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The Gradient of Polarity of *z* Gene Nonsense Mutations in *trp–lac* Fusion Strains of *Escherichia coli*

The polarity gradient of a series of nonsense mutations in the *z* gene of the *lac* operon has been determined in *trp–lac* fusion strains. The results described in this paper suggest that the general nature of the polarity gradient generated by nonsense mutations in a given cistron is a function of their location within this cistron and is not determined by the position of the cistron within the operon.

Nonsense mutations located in the first cistron of an operon are known to exhibit polar effects on the expression of later cistrons in the operon and these polar effects form a steep gradient dependent on the distance between each mutation and the subsequent translation initiation site: the greater this distance, the lower the rate of synthesis of the distal gene products (Newton, Beckwith, Zipser & Brenner, 1965; Yanofsky & Ito, 1966; Newton & Zipser, 1967; Fink & Martin, 1967; Zipser, Zabell, Rothman, Grodzicker & Wenk, 1970). The observation that nonsense mutations located in operator distal cistrons of the *his* operon do not generate significant polarity gradients suggests that the steepness of a polarity gradient might be in part related to the position in the operon of the cistron being studied (Fink & Martin, 1967). If this model were correct, nonsense mutations in operator-proximal cistrons would generate steep gradients while nonsense mutations in internal cistrons would generate shallow or no gradients. To test this possibility, we have examined eleven nonsense mutations in the *z* gene of the *lac* operon in order to determine the gradient of polarity in three situations: (1) in the "normal" case in which the *z* gene is the operator-proximal cistron of the *lac* operon; (2) in a strain in which the *lac* operon has been fused by means of a deletion to the *trp* operon in such a way that *z* is a *trp* operator-proximal cistron of the fused *trp–lac* operon; and (3) in another *trp–lac* fusion strain in which the *z* gene is a *trp* operator-distal cistron of the fused *trp–lac* operon. As will be described in this paper, the normal steep gradient generated by *z* nonsense mutations is found in all three situations suggesting that the relative position of a cistron

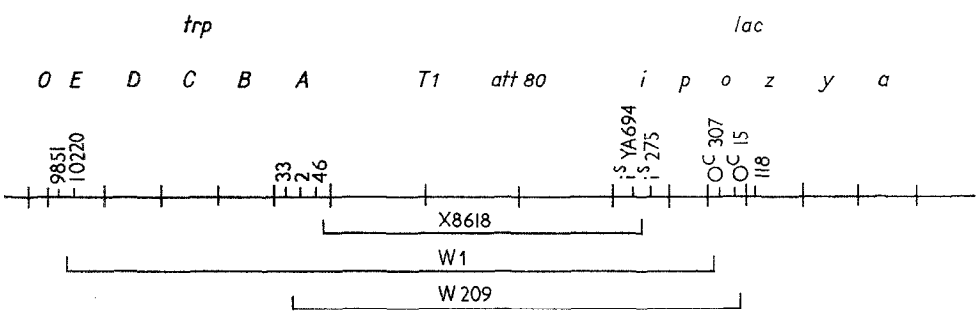

FIG. 1. Deletions fusing the *trp* and *lac* operons. W1 is described in Reznikoff *et al.* (1969). W209 and the *trp* end of X8618 were mapped as described in Reznikoff *et al.* (1969). The *i* end of X8618 was mapped by J. Miller (unpublished results).

within an operon does not significantly affect the polarity gradient generated by its nonsense mutations.

The fusion strains used in these experiments are *trpR*$^-$ (*trp* constitutive) derivatives of W1 (described in Reznikoff, Miller, Scaife & Beckwith, 1969) and W209 (to be described in a subsequent communication by Mitchell, Reznikoff, Beckwith & Michels) (see Fig. 1). In both cases, the fusion deletion extends from a structural gene in the *trp* operon to the *lac o* region. The expression of the *lac z* gene therefore is due to transcription initiated at the *trp* promoter. The isogenic control strain (*trpR*$^-$ X8618) contains a similar *T1*^{r}*-trp*$^-$ deletion as W1 and W209, except that the deletion ends in the *lac i* gene so that transcription of the *lac* operon initiates at the *lac* promoter (see Fig. 1). This strain was isolated by J. Miller, and is similar to strains described in Miller, Beckwith & Muller-Hill (1968). The nonsense mutations used have been described before (Newton *et al.*, 1965; Grodzicker & Zipser, 1968; Zipser *et al.*, 1970). Their genetic locations are shown in Figure 2.

The results are shown in Table 1 and in Figure 2. The polarities of the *z* nonsense

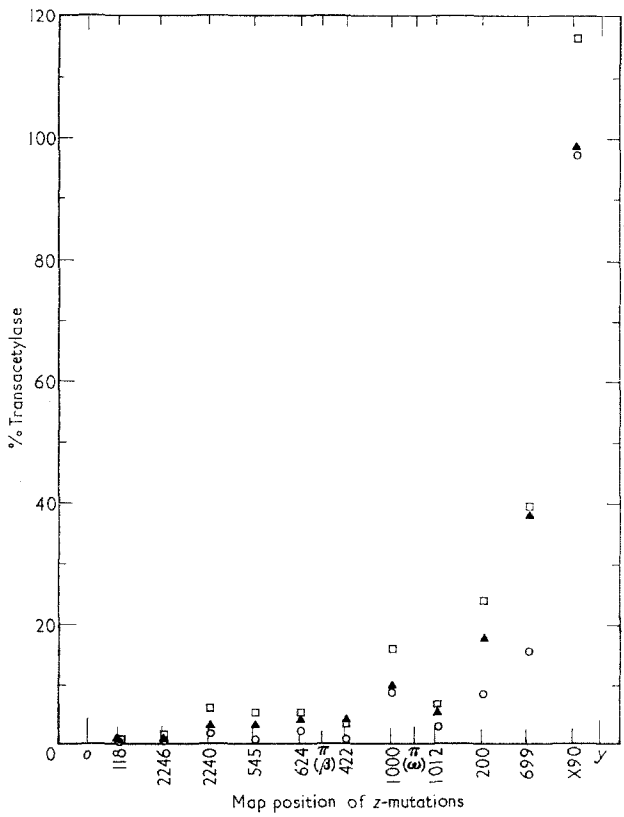

FIG. 2. The polarity of *z* nonsense mutations in the *trp–lac* fusion strains. The results shown here are taken from Table 1. The abscissa represents the genetic order of the *z* nonsense mutations studied and is not intended to represent the actual genetic distances between them. The percentage transacetylase found in X8618-nonsense mutation strains is shown with a □, that found in W1-nonsense mutation strains is shown with a ○, and that found in W209-nonsense mutation strains is shown with a ▲.

TABLE 1

Thiogalactoside transacetylase activity of z *nonsense mutations in the fusion strains* trpR⁻ *X8618,* trpR⁻ *W1 and* trpR⁻ *W209.*

Strain	Transacetylase activity	% Wild-type activity
R⁻ W1	11·5	100
R⁻ W209	14·6	100
R⁻ X8618	13·2	100
R⁻ W1-U118	0·05	0·4
R⁻ W209-U118	0·09	0·6
R⁻ X8618-U118	0·13	1·0
R⁻ W1-AP2246	0·10	0·9
R⁻ W209-AP2246	0·08	0·6
R⁻ X8618-AP2246	0·22	1·7
R⁻ W1-AP2240	0·24	2·1
R⁻ W209-AP2240	0·44	3·0
R⁻ X8618-AP2240	0·76	5·8
R⁻ W1-NG545	0·12	1·0
R⁻ W209-NG545	0·49	3·4
R⁻ X8618-NG545	0·72	5·5
R⁻ W1-NG624	0·26	2·3
R⁻ W209-NG624	0·60	4·1
R⁻ X8618-NG624	0·68	5·2
R⁻ W1-NG422	0·13	1·1
R⁻ W209-NG422	0·58	4·0
R⁻ X8618-NG422	0·50	3·8
R⁻ W1-NG1000	1·05	9·1
R⁻ W209-NG1000	1·43	9·8
R⁻ X8618-NG1000	2·13	16·1
R⁻ W1-NG1012	0·34	3·0
R⁻ W209-NG1012	0·79	5·4
R⁻ X8618-NG1012	0·87	6·6
R⁻ W1-NG200	1·00	8·7
R⁻ W209-NG200	2·63	18·0
R⁻ X8618-NG200	3·20	24·2
R⁻ W1-NG699	1·80	15·7
R⁻ W209-NG699	5·55	38·0
R⁻ X8618-NG699	5·20	39·4
R⁻ W1-X90	11·2	97·4
R⁻ W209-X90	14·4	98·6
R⁻ X8618-X90	15·4	116·7

The cultures were grown in 1% Casamino acids, 1mM-$MgSO_4$, 4mM-sodium citrate, 100 μg tryptophan/ml., 200 μg proline/ml., 6·7 μg vitamin B_1/ml., in M9 (Adams, 1959). The assays were done using the method described in Michels & Zipser (1969). Each strain was assayed at least twice and in most cases more often. The percentages given represent the percentage activity found in the fusion strain containing the nonsense mutation as compared to the activity found in the appropriate wild-type fusion strain. No correction has been made for the non-linear relationship between enzyme activity and protein concentration described in Michels & Zipser (1969). Therefore, while these percentages may not accurately represent the percentage of transacetylase *protein,* differences between the percentages are real.

mutations in the control strain are consistent with those previously published (Newton *et al.*, 1965; Grodzicker & Zipser, 1968; Michels & Zipser, 1969; Zipser *et al.*, 1970). In the fusion strains, all of the mutations were at least as polar as in the control. The fusion strains also showed qualitatively the same steep gradients of polarity.

The results described in this paper suggest that the general nature of the polarity gradient generated by nonsense mutations in a given cistron is a function of their location within this cistron and is not determined by the position of the cistron within the operon. It is possible that the failure to observe steep polarity gradients in the internal cistrons of the *his* operon is due to an insufficient number of nonsense mutations in these cistrons.

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