strains*

| Strain | Clone | Segregation frequency* — UV | + UV |
|---|---|---|---|
| CTS71 | 1 | $^0/_{267}$ | $^2/_{108}$ |
| | 2 | $^0/_{193}$ | $^4/_{245}$ |
| | 3 | $^0/_{215}$ | $^5/_{176}$ |
| CTS194 | 1 | $^0/_{301}$ | $^1/_{32}$ |
| | 2 | $^0/_{195}$ | $^1/_{118}$ |
| | 3 | $^0/_{172}$ | $^2/_{158}$ |
| CTS43 | 1 | $^1/_{310}$ | $^{14}/_{124}$ |
| | 2 | $^0/_{203}$ | $^8/_{248}$ |
| | 3 | $^3/_{412}$ | $^{11}/_{519}$ |
| CTS84 | 1 | $^0/_{197}$ | $^{21}/_{28}$ |
| | 2 | $^0/_{186}$ | $^{15}/_{16}$ |
| | 3 | $^1/_{162}$ | $^3/_{32}$ |

* The segregation frequency is given as a fraction from the raw data of a segregation assay.

mean that the level of enzyme activity in CTS strains would be proportional to the proportion of cells with CSP integrated, *i.e.*, inversely proportional to the segregation frequency. Also, if the integration of CSP occurs in a regulatory region of the appropriate *cys* gene, then wherever an active enzyme is detected it should possess wild-type properties.

These predictions were tested in CTS strains derived from Cym$^-$ mutants deficient either in the sulphate-activating enzymes, PAPS synthetase (*cysCD*) or sulphite reductase (*cysGII*). The levels of these two enzymes were determined in wild type, CTS71, 194, 84 and representative CymTrz$^r$ double mutants bearing the same three Cym$^-$ mutations as the CTS strains that were unsuppressed when grown under both derepressing (SF + djenkolic acid) and repressing (MM and cysteine) conditions (Table 3). The restored enzyme activities in the CTS strains were high (74% and 95% of wild-type activity for PAPS synthetase in CTS71 and 194 and 53% for sulphite reductase in CTS84). This is in contrast to the low activity of the respective enzymes in the unsuppressed double mutants. Wherever high enzyme activity was detected, it was repressed by cysteine.

The effect of segregation on restored enzyme activity was examined in CTS21 and 151. Then clones of CTS21 and 5 of CTS151 were grown on SF + djenkolic acid. First, the segregation frequencies of these cultures were determined and then, immediately afterwards, their PAPS synthetase and sulphite reductase activities were measured. There was a significant negative correlation between restored enzyme activity and segregation frequency in both strains (Figure 2).

Different properties of each of the two enzymes whose activities were restored in the CTS strains were examined. The heat labilities of PAPS synthetase activities of CTS71, 194 and 21 (segregation frequency of 39%) were indistinguishable from that of wild type (Figure 3), as were the $Km_{(NH_2OH)}$ values of the sulphite reductase of CTS84 and 151 (segregation frequency of 32%) calculated from Lineweaver-Burke plots (Figure 4). These data strongly suggest that the restored enzyme activities in CTS strains are determined by wild-type proteins. This, coupled with less than wild-type enzyme activity in the stabilized and nonsegregating (from extrapolation) unstable strains, supports the idea that

TABLE 3

*Restored enzyme activities in CTS strains*

| Strain | PAPS synthetase* after growth in: SF + DJA | MM + CYS | $SO^=_3$ reductase* after growth in: SF + DJA | MM + CYS |
|---|---|---|---|---|
| Wild type | 100 | 0 | 100 | 0 |
| CTS71 | 74.2 | 1.2 | 97 | 1.2 |
| CTS194 | 95.4 | 0.4 | 108.3 | 0 |
| CTS84 | 105 | 1.1 | 53 | 0.8 |
| CymAa7Trz62 | 0.7 | 0 | 136.1 | 0.01 |
| CymBb8Trz113 | 118 | 0.3 | 0.1 | 0 |

* Activities are expressed relative to wild type.
CYS = cysteine.
DJA = djenkolic acid.

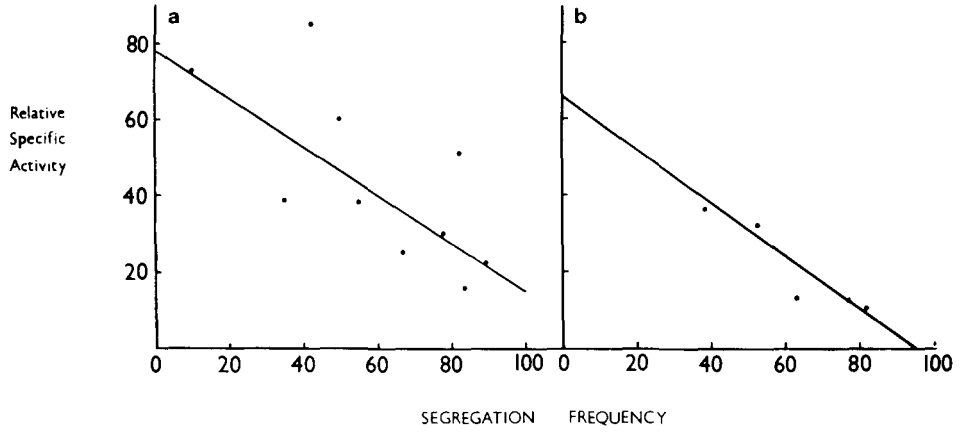


FIGURE 2.—The relationship between enzyme activity and segregation frequency. (a) PAPS synthetase in CTS21. (b) Sulphite reductase in CTS151. Each point represents the mean of three replicate assays within the same experiment; these never differed by more than 15%. The equations of the straight lines were calculated by the method of least squares. In both (a) and (b), $P = < 0.05$ from regression analysis of variance.

Cym$^-$ and the integrative events involved in its suppression are located in regulatory regions of *cys* genes. When CSP is integrated, it may reduce the level of expression of the relevant *cys* gene, but does not alter the nature of its product. This could be due to some disruption of the local template topology that may be required for optimum gene expression (SOBELL 1973).

*The presence of a deletion in the* cysALKptsHI *region of CTS strains*: KINGSMAN, SMITH and HULANICKA (1977) proposed that CSP is produced by the excision of at least part of the *cysALKptsHI* region of the chromosome. This means that the integrity of *cysK* would be disrupted and so render the Cym$^-$ mutant resistant to triazole (HULANICKA and KLOPOTOWSKI 1972). Most of the deletions in this region may arise in this way (CORDARO and ROSEMAN 1972). If CTS strains possess a deletion in the *cysALKptsHI* region, altered linkage relationships between genes in this region of their chromosomes should be detectable (ROTHMAN 1965; CHELALA and MARGOLIN 1974). This was tested by measuring linkage between *cysA* and *ptsI* in transduction between *cysA20ptsI31* and wild type, CTS21 and 71 as donors. *CysA*$^+$ recombinants were selected and replicated to MA and mannitol as their sole carbon source to determine their Pts phenotype. In all cases the linkage of *cysA* and *ptsI* was the same (about 40%), which is in agreement with CORDARO and ROSEMAN (1972) who found 46% linkage between these markers. These data suggest, in contradiction to prediction, that there is no deletion in the *cysALKptsHI* region of the chromosome of CTS strains.

## DISCUSSION

Unstable suppression of Cym$^-$ in CTS strains is a genetic rather than a metabolic phenomenon. The Cym$^+$ (suppressed) genotype can be selected and trans-

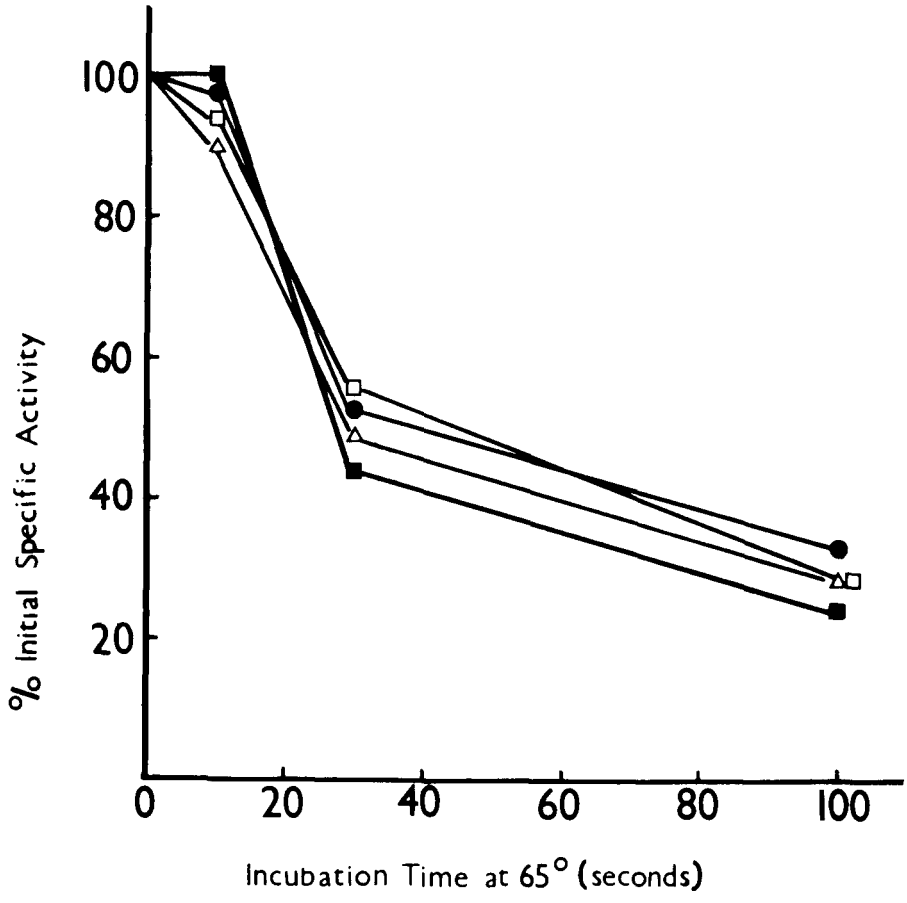


FIGURE 3.—The heat lability of PAPS synthetate in extracts of wild type, CTS21, CTS71 and CTS194. The extract of CTS21 was prepared from a culture with a segregation frequency of 39%. ● wild type; □ CTS21; △ CTS71; ■ CTS194. Each point is the mean of two replicate experiments; in no case did these differ by more than 10%.

mitted to other strains, together with other markers in the *cysCDHIJ* region (Table 1). The suppression is dependent on a functional *recA* system, and stability is markedly affected by stimulation of recombination with UV irradiation (Figure 1 and Table 2). Restored enzyme levels are relatively high and determined by wild-type proteins (Figures 2, 3 and 4 and Table 3). All these observations are in agreement with the model proposed by KINGSMAN, SMITH and HULANICKA (1978), but the absence of a deletion in the *cysALKptsHI* region of these strains is contrary to expectation. These authors showed clearly that mutation to triazole resistance was essential for the suppression of $Cym^-$. This observation and those of CORDARO and ROSEMAN (1972) led to the proposal that the suppressing element (CSP) originated in the *cysALKptsHI* region of the *S. typhimurium* chromosome. This aspect of the model now needs to be modified: there may be some unknown effect of isolation of $Trz^r$ derivatives

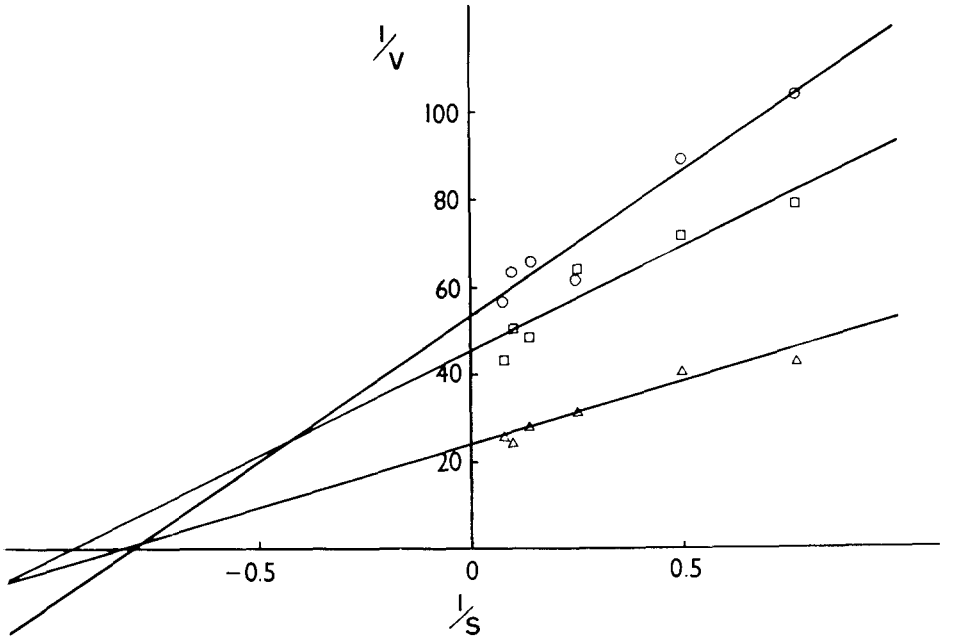


FIGURE 4.—Lineweaver-Burke plot of sulphite reductase activities with varying hydroxylamine concentrations in extracts of wild type, CTS84 and CTS151. In each case the equation of the straight lines was calculated by the method of least squares. This gave $Km_{(NH_2OH)}$ values for the three extracts as follows: wild type, △ 1.197 mM; CTS84, □ 1.046 mM; CTS151, ○ 1.157 mM. The extract from CTS151 was prepared from a culture with a segregation frequency of 32%.

that may account for the production of a DNA species able to integrate and correct the Cym⁻ lesions. Alternatively, the phenomena we have observed in CTS strains may be similar to the transposition of drug-resistance markers from some R-factors to other replicons (COHEN 1976) in which the donor replicon may not lose the transposed element (RICHMOND 1976). The estimated size of CSP of about 9 kb (KINGSMAN 1977) is about the same as the transposon Tn5 carrying Kanamycin resistance (STARLINGER and SAEDLER 1976). Thus, the *cysALKptsHI* region may donate a DNA fragment without a concommitant deletion. If this is the case, then the system is different from transposition and insertion-sequence-mediated recombination in *E. coli*, as these processes are *recA*-independent (COHEN 1976).

QURESHI, SMITH and KINGSMAN (1975) suggested that Cym⁻ mutants may be altered in regulatory regions of the various cys genes. This means that the integrative events involved in suppression of Cym⁻ occur in these regions. The observation that restored enzyme activities in CTS strains differ from wild type in quantity, but not quality, supports these suggestions. It seems unlikely that an insertion into a structural gene could result in the production of wild-type enzyme.

The restoration of enzyme activity in CTS strains may be due to recombina-

tion of the Cym⁻ site with a wild-type site on CSP (KINGSMAN, SMITH and HULANICKA 1978), or Cym⁻ may cause early termination of a *cysCDHIJ* polycistronic message (LOUGHLIN 1975) and CSP, by insertion, would perhaps provide a new promoter (*cf*, IS2, SAEDLER *et al.* 1974). These speculations await a detailed analysis of the identity of the inserted DNA.

Genetic instability in bacteria has frequently been compared with genetic instability in eukaryotes, in particular the unusual variations in *Zea mays* that led MCCLINTOCK (1956) to introduce the concept of controlling elements. Other examples of genetic instability attributable to controlling elements have been observed in *Antirrhinum majus* (HARRISON and FINCHAM 1968; HARRISON and CARPENTER 1973), *Glycine max* (PETERSON and WEBER 1969), interspecific hybrids of Nicotiana (SAND 1971) and *Nicotiana tobaccum* (DESHAYES 1973) and in Drosophila (GREEN 1973; RASMUSON and GREEN 1974; RASMUSON, GREEN and KARLSON 1974). These systems involve either one or two elements. In one-element systems, the element resides in or close to the affected gene. In the two-element systems, a separate locus, the regulatory element, controls the activity of the controlling element. PETERSON (1970) and GREEN (1973) have suggested that the closest analogues of controlling elements in prokaryotes are the IS-sequences (STARLINGER and SAEDLER 1976), while FINCHAM and SASTRY (1974) suggest that the IS-sequences are too simple to explain the facts and favor models based on the integrative properties of λ or Mu (GOTTESMAN and YARMOLINSKY 1968; HOWE and BADE 1975). Whichever of the systems is most appropriate, it seems that the properties of either IS-sequences or temperate bacteriophages are closest to the one-element systems, since in each case the insertable element controls its own activities.

The instability in CTS strains is genetically complex and involves several genetic entities. A mutation in *cysK* is required to generate the controlling element CSP (KINGSMAN, SMITH and HULANICKA 1978), which then acts at the Cym⁻ site to restore activity. The stability of this event is then controlled by the *recA* gene. While close analogies with other eukaryotic systems may be presumptuous, the phenomena observed in CTS strains may be useful in formulating ideas about the more complex, multi-element systems in eukaryotes.

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