Deletions Fusing the $i$ and $lac$ Regions of the Chromosome in $E. coli$: Isolation and Mapping

GHO, Djim, MILLER, Jeffrey H.

Abstract

Three hundred and fifty deletions ending within the $i$ gene (which codes for the lac repressor) at one terminus and within the $z$ gene at the other terminus have been isolated. Twenty-nine of these have been mapped in detail and have endpoints which are distributed throughout the $i$ gene. Two deletions which remove only the very end of the $i$ gene still permit the synthesis of altered repressor molecules retaining some properties of the wildtype lac repressor. In strains carrying these deletions, the synthesis of lac permease is under the control of the $i$ promoter. An additional 40 deletions have been found which apparently fuse the lac permease to an untranslated portion of the terminus of the $i$-message.

Reference


DOI: 10.1007/BF00266149
Deletions Fusing the *i* and *lac* Regions of the Chromosome in *E. coli*:
Isolation and Mapping

Djim Gho
Institut für Genetik der Universität zu Köln, Köln, Germany

Jeffrey H. Miller
Département de Biologie Moléculaire, Université de Genève, Genève, Suisse

Received March 3, 1974

Summary. Three hundred and fifty deletions ending within the *i* gene (which codes for the *lac* repressor) at one terminus and within the *z* gene at the other terminus have been isolated. Twenty-nine of these have been mapped in detail and have endpoints which are distributed throughout the *i* gene. Two deletions which remove only the very end of the *i* gene still permit the synthesis of altered repressor molecules retaining some properties of the wild-type *lac* repressor. In strains carrying these deletions, the synthesis of *lac* permease is under the control of the *i* promoter. An additional 40 deletions have been found which apparently fuse the *lac* permease to an untranslated portion of the terminus of the *i*-message.

Introduction

The lactose operon in *E. coli* is a cluster of three genes involved in the metabolism of lactose (Jacob and Monod, 1961). These genes are termed *z*, *y*, and *a*, and code for the structures of beta-galactosidase, permease, and thiogalactoside-transacetylase, respectively. Initiation of transcription of the *lac* operon message occurs in the promoter region, a small part of the DNA located at the beginning of the operon (Scaife and Beckwith, 1966). Maximum levels of transcription of the lactose operon require not only an intact promoter, but also a protein factor, CAP, which interacts with both cyclic-AMP and the promoter (Zubay et al., 1970; Emmer et al., 1970; Silverstone et al., 1970). Recently, evidence has been presented to show that the promoter region consists of two sites, one of which interacts with the CAP protein and the other presumably with RNA polymerase (Beckwith et al., 1972).

The lactose operon is under the control of a protein termed the repressor, which is coded for by the nearby *i* gene (Jacob and Monod, 1961). Normally, the repressor binds to the DNA at the beginning of the operon at a site termed the operator (Jacob and Monod, 1961; Gilbert and Müller-Hill, 1967). When complexed with certain lactose analogues, particularly IPTG (isopropylthio-beta-D-galactoside), the repressor is no longer able to bind to the operator and transcription is permitted to occur at the maximal rate (Jacob and Monod, 1961). IPTG is thus said to induce the operon.

Several experiments have demonstrated that the genes of one operon can be effectively fused to those of a second operon. The first description of such a
system was by Jacob, Ullmann, and Monod (1965), who fused the \textit{lac} genes to the \textit{pur} operon by isolating a strain carrying a deletion of the chromosomal material between the two operons. One end of the deletion cut into the \textit{pur} operon and the other endpoint terminated within the \textit{z} gene. The remaining intact \textit{lac} genes were then shown to be no longer under the control of the lactose operon, but instead to respond to the controls of the \textit{pur} operon (Jacob et al., 1965).

Fusions of the \textit{lac} genes to the \textit{trp} operon have also been reported (Miller et al., 1970a). Such strains have a wide use in the study of gene control (Beckwith, 1970). For instance, by placing the synthesis of beta-galactosidase under the control of the \textit{trp} operon it became possible to assay the \textit{trp} repressor by monitoring its ability to repress the production of beta-galactosidase \textit{in vitro} in a cell-free system programmed with DNA from the fusion strain (Zubay et al., 1972).

In this report we describe a series of strains in which the lactose permease and presumably thiogalactoside-transacetylase are now placed under the control of the \textit{lac i} gene. This has been done by isolating a set of strains carrying deletions with one end in the \textit{i} gene and the other terminus in the \textit{z} gene. The starting strain carried a mutation (\textit{iQ}) which results in a 10-fold increase in the level of \textit{lac} repressor (Müller-Hill et al., 1968). The increased level of expression of the \textit{i} gene is sufficient in these strains to allow the synthesis of enough permease to permit growth on certain galactosides, such as melibiose. We have mapped the endpoints of a set of these deletions in both the \textit{z} and \textit{i} genes. These strains provide a useful source of deletions for fine structure mapping of both the \textit{i} gene and the \textit{z} gene. The use of different galactosides dependent on yet higher levels of permease should enable one to use these strains to select additional mutations in the \textit{i} gene promoter. Also, these strains will facilitate studies aimed at determining the controls operating on regulatory genes.

\textbf{Materials and Methods}

\textbf{Strains}

<table>
<thead>
<tr>
<th>Strains</th>
<th>Sex</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG1</td>
<td>F'\textit{lacproA, B(iQ)L8)}</td>
<td>$\Delta$\textit{(lac-proB)$_{z11}$ strA thi}</td>
</tr>
<tr>
<td>DG2</td>
<td>F-</td>
<td>$\Delta$\textit{(lac-proB)$_{z11}$ swpE nal thi}</td>
</tr>
<tr>
<td>X8606N</td>
<td>F-</td>
<td>ara $\Delta$\textit{(lac-proB)$_{z11}$} $\Delta$(tonB-trp) (\textit{?} 80d\textit{lacI}) \textit{nal A strA thi}</td>
</tr>
<tr>
<td>X8601-X8661</td>
<td>F-</td>
<td>like above but \textit{nal}+</td>
</tr>
<tr>
<td>CSH 1-11</td>
<td>F-</td>
<td>\textit{lacZ trp strA} each strain carries a nonsense mutation in one of 27 intervals in the \textit{lacZ} gene (Zipser et al., 1970)</td>
</tr>
</tbody>
</table>

\textbf{Mapping Techniques}

In order to map the deletion endpoints in the \textit{i} gene, each diploid was crossed with a \textit{Nal}\textsuperscript{r} strain carrying a \textit{lac-proB} chromosomal deletion. Selection was for \textit{Lac}\textsuperscript{+}Pro\textsuperscript{+}\textit{Nal}\textsuperscript{r}, the donor strain being \textit{Nal}\textsuperscript{r} and in about half of the cases also \textit{Trp}\textsuperscript{r}. Prior to each cross the donor di-
ploids were grown to saturation and subcultured 3 times to allow recombinants to accumulate. In a typical cross 0.5 ml of exponentially growing donor cells was mixed with 0.5 ml of recipient cells which were at a density of 5–8 × 10^9/ml. The mating mixture was incubated in a large test tube and placed on a roller drum at 30 rpm at 37° for 30 minutes. At the end of this time the cells were spun down and washed twice in buffer and then resuspended in 1 ml of buffer. The number of recipient cells which had received the F'lacpro factor was determined by plating dilutions on glucose minimal medium containing nalidixic acid. Most of the mating mixtures contained between 5 × 10^7 and 2 × 10^8 recipient cells/ml carrying the F'factor at this point. Aliquots of 0.2 ml were plated onto the lactose-nalidixic acid selection plates, the entire volume being plated onto 5 plates. When low frequencies were being determined, mating mixtures were prepared in larger volumes, the entire mixture again being plated. As many as 10 independent determinations were used in cases where recombination frequencies were low. Verification of the i+ character of the lac+ recombinant F' factors was accomplished by replica on Xgal indicator medium (see Miller, 1972) with and without IPTG.

Deletions which left the i gene intact could be mapped against Δ605 by plating diploids directly on lactose minimal medium. As many as 10^10 cells were plated to establish a negative result. The deletion endpoints in z were mapped as described in Miller (1972, p. 159).

Results

Wild-type strains of E. coli K12 require the lac permease to transport melibiose into the cell at temperatures above 37° (Beckwith, 1963). Melibiose is a weak inducer of the lac operon, and although wild-type i+ strains can synthesize enough permease in the presence of melibiose to be able to utilize this sugar, strains which are i+ and contain a defect in the lac promoter are not able to synthesize sufficient levels of the permease (Scaife and Beckwith, 1966). Revertants to Mel+ are strains which synthesize at least several fold higher levels of permease. The major class of these revertants consists of i- mutants. However, a small proportion of the revertants are z-. Although double mutants are possible, these revertants appear even when no mutagen is employed. One explanation for this class of mutants is that these strains carry deletions which fuse the lac genes to another operon. We decided to isolate and test the z- revertants which synthesized higher levels of lac permease in order to find those which might be fused to the i gene itself.

The starting strain for this work contained a chromosomal deletion covering the lac-proB region. The lac region in this strain was carried on an F' factor which also carried the proA and proB loci. The lac region on the F' factor contained both the lac promoter mutation L8 and the i promoter mutation iQ, which results in a 10-fold increase in repressor synthesis (Müller-Hill et al., 1968). Single colonies were streaked onto sectors of plates containing melibiose as the sole carbon source and incubated for 48 hours at 37°. Revertants were picked, one per sector, and streaked onto glucose minimal medium containing the dye indoxyl-galactoside (Xgal; Davies and Jacob, 1968). This galactoside is a substrate for beta-galactosidase but not an inducer of the lactose operon. Colonies which are i-z+ appear dark blue, while those which are i+z- appear very pale blue. Upon prolonged incubation all strains which have any beta-galactosidase activity eventually form colonies with some blue color. Colonies which remain completely white after 72 hours incubation are z-, which can be verified by direct assay.

We examined 3,500 revertants, each originating from a different single colony. Of these, approximately 400 were found to be z- in addition to having gained the ability to utilize melibiose as sole carbon source at 37°.
Fig. 1 shows the *lac* region carried by the F' factor. We can envision four classes of deletions which would render the cell *z*− and still account for the reversion to *Mel*+ at high temperature. Class I represents a fusion to a new operon that lies outside the *lac* region, while class II represents those deletions which fuse the permease to the *i* gene itself. These two classes consist of strains which no longer have an intact *i* gene. Class III represents a hypothetical deletion which would end in the small region between the end of the *i* gene and the beginning of the *lac* promoter and yet still be fused to the *i* message. This would be possible provided the end of the *i* message does not coincide with the end of the *i* gene, but extends for some distance. It has been suggested that the *trp* message may have such a structure (Mitchell et al., quoted in Reznikoff, 1972). Class IV represents a deletion ending in the operator, thus accounting for the constitutivity which would allow the increased levels of permease. Such deletions have already been reported (Eron et al., 1970). Classes I and II can be differentiated from classes II and IV since only the latter 2 classes will retain a function *i* gene. We can test this by examining diploids containing an *i−z+ lac* region together with each of the above F' factors.

Fig. 2 shows the strain used to construct these diploids. In addition to containing the same *lac-proB* deletion as the parent strain, this strain carries a transposed *lac* region with a deletion cutting into the beginning of the *i* gene. Each F' factor carrying the respective mutations was crossed into this strain and the diploids were replicated onto Xgal indicator plates. We found 350 of the mutants to be *i−* and assigned these to classes I and II. Forty mutants were clearly *i+* and were assigned to classes III and IV. Seven mutants gave a result intermediate between *i+* and *i−*. These diploids gave uniform colony color when streaked for single colonies on indicator plates, ruling out the possibility that the intermediate level was an artefact due to a mixture of blue and white colonies. These results were also verified by direct assay (see Table 1). The most reasonable explanation for this category of deletion is that it represents a subclass of type II in which the deletion has cut out only a very small part of the *i* gene resulting in the synthesis of a repressor with a partially impaired ability to repress an *i−o+z+ lac* region in
Deletions Fusing the i and lac Regions of the Chromosome in *E. coli*

### Table 1

<table>
<thead>
<tr>
<th>Deletion carried by F'lacpro in i-z+ background (see Fig. 2)</th>
<th>Specific activity of Repressor (IPTG binding)</th>
<th>Ratio of specific activity of beta-galactosidase with and without IPTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>F- control</td>
<td>---</td>
<td>1.0</td>
</tr>
<tr>
<td>F'iQz- control</td>
<td>1.5</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Δ85</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Δ168</td>
<td>0.8</td>
<td>45</td>
</tr>
<tr>
<td>Δ203</td>
<td>1.4</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Δ207</td>
<td>1.0</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>

Repressor activity *in vitro* and *in vivo* from i-z fusions. Deletions 85 and 168 are type II fusions and deletions 203 and 207 are type III deletions (see Fig. 1). Both beta-galactosidase and repressor were assayed as described in Miller (1972; Unit VII). For the determination of beta-galactosidase cells were grown with and without IPTG (5 X 10⁻⁴ M). The specific activity of repressor is expressed as

\[
\text{% of excess IPTG inside dialysis sac} = \frac{\text{mg protein/ml}}{\text{after equilibrium dialysis.}}
\]

trans. A similar deletion has already been described (Miller *et al.*, 1970). These deletions were also detected by screening for partial IPTG binding activity amongst the i-z- strains.

### Mapping the Deletion Endpoints in the i Gene

In order to map the end points within the i gene, each mutation was crossed into a set of strains carrying deletions of varying lengths extending into the i gene. The isolation of these deletions has been described (Miller, 1970; Miller *et al.*, 1970a), as has their relative order within the i gene (Ganem, 1972; Platt *et al.*, 1972; Pfahl, 1972; Ganem *et al.*, 1973). The i deletion strains also carried a chromosomal lac-proB deletion, and thus single colonies grown on minimal medium in the absence of proline were diploids carrying the respective lac, proA, B episomes. The dotted lines in Fig. 3 indicate one type of recombination event which would convert the lac region on the episome to i+z+y+. Although the chromosomal lac region in this stain is lac+, we can cross the F' factors into a second lacpro deletion strain and examine the recipient cells for both the Lac+ and i+ character, after selecting for Lac+ colonies (see Materials and Methods).

Fig. 4 shows the deletion end points in the i gene. The representative data given in Table 2 demonstrate the ease with which one can determine the location of an i-z endpoint with respect to the existing deletion groups in the i gene, since recombination frequencies are high enough to be easily detectable for most diploids. Because this mapping system can detect recombinants which appear at frequencies as low as 10⁻², even very close deletion pairs can give positive results. The low frequencies that inevitably occur when two endpoints are extremely close do create some ambiguity concerning the absolute endpoints of the deletions, but not their relative order.
Fig. 3. Recombination between the chromosome and Flacpro factor. The dotted lines depict one type of exchange which can produce a recombinant i+z+ episome starting from the mapping diploids described in the text.

Fig. 4. Deletion endpoints in the i gene. Twenty-nine i-z deletions (top of Figure) have been mapped against 22 tonB-i deletions (bottom of Figure). These latter deletions have been described previously (Miller et al., 1970) as has their relative map position (Ganem, 1972; Platt et al., 1972; Pfahl, 1972; Ganem et al., 1973). Twenty-seven of the i-z deletions were selected at random from a collection of 350. Deletions 168 and 85 (*) were mapped only after they were found to allow the synthesis of repressor retaining partial activity.

Table 2

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Frequency of i+z recombinants per 10⁷ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>114 × 646</td>
<td>428</td>
</tr>
<tr>
<td>114 × 612</td>
<td>48</td>
</tr>
<tr>
<td>114 × 625</td>
<td>83</td>
</tr>
<tr>
<td>114 × 617</td>
<td>1</td>
</tr>
<tr>
<td>114 × 660</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>114 × 604</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>114 × 632</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>107 × 632</td>
<td>380</td>
</tr>
<tr>
<td>107 × 610</td>
<td>210</td>
</tr>
<tr>
<td>107 × 628</td>
<td>60</td>
</tr>
<tr>
<td>107 × 608</td>
<td>7</td>
</tr>
<tr>
<td>107 × 618</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>107 × 601</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Recombination frequency of i-z deletions with tonB-i deletions. (Distances not drawn to scale.)
Deletions Fusing the \textit{i} and \textit{lac} Regions of the Chromosome in \textit{E. coli}

**Fig. 5. Deletion endpoints in the \textit{z} gene.** \textit{F}\textsuperscript{-} strains carrying point mutations in one of 27 intervals in the \textit{z} gene (Zipser et al., 1970) have been used to map the \textit{i}-\textit{z} deletion endpoints. Here the number of the mutation has been given as the number of the interval it defines. The original mutation numbers (Zipser et al., 1970) are as follows: 1-2246, 3-2163, 4-2141, 6-2403, 8-2343, 10-2365, 11-2388, 14-2116, 18-2173, 20-2365, 21-2394, 27-143B

**Fig. 6. Deletion endpoints in the \textit{i} and \textit{z} genes**

From 27 \textit{i}-\textit{z} deletions picked at random from the collection of 350, at least 13 different endpoints in the \textit{i} gene could be demonstrated. There is one group of 8 deletions (A3, A4, A9, A11, A14, A15, 120 and 135) whose \textit{i}-terminus is in the same deletion interval.

**Mapping the End Points in the \textit{z} Gene**

In order to map the end points in the \textit{z} gene, a series of nonsense mutations was used. These have been described and were previously used to subdivide the \textit{z} gene into 27 intervals (Zipser et al., 1970). The number of each nonsense mutation corresponds to the number of the deletion interval in which it is assigned. Recombination between deletions and point mutations was determined by spot
test (see Materials and Methods). The results are depicted in Fig. 5. Here we find that the 27 end points are distributed amongst 7 deletion intervals, with a large cluster in the interval between 3 and 4. Although the fact that 14 of the 27 randomly selected deletions end in this interval would seem to indicate preferential clustering, it has been shown that this interval is very large and could constitute 20% of the z gene (Morrison and Zipser, 1970).

Comparing Figs. 4 and 5, we can see that of the 27 deletions at least 19 of them are distinctly different. Although it is possible that some of the deletions represent preferred repeats (for instance the group of four consisting of A3, A9, A11, and A15), still more fine structure mapping should be completed before such conclusions can be drawn. The combined i−z map depicting the full length of these deletions is presented in Fig. 6.

Mapping the Deletions from Class III

The deletions which still possess an intact i gene and yet are z− and still synthesize high levels of permease were also mapped. Surprisingly, 38/40 end in the same interval in z (between 3 and 4 in Fig. 5). The left end of these deletions was placed to the left of the endpoint of deletion 605, which has been shown to lie between the operator and part of the promoter (Miller et al., 1968). It is probable, therefore, that these deletions cut into or beyond the lac promoter and CAP binding site. This would place them in the small region between the end of the i gene and the beginning of the lac promoter. No deletions ending in the operator (class IV) were found.

Discussion

We have described the isolation of a large set of mutants synthesizing higher levels of lac permease in a strain originally carrying both the iQ mutation and the lac promoter mutation L8 on an F' factor. In this study we specifically examined those which had become z− because several interesting types of fusion strains were possible, such as i−z internal deletions and o−z deletions. Another possibility would be fusions of the permease to the i message resulting from a deletion endpoint in the space between the end of the i gene and the beginning of the z gene. This class could be found provided that the i gene message continued for some distance after the translation termination signal at the end of the i cistron.

In order to distinguish between these possibilities, tests for repressor activity in vivo were performed. To further characterize those strains which were i−, detailed mapping studies were carried out on a random collection of 27 such deletion strains. As shown in Figs. 4—6, these revertants carried deletions which cut out entire sections of both the z gene and the i gene. In addition, 40 revertants which were i+ were also demonstrated to harbor deletions, since they were missing the initial segment of the z gene.

In Fig. 1 two types of i−z deletions are depicted. The first class would consist of fusions that extend beyond the i gene, while the second class includes those fusions that end within i. Our results show that at least 26/27 i−z deletions tested are of the latter category. In these strains the permease is directly under the control of the i gene promoter.
Two deletions (85 and 168) which still allow the synthesis of proteins with IPTG binding activity and in one case partial operator binding activity in vivo (see Table 1) were also mapped. These were shown to map at the very end of the i-gene, as expected. The properties of a similar deletion, LI (Miller et al., 1970) clearly prove that the carboxyl-terminal end of the lac repressor is not completely essential for operator binding. The finding of deletions such as 168 (Table 1) merely serves to verify this conclusion.

The clustering of the class III deletion endpoints in the z gene is striking. It is possible that there is a deletion hot spot which is responsible for 38/40 of these deletions ending in the same interval. Another alternative is that the clustering of endpoints reflects a prejudice which is related to the levels of permease required for growth on melibiose. If these strains were really fused to the i gene message, they would still require a strong translation initiation signal, since the natural termination signal for translation at the end of the i cistron (which is still in these strains), would still operate. Perhaps the deletion endpoints in z are therefore limited to those regions near an in-phase reinitiation point, such as those described by Zipser and coworkers (Zipser et al., 1970). It is interesting to note that these clustered endpoints do fall in the region adjacent to the strongest internal translation reinitiation point described in the z gene (Zipser et al., 1970).

These new i—z deletion strains provide an independent confirmation of the order of the tonB-i deletions used to map the i gene (Ganem, 1972; Platt et al., 1972; Pfahl, 1972; Ganem et al., 1973). Also, using the i—z fusions we can separate several of the tonB deletions which previously mapped together, for instance 618 and 601 (see Fig. 4). These strains should have additional uses, such as allowing selection of i promoter mutants and permitting studies of the control mechanisms operating on repressor synthesis.

Acknowledgements. We would like to thank Marlies Becker and Hanne Neubauer for technical assistance. Much of this work was done in the laboratory of Prof. B. Müller-Hill and supported by grant SFB74 from the Deutsche Forschungsgemeinschaft. JHM was supported by a grant from the Swiss National Fund (F. N. 3.800.72).

References

Davies, J., Jacob, F.: Genetic mapping of the regulator and operator genes of the lac operon. J. molec. Biol. 36, 413–416 (1968)

Communicated by W. Gilbert

Dr. Djim Gho
Institut für Genetik
der Universität
Weyertal 121
D-5000 Köln 41
Federal Republic of Germany

Dr. Jeffrey H. Miller
Département de Biologie Moléculaire
Université de Genève
30, quai École de Médecine
Ch-1211 Genève
Switzerland