THE FINE STRUCTURE OF THE GENE CONTROLLING THE SYNTHESIS OF DNA POLYMERASE IN BACTERIOPHAGE T4B

S. I. ALIKHANIAN AND V. Z. POGOSOV

Institute for Genetics and Selection of Industrial Microorganisms, Moscow, USSR, 8-182, Box 379

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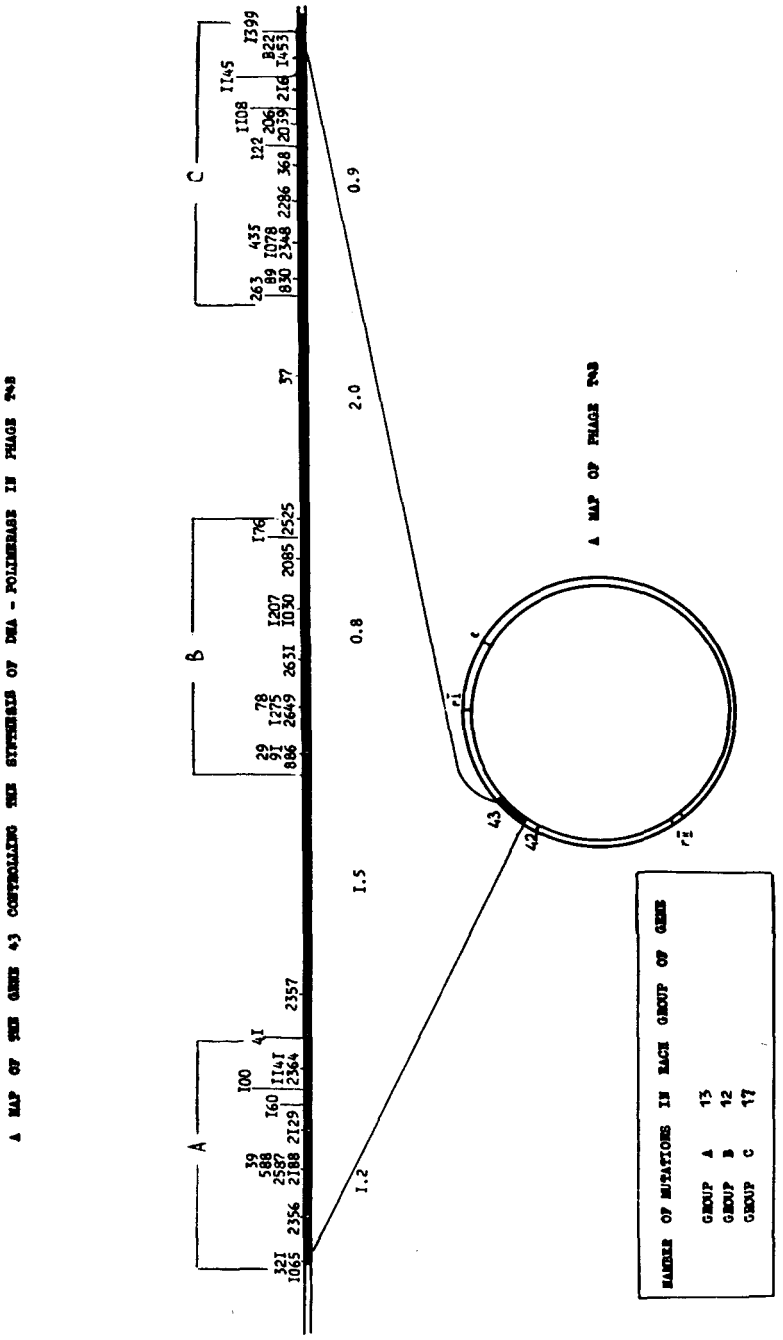
IN bacteriophage T4D gene 43 controls the synthesis of the viral specific DNA polymerase. In order to study the fine structure of that gene in the related strain T4B, we obtained about 3,000 amber mutants induced by hydroxylamine. Fifty-two of these mutants proved by preliminary test methods to be defective in gene 43. Verification of this defect was obtained by quantitative complementation tests with a T4D gene 43 reference mutant, *am*B22. Forty-four of these mutants were mapped by two-factor crosses. A genetic map of gene 43 is shown in Figure 1. On the genetic maps of phage T4D gene 43 is localized between genes 42 and 62. As indicated in the figure, in phage T4B the location of gene 43 is the same.

The 44 amber mutations in T4B fall within a segment 6.4 recombination units in length but appear clustered into three groups; group A which contains 13 mutations; group B which contains 12 mutations, and group C containing 17 mutations. The length of these clusters and the distances between them, expressed in recombination units, are shown in Figure 1. In each group of mutations the order of localization corresponds to the data of recombinant analysis.

This clustering could be due to the occurrence of 3 segments in the gene rich in G-C base pairs, and containing a number of triplets coding for trytophane or glutamine.Since most of our mutants were isolated using the *Su*$^+$-*1* suppressor strain (CR-63) we obtained new mutants active on other suppressor strains of *E. coli*, using hydroxylamine as a mutagenic agent, hoping that the use of different suppressors would expand the spectrum of mutations obtained. Nine new gene 43 amber mutants growing on strain C600 (*Su*$^+$-*2*) and 6 mutants growing on strain CA265 (*Su*$^+$-*3*) were isolated. The mutational defects again fell in one of the three previously identified clusters.

At ten of the sites identified there are two or more independent mutations. These include several instances of mutations which respond to different suppressors: i.e. *Su*$^+$-*3*–321 at the same site as *Su*$^+$-*1*–1065; *Su*$^+$-*3*–39 at the same site as *Su*$^+$-*1*–588, 2587 and 2188; *Su*$^+$-*2*–29 at the same site as *Su*$^+$-*1*–91 and *Su*$^+$-*1*–886.

The behavior of these repeat mutations on different *Su*$^+$-strains (amber, ochre) at 27°C and 42°C was examined to determine the type of acceptability of amino acid substitutions for restoration of DNA polymerase function. These studies



show that different mutations falling at the same apparent site may differ from one another in their response to different suppressors and thus cannot be regarded as identical alleles even though the resolving power of recombination analysis

(about 0.01 to 0.02% recombination) should be sufficient to insure location of such mutations to one site.

To explore more fully the functional properties of our amber mutants, a non-permissive strain was co-infected with various amber mutants together with a wild-type phage. Since between amber mutants there is no interallelic complementation, we assumed that mixed infection with amber and wild type would result in a reduced phage yield due to the non-functional protein fragments produced by the amber genomes. The mixed infections of *E.. coli* strain B with *am*-mutants, defective in regions A, B or C of gene 43, together with wild-type phage were performed at different multiplicities of infection; 5:5, 10:1 and 1:10. The results of these experiments are summarized in Figure 2. As is evident from this figure, the phage yields obtained in one-step growth experiments differ, dependent on the location of the amber mutation within the gene. Amber mutants defective in region A give the highest phage yield, while mutants defective in region C produce the lowest phage yield. This trend was found for all multiplicities examined.

While this unexpected result is not yet understood, it is possible that the degree of dominance of the mutant allele, which depends on the location of the amber mutation within the gene, is a manifestation of the direction of gene transcription. Conceivably the DNA polymerase is a multimeric protein and large polypeptide fragments inhibit the activity of normal polypeptide chains. On this notion we would infer that gene 43 is transcribed in the direction 1065 to 1399.

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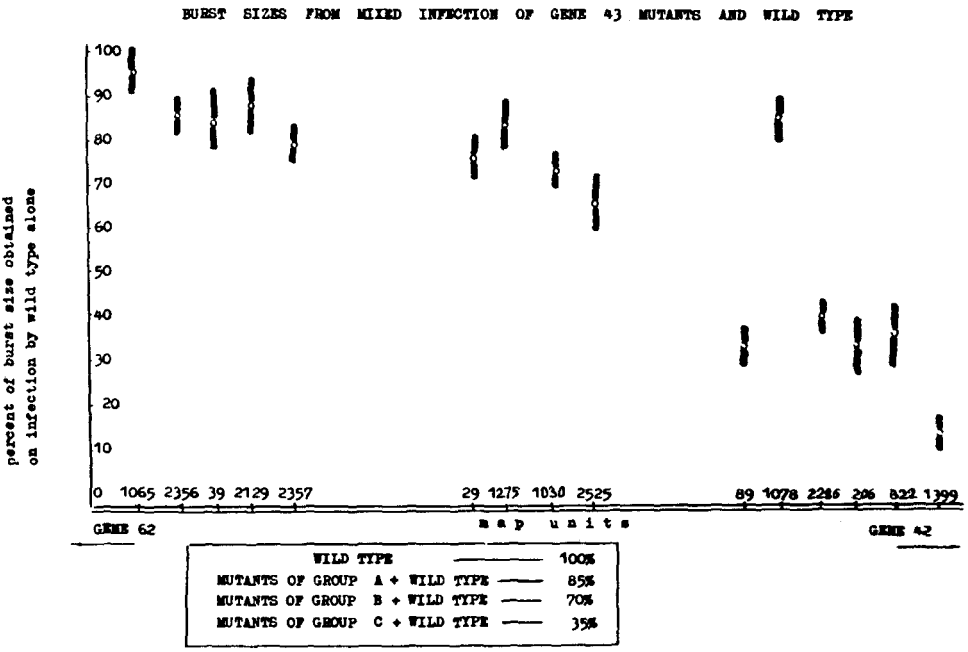

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SUMMARY

In the DNA polymerase gene of bacteriophage T4B amber mutations induced by hydroxylamine are not distributed randomly throughout the gene but are localized to three distinct clusters. Mutants occur which differ in their response to different suppressor strains but which fail to recombine. Variability in the degree of dominance of different amber mutants is found which correlates with the location of the mutant site in the gene. This polarized dominance may relate to the direction of transcription of the gene.