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Location of the Gene Specifying Cytosine Deaminase in *Escherichia coli*

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Summary. The gene specifying cytosine deaminase (*cod*) is shown to be located at approximately 86 minutes on the linkage map of *E. coli*. The corresponding gene in *S. typhimurium* has been reported to have a different location.

In *Escherichia coli* and *Salmonella typhimurium* cytosine has been shown to be deaminated to uracil by cytosine deaminase (Chargaff and Kream, 1948; Hayashi and Kornberg, 1952). In a recent report Beck *et al.* (1972a) have shown that in *S. typhimurium* the gene specifying cytosine deaminase (*cod*) is located between the *arg*E and *str*A loci at approximately position 108 minutes on the linkage map (Sanderson, 1970). Since our own work with the *cod* gene in *E. coli* indicates that it is located at a different position (at approximately 86 minutes) on the linkage map (Taylor, 1970), it seemed useful to describe the data on which this conclusion is based.

Isolation of cod Mutants. Neuhard and Ingraham (1968) have shown that mutants of *S. typhimurium* lacking cytosine deaminase activity are found among colonies resistant to 5-fluorocytosine (FC). This cytosine analogue inhibits cell growth only after deamination to fluorouracil (FU), the reaction being catalysed by cytosine deaminase. Mutants lacking cytosine deaminase will therefore be characteristically resistant to FC but remain sensitive to FU. Such mutants were isolated in strain P226 (Hfr R4 *metB*$^-$) as colonies growing on minimal agar plates containing 20 μg/ml FC. A proportion of these colonies were sensitive to FU (0.25 μg/ml) and in one such mutant (SA74) no cytosine deaminase activity was detectable. The enzyme activity was determined as described by Neuhard (1968).

The *cod*$^-$ mutation in SA74 was then transfered to strain P203 (F$^-$ *thr*$^-$ *leu*$^-$ *thi*$^-$ *argB*$^-$ *his*$^-$ *trp*$^-$ *xyl*$^-$ *str*^{r}) by P1 transduction (Ahmad and Pritchard, 1969), selecting FC-resistant mutants. No FC-resistant colonies appeared on a control plate spread with an equal number of cells of P203 not pretreated with P1 indicating that the colonies on the P1 treated plates had not arisen by *de novo* mutation to FC-resistance. In addition all the transductants that were tested were found to be FC-resistant and FU-sensitive.

In order to facilitate the mapping of the *cod* gene, a *pyrE* mutation was introduced into a *cod*$^-$ derivative of P203 (SA161). Such a double mutant should not be able to grow on cytosine as sole pyrimidine source since cytosine can only

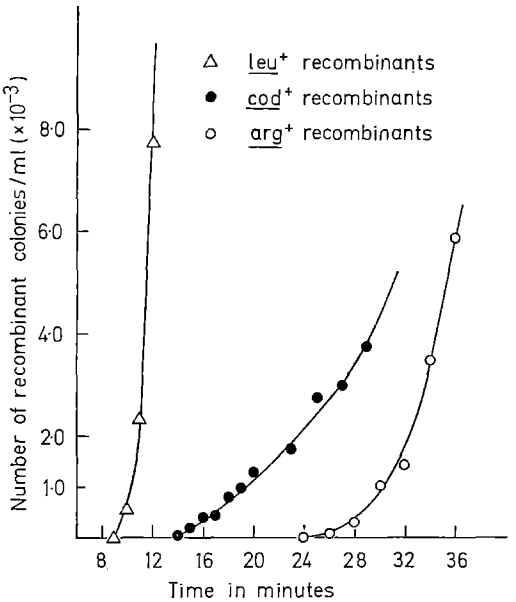

Fig. 1. Relative time of entry of *leu*+, *cod*+ and *arg*+ in an interrupted mating experiment

be used as a pyrimidine source after prior deamination to uracil (Beck *et al.*, 1972b).

The *cod*− *pyrE*− derivative of SA161 was obtained by mating it with strain SA83 (Hfr C *met*B− *pyr*E−) selecting for *xyl*+ recombinants using 0.4% xylose as sole carbon source. The *xyl*+ colonies were screened to isolate strains which were unable to grow in the absence of uridine (*pyrE*−) the uridine requirement of which could not be satisfied by cytosine (*cod*−). Such strains should retain the ability to grow in presence of cytidine as pyrimidine source since cytidine and cytosine are deaminated by a different deaminase (Wang *et al.*, 1950). One such recombinant strain (SA168) was used in subsequent experiments. Mutants of *S. typhimurium* having these properties have also been reported (Beck *et al.*, 1972a).

Mapping of the cod Gene. The location of the *cod* gene was determined from an interrupted mating experiment with Hfr P4X *thi*− *str*s (orientation of transfer *pro*B—*leu*—*thr*) and SA168 using streptomycin to prevent growth of the male strain on the plates. The number of colonies able to grow on the medium containing cytosine as sole pyrimidine source (*cod*+) or in the absence of arginine (*argB*+) or in the absence of leucine (*leu*+) was recorded after 48 hrs incubation. During the first 32 minutes after mating, an insignificant number of colonies appeared on the plates containing no pyrimidine indicating that the *pyrE*+ allele had not been transfered to a significant number of the F− cells. The results obtained (Fig. 1) indicate that the time of entry of *cod*+ is about 14 minutes after the mixing of Hfr and F− cells, whereas *leu*+ and *arg*+ appear after 9 and 24 minutes respectively. From these results we concluded that the *cod* gene is located at about 86 minutes on the *E. coli* linkage map.

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