Identification of Nonsense Mutations in the *cysJ* gene of *Salmonella typhimurium*

By R. E. LOUGHLIN

Department of Biochemistry, University of Sydney, New South Wales 2006, Australia

(Received 20 January 1976)

In *Salmonella typhimurium*, the structural genes which convert sulphate to sulphide form a cluster which maps at 90 minutes on the linkage map (Sanderson, 1972). It has been proposed previously that three of these genes, *cysJ*, *cysI* and *cysH*, form an operon with a promoter at the *cysJ* gene end (Loughlin, 1975). Part of the evidence supporting this proposal was the observation that extracts of four *cysJ* point mutants (*cysJ*148, 223, 304 and 630) lacked not only NADPH-cytochrome *c* reductase activity coded by the *cysJ* gene, but also lacked reduced methyl viologen-sulphite reductase and NADPH-3'-phosphoadenylyl sulphate reductase activities corresponding to the *cysI* and *cysH* genes respectively. These mutants thus exhibited polarity. As they were revertible by *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine they could not be frameshift or deletion mutants and thus they are likely to be nonsense mutants.

In the work reported here, the procedure and tester strains of Berkowitz *et al.* (1968) were used to test whether the four *cysJ* point mutants have nonsense mutations. The procedure consisted of replica plating revertants of the mutants on to lawns of the tester strains on minimal medium containing lactose as sole carbon source. The revertant clones receive from each tester strain an F'*lac* plasmid with a nonsense mutation in the β -galactosidase (*lacZ*) gene. Since *Salmonella* is unable to ferment lactose, only those revertants which have nonsense suppressors able to suppress both the original mutation and the nonsense mutation on the F'*lac* plasmid can grow on the lactose plates. As a check on the tester strains, revertants of known histidine nonsense mutants were included as controls.

From the results shown in Table 1 (after 6 days incubation) it may be concluded that *cysJ*148, *cysJ*223 and *cysJ*304 have nonsense mutations of either the amber or ochre type, and since good responses were observed after 2 days incubation with SB391 and SB392, it seems probable that *cysJ*148, 223 and 304 have amber mutations.

Thus three of the four mutants previously shown to exhibit polarity are now shown to have nonsense mutations. This evidence lends further support to the proposal (Loughlin, 1975) that genes *cysJ*, *cysI* and *cysH* form an operon which is transcribed from the *cysJ* gene end.

This work was supported by a University of Sydney Research Grant.

Table 1. *Responses of revertants of cysJ, hisC and hisG mutants to F' lac tester strains*

Revertants of the various mutants were replica plated on to lawns of the tester strains as described by Berkowitz *et al.* (1968). The growth responses were recorded after 6 days at 37 °C. The results after 2 days were essentially the same except that no growth was evident with SB393. The medium used has been described previously (Berkowitz *et al.*, 1968; Loughlin, 1975). Tester strains were generously provided by D. Berkowitz and by E. Balbinder; and the histidine mutants were kindly provided by J. R. Roth.

Mutant	Tester strains				
	SB391 (amber, UAG)	SB392 (amber, UAG)	SB393 (ochre, UAA)	TA216 (UGA)	TA217 (UGA)
<i>cysJ148</i>	+++	+++	+	—	—
<i>cysJ223</i>	+++	+++	+	—	—
<i>cysJ304</i>	+++	+++	+	—	—
<i>cysJ630</i>	—	—	+	—	—
<i>hisC31</i> (UAG)	+++	+++	+	—	—
<i>hisC121</i> (UAG)	+++	+++	+++	—	—
<i>hisC151</i> (UAA)	—	—	+++	—	—
<i>hisG200</i> (UGA)	—	—	+	+++	+++

—, No detectable growth; +, detectable, light growth; + + +, heavy growth.

REFERENCES

- BERKOWITZ, D., HUSHON, J. M., WHITFIELD, H. J., JR, ROTH, J. & AMES, B. N. (1968). Procedure for identifying nonsense mutations. *Journal of Bacteriology* **96**, 215–220.
- LOUGHLIN, R. E. (1975). Polarity of the *cysJHI* operon of *Salmonella typhimurium*. *Journal of General Microbiology* **86**, 275–282.
- SANDERSON, K. E. (1972). Linkage map of *Salmonella typhimurium*, edition IV. *Bacteriological Reviews* **36**, 558–586.