DEPENDENCE OF β-GALACTOSIDE-TRANSACETYLASE ACTIVITY ON THE TEMPERATURE OF GROWTH IN E. COLI STRAINS WITH POLAR MUTATIONS IN THE OPERATOR-PROXIMAL PART OF THE β-GALACTOSIDASE GENE

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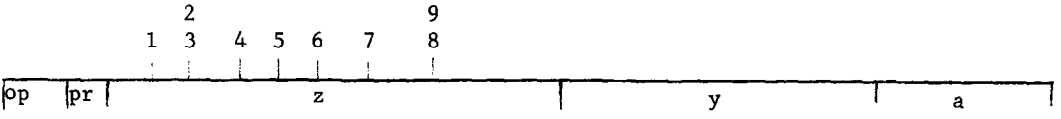
The order of genes in the lac operon of E. coli starting from the operator end is the following: z (specifying β-galactosidase), y (specifying β-galactoside permease) and a (specifying β-galactoside-transacetylase, subsequently abbreviated as acetylase) (Fig. 1). Mutations in the z gene which not only abolish the expression of z but also that of y and of a belong to the class of mutations designated as polar (Jacob and Monod, 1961; Franklin and Luria, 1961; see also Beckwith, 1967). In this communication we present data showing that the acetylase activity (and thus the extent of polarity) in E. coli strains with polar mutations in the z gene depends upon the temperature at which the cells are grown. This dependence was found to be pronounced in the case of six mutants tested, which map in the operator proximal region of the z gene. In the case of three other mutants tested, which map in a more distal region from the operator, the dependence was less pronounced, comparable to that of wild type E. coli.

Results

The acetylase activity (units of acetylase measured at 28°/mg protein) in extracts from cells grown at 26° was compared to that in extracts from cells grown at 41° (Table 1). The ratio of acetylase activities (activity in extracts from cells grown at 26°/activity in extracts from cells grown at 41°)

Figure 1



Approximate order of map positions of mutants in the z gene of E. coli

op = operator; pr = promotor z = β-galactosidase gene

y = β-galactoside-permease gene a = β-galactoside-transacetylase gene

The strains to which the numbers refer are indicated in Table I. The map positions of the mutants were taken from the following references: Jacob et al. (1965); Newton et al. (1965); Scaife and Beckwith (1966); Zipser and Newton (1967); and Ullman et al. (1967). The map position of 3000 X71 was determined by A. Newton.

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was about 2 in the wild type E. coli 3000 (see also Zabin, 1963). In extracts of cells with polar mutations in the z gene the ratio depended on the site of the mutation. It was higher than 5 (up to 13) in the mutants mapping in the operator proximal region, and decreased to about 2 in the mutants mapping further away from the operator.

A large percentage of the polar mutants in the z gene were found to be either amber or ochre nonsense mutants (see Newton et al., 1965). A characteristic of such amber or ochre nonsense mutants is that amber or ochre suppressors partially restore the activity of the gene in which the polar mutation occurred, and increase the expression of genes more distal from the operator than the mutated gene. The extent of polarity of amber or ochre nonsense mutants in the z gene was found to decrease with the distance of the mutations from the boundary between the z and y genes (Zipser and Newton, 1967).

Table 1

β-galactoside-transacetylase activity in E. coli strains grown at 26° and in the same strains grown at 41°

No.*	Description	Type of mutation causing polarity**	β-galactoside-transacetylase*** activity (unit/mg protein) in extracts of cells grown at (a) 26°	(b) 41°	ratio of β-galactoside-transacetylase activities (a)/(b)
-	3000	none	600	300	2
1	3000 U118	0	5	<1	> 5
2	3000 X84	FS	65	5	13
3	30ω0	FS	70	8	9
4	3320	0	6	<1	> 6
5	3000 U131	A	8	1,5	5
6	30B0	?	200	30	7
7	3000 X82	A	60	25	2,4
8	3000 YA404	0	65	32	2
9	3000 X71	FS(?)	220	100	2,2
2	2000 X84 W31****	0	210	50	4,2

*The numerical order of the strains is the same as the order of their map positions in the β-galactosidase gene. No. 1 is the nearest to the operator.

**The type of mutation causing polarity was taken from the references listed in the legend to Figure 1. 3000 X71 was obtained from A. Newton, all other strains were from the collection of F. Jacob. 0 - ochre, A - amber, FS - frameshift. The frequency of reversion to lac+ and melibiose+ of 3000 X84 and 30ω0 have been shown to be increased by ICR 180 (M. Malamy, A. Newton, personal communication). The latter compound is a mutagen known to cause frameshift mutations (Ames and Whitfield, 1966). 3000 X71 was not suppressed by either amber or ochre suppressors. Its rate of reversion was slightly increase by ICR 191 (A. Newton, personal communication). Therefore it is tentatively designated as FS. The nature of the polar mutation in 30B0 was not determined.

***β-galactoside-transacetylase activity was assayed by the Cohn modification of the method of Alpers et al. (1965) (Jacob et al., 1965). Protein was determined according to the method of Lowry et al. (1951).

****2000 X84 W31 contains the same polar mutation as 3000 X84 and a suppressor (su-A, see Scaife and Beckwith, 1966). The increasing effect upon acetylase activity of low temperature and of suppressor are additive if not synergistic. The ratio of activities is higher than in the wild type.

The strains were grown to log phase in aerated flasks in a minimal medium (Jacob et al., 1965) supplemented with 1 mg/l of thiamine, 8 g/l glycerol and 10^{-3}M isopropylthiogalactopyranoside at either 26° or 41°. The doubling time of the cells was between 75 and 120 minutes at 41° and between 170 and 200 minutes at 26°.

A comparison of the acetylase activities in 3000 U118, 3320 and 3000 U131 with those in 3000 X82 and 3000 YA404 reveals that the amber and ochre mutants

tested in this study also show such a dependence of polarity upon the mapping site at both temperatures (Table 1 and Figure 1).

Certain polar mutants (e.g. 3000 X84, 30⁄0) do not respond to amber or ochre suppressors (see Scaife and Beckwith, 1966). There are indications that 3000 X84 and 30⁄0 are actually frameshift mutants (see legend to Table 1). It can be seen that the acetylase activities in these two mutants are much higher both at 26° and 41° than the corresponding activities in polar nonsense mutants mapping at either side of 3000 X84 and 30⁄0 (Table 1 and Figure 1). Thus it seems that whereas (as discussed earlier) the ratio of acetylase activities depends primarily upon the mapping site of the polar mutation, the actual acetylase activity depends (among other factors) also upon the type of mutation causing polarity.

Discussion

Acetylase is believed to be a dimer consisting of identical subunits (Zabin, 1966). It is possible that dimerization (i.e. acetylase formation) is impaired at low monomer concentrations. In that case the low acetylase activities measured might not reflect accurately the amounts of acetylase monomer formed.

It might be asked whether the more pronounced dependence upon temperature (of acetylase activity) in operator proximal mutants than in distal ones is not simply due to the fact that there is less acetylase in the proximal than in the distal mutants. That this is not the (or not the only) cause of the observed phenomena is indicated by the data in Table 1: e.g. 3000 X84 an operator proximal mutant and 3000 YA404 a distal mutant have both the same acetylase activities at 26° (65 units/mg protein). The acetylase activities in the two mutants at 41° are very different however (5 units/mg protein in 3000 X84 and 32 units/mg protein in 3000 YA404).

It remains to be seen whether the pronounced temperature dependence of polarity is restricted to mutants in the operator proximal part of the *z* gene, or if it is a more general phenomenon observable also for mutations

in the beginning region of the y gene of lac or in other operons too. Since the polar mutations resulting in the decrease of acetylase activity occurred in a gene not specifying acetylase, the acetylase in the mutants is presumably the wild type enzyme. Consequently, it is not the enzyme itself or its synthesis (e.g. the folding or dimerization of its peptide chain into an active form) which is thermosensitive, but rather an intermediate component or a phase of expression preceeding the translation of the part of the lac messenger which specifies acetylase. The identification of this presumed thermosensitive compound or phase, will, however, require further studies.

Summary

The β-galactoside-transacetylase activity in E. coli with polar mutations in the z gene depends upon the temperature at which the cells were grown. This temperature dependence was found to be more pronounced in mutants in the operator proximal region, than in those in the distal region of the z gene.

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