MUTAGENIC DNA POLYMERASE

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Received August 23, 1965

An experiment will be described which shows that DNA polymerase helps select the base in DNA replication.

After elucidating the structure of DNA (1953a), Watson and Crick proposed a template mechanism for the replication of DNA (1953b). This hypothesis invokes the same hydrogen bonds which hold the finished DNA strands together as the agents for the selection of precursor nucleotides to complement those of the parental DNA strand. The enzyme for this polymerization process was subsequently discovered,Kornberg (1960), and was not previously known to play a selective role. Nor was it implicated in mutagenesis.

In another selective polymerization directed by a nucleic acid, protein synthesis, the ribosomes-which may be considered as enzymes-affect the specificity of the process, Gorini and Kataja (1964). This suggested that the enzymes involved in nucleic acid synthesis might also affect the specificity. Since enzymes may change their substrate specificity when their gene is mutated, Hotchkiss and Evans (1960), a mutant DNA polymerase might therefore cause errors in DNA synthesis. These errors can be detected as an increase in the frequency of mutations.

When a mutant DNA polymerase was discovered a direct test became possible. This discovery was made by Epstein and Edgar and their colleagues (1963) who mapped the genes of coliphage T4. They have found about 70 genes, of which several are involved in DNA replication. One of these, gene 43, (Edgar *et al*, 1964) has been identified as a structural gene of DNA polymerase by de Waard *et al* (1965). Dr. Edgar generously gave us thirteen temperature sensitive mutants of this gene. We have studied the effect of two ts alleles of this gene on mutagenesis.

Materials and Methods

The rII system of coliphage T4 was described by Benzer (1955). The reversion frequency of rII mutants is readily determined since rII will not grow or plate on *E. coli* K 12 (λ) - (K112-12(λh). This characteristic was used in the identification of double mutants containing rHB118 and either of two ts DNA polymerase mutations, L-56 and L-141. The double mutants rHB118/L-56 and rHB118/L-141 were constructed by genetic crosses. The rII and the ts mutation of these double mutants was characterized by genetic recombination. Crosses of the double mutant with hetero alleles of cistrons rIIA and 43 gave wild type. No recombination was observed in crosses with the alleles purported to be in the double mutants.

Results

A highly variable plaque morthology was the first indication of the high mutagenicity of one of these DNA polymerase mutants. The mutant L-56 when grown and plated on E. coli B produces mostly mottled plaques and a scattering, 3% each, of rI and rII plaques. Lysozyme mutations were also seen. However, another gene 43 mutant, L-141, gave homogenous appearing plaques.

To quantitate mutagenesis, the back mutation to r^+ of rHB118/L-56 and rHB118/L-141 was studied and compared to rHB118 with the normal DNA polymerase gene. The results, Table I, show that as with the forward mutations L-56 is highly mutagenic: the reversion frequency increased 2000 fold. The temperature at which the lysate was prepared had an effect. Higher temperature, 37°C vs. 27°C, decreased the yield of plaque forming units and increased the reversion frequency.

In contrast to this rHB118/L-141 had an almost normal reversion frequency. Thus one ts allele is highly mutagenic, another is not. This indicates that mutagenesis is not due to impaired DNA synthesis or temperature sensitivity as such.

A cross of rHB118/L-56 with wild type T4 allowed the reisolation of rHB118 as judged by recombination, amber character and the now normal reversion frequency. Independent isolates of rHB118/L-56 and rHB118/L-141 gave similar results to those above. In order to check that the plaques of rHB118/L-56 on E. coli K-12(λ) represented true reversion we analysed thirty plaques. These were replated on E. coli K-12(λ)$_+$ and their plaque morphology was studied on E. coli S/6. All were r.

Table I

Mutant	Grown at	Plaques per ml at 30°C on K 12 (λ)	Plaques per ml at 30°C on B	Reversion frequency $\frac{K}{B} \times 10^7$
rHB 118	37°C	95	1.72×10^{10}	0.06
rHB 118/L-56	27°C	1.82×10^5	2.19×10^{10}	83
rHB 118/L-56	37°C	1.75×10^5	0.83×10^{10}	210
rHB 118/L-141	27°C	140	0.4×10^{10}	0.35
rHB 118/isolated from rHB 118/1-56 x T4	37°C	100	1.01×10^{10}	0.10

Preliminary results show that L-56 increases the reversion frequency of other base analogue revertible rII mutatious but has no effect on the reversion of deletions or of a frame shift rII mutation. Other gene 43 ts mutants also are mutagenic. The rII mutants produced by gene 43 ts mutants are being studied.

Discussion

The results show that one mutant of gene 43 (DNA polymerase) is mutagenic, another is not. Thus a DNA polymerase mutant has the

properties of a mutator gene. This implies that the polymerase is involved in the selection of the base in DNA replication

In the template hypothesis this may mean that a defective enzyme will cause polymerization of imperfect base pairs more often than a normal enzyme with stricter requirements.

However, the results may be an indication that the replicating enzyme is involved more directly in the selection of the base. It is possible that the information of the parental DNA strand is transmitted sequentially by the enzyme to an allosteric site where selection of the nucleotide bases and synthesis of the daughter strand occurs. Such an enzymic mechanism may permit selection by criteria other than the relatively weak hydrogen bonds postulated in the template hypothesis and account for the high accuracy of DNA replication. The effect of mutagens may be on the polymerase to induce errors in transmission and thus replication. This would be partly similar to the effect of streptomycin which acts on the ribosomes to produce errors in protein synthesis.

From this unproven model several predictious may be made. One is that different DNA polymerase mutatious may cause different types of mutation.

Acknowledgments

I thank Dr. R.S. Edgar of California Institute of Technology for the mutants of gene 43 and good advice. I thank Dr. S.P. Champe, of Purdue University, for rII mutants, and from this laboratory, Drs. C.I. Davern and P. Lengyel for encouragement, J.D. Karam for help with some experiments

This research was supported by research grant GM 12371-01 from the National Institutes of Health, United States Public Health Service.

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