SUPPRESSORS OF MUTATIONS IN THE rII GENE OF BACTERIOPHAGE T4 AFFECT PROMOTER UTILIZATION

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ABSTRACT

Homik, Rodriguez and Weil (1976) have described T4 mutants, called sip, that partially suppress the inability of T4rII mutants to grow in λ lysogens. We have found that mutants sip1 and sip2 are resistant to folate analogs and overproduce FH2 reductase. The results of recombination and complementation studies indicate that sip mutations are in the mot gene. Like other mot mutations (Mattson, Richardson and Goodin 1974; Chace and Hall 1975; Sauerbier, Hercules and Hall 1976), the sip2 mutation affects the expression of many genes and appears to affect promoter utilization. The mot gene function is not required for T4 growth on most hosts, but we have found that it is required for good growth on E. coli CTr5X. Homik, Rodriguez and Weil (1976) also described L mutations that reverse the effects of sip mutations. L decreases the folate analog resistance and the inability of sip2 to grow on CTr5X. L itself is partially resistant to a folate analog, and appears to reverse the effects of sip2 on gene expression. These results suggest that L affects another regulatory gene related to the mot gene.

Different T4-coded proteins are synthesized in a characteristic sequence after infection of Escherichia coli. The control of this selective gene expression appears to be mainly at the level of transcription (Guha et al. 1971). O'Farrell and Gold (1973) have shown that two types of promoters can account for most early transcription in T4-infected cells. They defined early promoters as those recognized immediately after infection and middle ("quasi-late") promoters as those that can be recognized only after a delay of about 2 min at 30°. Some genes, such as rIIB and 43, are transcribed using both types of promoters, with the middle promoters being closer to the genes.

Mattson, Richardson and Goodin (1974) have isolated a T4 mutant (ts G1) affecting the expression of many early genes. The tsG1 mutation maps between genes 52 and t and defines a new gene called mot (Figure 1). Mattson, Van Houwe and Epstein (1978) have shown that the mot gene codes for a protein and that cells infected with mot mutants appear to have a transcriptional defect.

Hercules and Sauerbier (1974) have found by "UV mapping" that tsG1 prevents the utilization of some middle promoters. They irradiated T4 phage with increasing doses of UV light and then infected the cells with these phage.

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A few minutes after infection by wild-type T4, synthesis of the products of gene 43 and 45 shows less sensitivity to UV irradiation of the phage. This implies that a new promoter recognized at this time is closer to genes 43 and 45, but this new promoter does not seem to be recognized in the case of tsG1. The results of Pulitzer, Coppo and Caruso (1979) indicate that many, though not all, early genes, including the rIIB gene, are controlled both by recognition of mot-dependent middle promoters and by anti-termination of mot-independent transcription at rho-sensitive sites. Linder and Sköld (1980) reached similar conclusions and showed that expression of the gene for dTMP kinase is mot-dependent.

The T4 regulatory mutant farP85* was isolated on the basis of resistance to folate analogs (Johnson and Hall 1973). The farP85 mutation, which maps between genes 52 and t, causes overproduction of the T4-induced dihydrofolate (FH4) reductase and alters expression of other genes (Chace and Hall 1975). Sauerbier, Hercules and Hall (1976) have shown by UV mapping that farP85, like tsG1, is defective in the utilization of middle promoters and fails to complement tsG1, indicating that farP85 is in the mot gene.

Homyk, Rodriguez and Weil (1976) described T4 mutants, called sip, that partially suppress the inability of T4rII mutants to grow in λ lysogens. These mutations also map between genes 52 and t and are similar to suppressors of rII mutants described by Freedman and Brenner (1972). Homyk, Rodriguez and Weil (1976) also described L mutations that reverse the effects of sip mutations.

We have found that sip mutants have a phenotype very similar to that of mot mutant farP85, including defective promoter utilization, and that they fail to complement farP85, indicating that sip mutations are also in the mot gene. L reverses the effects of sip2 on gene expression and appears to affect another regulatory gene related to the mot gene.

**MATERIALS AND METHODS**

*Bacteriophage strains:* T4Do, an osmotic-shock-resistant derivative of T4D, is the standard bacteriophage type from which farP85 was isolated (Johnson and Hall 1973) and with which all mutants have been compared. T4 amber mutants (Epstein *et al.* 1963), isolated at the Cali-

* Abbreviation used: *far* = folate-analog-resistant. Different *far* mutants contain mutations in a variety of T4 genes (Johnson and Hall 1973).
fornia Institute of Technology, and rII deletion mutants, isolated by Benzer (1961), were obtained and used to identify bands on gels as previously described (Chace and Hall 1975). Mutants r1589-sip1, sip2, r1589-sip2-L, and L?, all in T4D, were obtained from T. Homyk. The sip1 single and the sip2-L, double mutants were constructed by crossing the r1589 out of the strains above.

**Bacterial strains**: E. coli B (nonpermissive for am mutants) and E. coli CR63 (permissive for am mutants) were used for preparing phage stocks and phage crosses. E. coli OK305 is a derivative of E. coli B that requires pyrimidine for growth and is deficient in cytidine deaminase activity (Hall, Tessman and Karlström 1967). E. coli CTr5X is a derivative of a clinical isolate and is nonpermissive for pesT mutants of T4 (Depew and Cozzarelli 1974).

**Chemicals**: Chemicals were purchased from sources previously described (Chace and Hall 1975), except that sodium dodecyl sulfate (SDS) and acrylamide were from Bio-Rad. Pyrimethamine was supplied by James J. Burchall of Burroughs Wellcome and Co.

**Media**: Broth medium, synthetic medium, and agar plates containing about 30 ml of broth or synthetic medium were prepared as described by Goscín and Hall (1972). E. coli OK305 was grown on synthetic medium supplemented with uracil (20 μg/ml). M9 medium was prepared as described by Chace and Hall (1975).

**Phage crosses**: Crosses were performed as described by Hall, Tessman and Karlström (1967).

**Preparation of cell extracts and enzyme and protein assays**: All were performed as described by Chace and Hall (1975).

**SDS-polyacrylamide gel electrophoresis**: This was performed on extracts of infected cells as described by Chace and Hall (1975), except that the cells were not subjected to UV radiation and the gels (12.5%) were run at 15 mA for 5 hr. Autoradiograms of the dried gels were prepared using Kodak XRP-5 film and were evaluated with a Helena Laboratories densitometer (Quick Scan Jr. TLC with high resolution optics) and integrator (Quick Quant II-T).

**UV irradiation**: Irradiation of phage and cells in the “UV mapping experiments” was performed as described by Sauerbier, Hercules and Hall (1976).

**RESULTS**

**Further characterization of sip mutants**: Since sip mutations map between genes 52 and t (Homyk, Rodriguez and Weil 1976), it seemed possible that they are in the mot gene. We have found that sip1 and the mot mutant farP85 show less than 0.5% recombination, suggesting that they are in the same gene (Figure 1). Comparison of sip mutants with farP85 has revealed many similarities. Table 1 shows that sip mutants are folate-analog-resistant and grow poorly on E. coli

**Efficiency of plating of mot and L mutants on different E. coli strains and media**

<table>
<thead>
<tr>
<th>Phage</th>
<th>EOP on:</th>
<th>B Broth 30°</th>
<th>OK305 Syn + Pyr 37°</th>
<th>CTr5X Broth 30°</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4D0</td>
<td>1</td>
<td>0.03</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>farP85</td>
<td>1</td>
<td>&gt; 1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>sip1</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>sip2</td>
<td>1</td>
<td>1</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>sip2-L</td>
<td>1</td>
<td>0.1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>1</td>
<td>0.1</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

* Synthetic medium containing uracil, 20 μg/ml, pyrimethamine, 75 μg/ml, and sulfanilamide, 160 μg/plate, added in the top agar.
Enzyme synthesis after infection of E. coli B by mot mutants

<table>
<thead>
<tr>
<th>Phage</th>
<th>Relative specific activity†</th>
<th>( \text{FH}_2 \text{ reductase} )</th>
<th>( \text{dTMP kinase} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected cells</td>
<td>0.2</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>T4Do</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>farP85</td>
<td>1.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>sip1</td>
<td>1.5</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>sip2</td>
<td>1.4</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

* Cells were infected at a multiplicity of 4 to 6 phage per cell for 15 min at 37°.
† Expressed relative to the specific activity present in extracts of cells infected by wild-type phage (T4Do).

CTr5X, as does farP85. Like farP85-infected cells, sip-infected cells overproduce dihydrofolate reductase and underproduce dTMP kinase (Table 2). Figure 2 shows that sip2-infected cells are delayed in the synthesis of the products of genes 43, 32 and 45 and overproduce the rIIA gene product, just as cells infected with other mot mutants (Mattson, Richardson and Goodin 1974; Chace and Hall 1975).

To test whether sip mutants also affect promoter utilization, we have used the "UV mapping technique" of Hercules and Sauerbier (1973 and 1974). We prepared autoradiograms of SDS-polyacrylamide gels to analyze proteins synthe-

![Figure 2](image-url)
The UV sensitivities of the expression of some early genes was determined by quantitating the amounts of proteins in specific bands using a sized 6 to 9 min after infection of *E. coli* B at 37 °C by *sip2* that had been UV irradiation densitometer and is shown in Figure 3B. The same type of analysis was used for wild-type phage and the results are shown in Figure 3A. The expression of the genes coding for proteins C and D shows similar UV sensitivity for *sip2* and wild-type phage. In contrast, the expression of the *rIIB* gene is much more UV sensitive for *sip2* than for wild-type phage. About 2 min after infection by wild-type phage, a nearby middle promoter for the *rIIB* gene begins to be used; whereas, a more distant promoter is used earlier (O'Farrell and Gold 1973; Schmidt et al. 1970). One would expect a decrease in the UV sensitivity of *rIIB* gene expression after the middle promoter starts being utilized. Hercules and SauriBier (1973) have shown that deletion of this promoter apparently results in increased UV sensitivity of *rIIB* gene expression. This suggests that *sip* mutants are defective in the utilization of the *rIIB* middle promoter. SauriBier, Hercules and Hall

**Figure 3.**—Rates of synthesis of T4 early proteins as a function of UV dose to the infecting phage. (A) T4Do; (B) *sip2*; (C) *sip2-L2* (double mutant); (D) *L2*. These rates were determined by densitometric tracings of autoradiograms of SDS-polyacrylamide slab gels after infection of *E. coli* B by phage that have been UV-irradiated for 0, 10, 20 or 30 sec. Proteins were pulse-labeled with a mixture of 14C-labeled amino acids from 6 to 9 min after infection at 37 °C. Proteins C and D, which are the two darkest bands just above gene 32 protein in Figure 2, were not identified, but were evaluated as references.
D. H. HALL AND R. D. SNYDER (1976) have found that mot mutants are defective in the utilization of the middle promoters for genes 43 and 45. The expression of genes 43 and 45 appears to be more UV sensitive for sip2 than for wild-type phage, but we have been unable to quantitate this because the gene 43 product is made in relatively small amounts, and the gene 45 product is not resolved well on our gels.

**Complementation studies:** Since sip and mot mutations cause very similar phenotypes and are very close on the genetic map, complementation studies were done to determine if they are in the same gene. These studies were performed by mixedly infecting *E. coli* B with sip2 and farP85 and determining whether the synthesis of the products of gene 43 and 32 is delayed as it is after infection by either mutant alone. We find that the mixed infection shows the characteristic delays (Figure 4). This apparent lack of complementation could be due to the dominance of one of the mutations. SAUERBIER, HERCULES and HALL (1976) have shown that farP85 is recessive to wild type. Figure 4 also shows that in a mixed infection with an amber mutant in gene 43 (or 32) and sip2, the synthesis of the product of gene 43 (or 32) is not delayed. Note that in these infections, the gene 43 (or 32) product must be made from the sip mutant DNA. This shows that sip2 is recessive and that the sip+ product can act in trans to turn on the expression of genes 43 and 32. Since sip2 and farP85 do not complement each other, we conclude that sip2 is in the mot gene.

**Further characterization of an L mutant:** HOMYK, RODRIGUEZ and WEIL (1976) described L mutations that reverse the effects of sip mutations on phage growth. Table 1 shows that L decreases the folate-analog-resistance and the inability to grow on CT5X of sip2. L itself is partially resistant to the folate ana-
log. $L_2$ also reverses the effects of sip2 on the expression of genes 43, 32, 45 and rIIA (Figure 2). That is, the sip-$L_2$ double mutant has expression of these genes similar to that of wild-type T4. The $L_2$ single mutant also has gene expression similar to wild type. Figure 3C shows that $L_2$ reverses the increased UV sensitivity of rIIB gene expression shown by sip2. The $L_2$ mutation by itself does not appear to affect the UV sensitivities of expression of the genes studied (Figure 3D).

**DISCUSSION**

We have shown that sip mutations are in the mot gene. The mot gene affects the expression of many genes at the level of utilization of middle promoters (Sauerbier, Hercules and Hall 1976; Pulitzer, Coppo and Caruso 1979). The high UV sensitivity of rIIB gene expression 6 to 9 min after infection by sip2 is apparently due to failure to utilize a mot-dependent middle promoter.

It is not clear why sip (mot) mutations suppress rIIs mutations. Homyk, Rodriguez and Weil (1976) detected partial suppression of rII deletion mutants and suggested that sip mutations must somehow compensate for the complete absence of rII products. Since the rII products are in the membrane (Huang 1975), other membrane proteins might partially substitute for them. In fact, Chace and Hall (1975) observed that the products of genes 39 and 52, which are also membrane proteins (Huang 1975), are over-produced by some mot mutants. The products of genes 39 and 52 are also needed for normal synthesis of DNA (Epstein et al. 1963) and are subunits of a T4 DNA topoisomerase (Liu, Liu and Alberts 1979; Stetler, King and Huang 1979).

The mutation $L_2$ effectively suppresses all the phenotypes of sip2 by changing the expression of many genes back to normal. Since mot mutations may affect transcription by altering the structure of the T4 DNA (Thermes et al. 1976), a suppressor mutation like $L_2$ might cause a compensatory change in the DNA structure, thereby restoring normal transcription. It has been suggested (see, for example, Mosig et al. 1979) that, inside the cell, T4 DNA exists in a membrane-associated complex with many proteins required for replication. Obviously, mot and $L$ mutations could cause changes in the structure of the DNA, which might cancel each other out, by altering any of the proteins in the complex or in the membrane. The $L_2$ single mutant is difficult to distinguish from wild-type T4.

The only phenotype we have observed is partial resistance to a folate analog. Preliminary results indicate that $L_2$ maps near the rII genes. Many genes in this region have products that are involved in membrane functions (Wood and Revel 1976).

This is the first report of the finding that mot mutants grow very poorly on *E. coli* CTr5X. CTr5X is a derivative of a clinical isolate and is nonpermissive for T4 mutants (pseT) that fail to induce a 3' phosphatase (Depew and Cozzarelli 1974). After infection of CTr5X by a pseT mutant, T4 DNA metabolism is altered, and there is a DNA packaging defect. Recently, Sirotnik et al. (1978) have shown that late genes are not expressed in such an infection. They suggest that the pseT gene product is involved in forming a DNA structure that is required for
late transcription and that CTrr5X might be deficient in a gene product that can substitute for the pseT gene product. In vitro transcription studies by THERMES et al. (1976) suggested that the tsG1 (mot) mutation blocks promoter recognition by affecting the DNA template, not the RNA polymerase. Thus, both mot and pseT mutations may affect proteins that somehow alter DNA structure.

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LITERATURE CITED


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