MUTATIONAL ACTIVATION OF A CISTRON FRAGMENT[1]

JOHN W. DRAKE

Department of Microbiology, University of Illinois, Urbana, Illinois

Received January 25, 1963

ENZYMES often consist of more amino acids than are necessary for full catalytic activity. As an extreme example, papain may be degraded with aminopeptidase until only 76 of its original 180 amino acid residues remain, and yet retain full activity (HILL and SMITH 1960). Can analogous alterations of genes occur? A deletion penetrating into but not inactivating a gene was reported by CHAMPE and BENZER (1962). The mutation *r*1589, which originates within the A cistron of the *r*II region of bacteriophage T4, also extends into the adjacent B cistron (Figure 1). The function of the B cistron (ability to support bacteriophage multiplication in *Escherichia coli* K(λ)) is fully expressed in this mutant.

The interpretation of the functional B cistron in the mutant *r*1589 assumes a drastic alteration in the gene product (CHAMPE and BENZER 1962). Instead of two separate proteins, a compound protein is believed to be produced, one end containing a sequence of amino acids coded by the undeleted fragment of the A cistron, and the other end a sequence coded by the residual fragment of the B cistron. The portion of the code that terminates one protein, and originates the

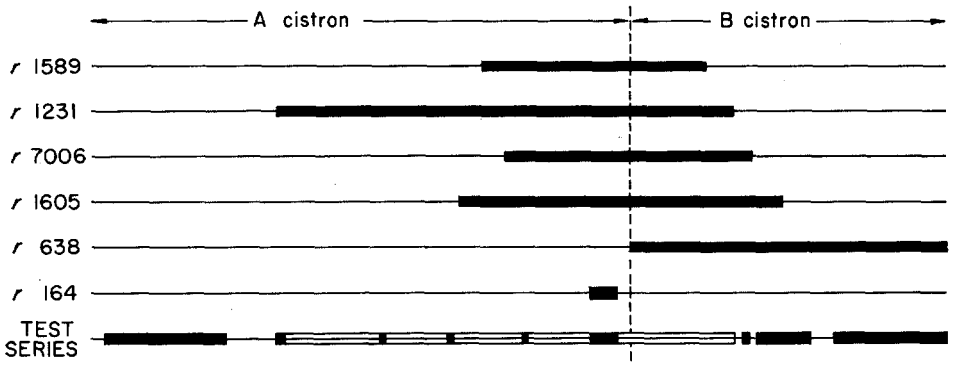

FIGURE 1.—Map of the *r*II region showing the mutants used in this study. The top four mutants are those examined for the presence of B cistron function. The next two (*r*638 and *r*164) were used in selection procedures and in the assay of B function. The bottom map groups together the mutants used to confirm the composition of suppressed *r*1231, which is indicated by the open bar; from the left, they are: *r*1364, *r*UV268, *r*UV248, *r*UV365, *r*UV183, *r*164, *r*UV24, *r*1299, and a graphical composite of *r*W8–33 and *r*187, which actually overlap each other.

[1] Supported by grant E59 from the American Cancer Society and grant E3203 from the National Institutes of Health.

next, is assumed to be lost. Two hemoblobin variants have been described with similarly compound polypeptides (BAGLIONI 1962). Each consists of a sequence of amino acids characteristic at one end of δ hemoglobin, and at the other end of β hemoglobin. Since the genes for δ and β hemoglobin are linked, deletions originating in the δ gene and terminating in the β gene create new cistrons coding the compound hemoglobin variants.

The measurement of B activity in *r*II mutants depends upon the fact that both the A and the B cistrons must be intact for T4 to multiply in *E. coli* K(λ). If an *r*II cistron contains a mutational damage, its function may be replaced by a second, co-infecting phage particle containing an undamaged cistron (complementation). K(λ) cells infected with *r*638, a deletion which covers the B cistron entirely but does not extend into the A cistron (Figure 1), will therefore yield progeny phage if simultaneously infected with another particle carrying a functional B cistron.

Three other *r*II deletions (*r*1231, *r*NB7006, and *r*1605) also cross the divide between the A and B cistrons, but cover neither cistron completely (Figure 1). None can express B activity. Following a suggestion of DR. SIDNEY BRENNER, methods were developed to detect latent B activity in these mutants. Since *r*II deletions appear to be true losses of genetic material (NOMURA and BENZER 1961), a minimum estimate can therefore be made of the portion of the B cistron which is dispensable.

For a segment of the B cistron to be active, two requirements arise. First, the deleted segment must be expendable, at least for the growth of T4 on K(λ). Second, the new (mutated) configuration of the DNA must still be transcribed into an active gene product. For the transcription process to succeed, the deletion must result in the loss of exactly $3n$ DNA base pairs (n an integer, probably 1; CRICK, BARNETT, BRENNER, and WATTS-TOBIN 1961). Furthermore, if a new coding unit arises at the junction between the severed DNA strands, it must be an acceptable unit. The failure of some of the above deletion mutants to exhibit B activity may therefore represent a breakdown of the transcription process, rather than a loss of an essential portion of the B cistron. If the number of deleted base pairs is not equal to $3n$, it should be possible to introduce a new mutation, close to one end of the deletion, restoring the number of missing base pairs to $3n$ (CRICK *et al.* 1961). Such mutations may be induced with proflavin. If, on the other hand, an unacceptable coding unit exists, it may be transformed into an acceptable unit by mutagenesis with a base analogue (BRENNER, personal communication).

MATERIALS AND METHODS

Standard conditions for handling the T4*r*II system and for mutagenesis have been described (BENZER and CHAMPE 1961; FREESE 1959; DRAKE 1963). All stocks were originally provided by DR. SEYMOUR BENZER and DR. SIDNEY BRENNER.

Assay of B function was performed qualitatively by complementation spot

tests. One drop each of *r*638 and of test phage at 4×10^9 particles/ml were mixed with two drops of K(λ) at about 2×10^8 cells/ml. After about ten minutes at room temperature, the mixture was spotted with a sterile paper strip onto a plate seeded with K(λ). A positive response consisted of a large number of minute plaques, resulting from the continued multiple infection of K(λ) with *r*638 (contributing an active A cistron) plus the test phage (contributing an active B cistron).

Quantitative measurements for B activity were performed by simultaneous infection of K(λ) with *r*638 (about four particles per cell) and the test phage (0.1 particle per cell). Unadsorbed phages were inactivated with antiserum. Lysis was completed after 30 minutes by shaking with chloroform, and the burst size was measured by plating on *E. coli* B.

Activation of B function. Cells infected with a deletion mutant were exposed to proflavin, and stocks were grown from the resulting lysates. Log phase K(λ) at 4×10^8 cells/ml were simultaneously infected with five particles per cell of the treated stock and of *r*638. Unadsorbed phages were inactivated with antiserum. Lysis was completed with chloroform after 30 minutes, and the lysates were assayed on B cells. Burst sizes were extremely small, 10^{-4} to 10^{-2} per cell. The lysates were regrown into stocks and used to reinfect K(λ) (about ten particles per cell) as above. This process was repeated either until the burst size rose to about one, or for several more passages than were required to obtain activated *r*1231.

When this process failed to yield activated B cistrons, infected cells were exposed to proflavin and 5-bromouracil simultaneously. A large proportion (roughly 20 percent) of the treated phages exhibited alterations in plaque morphology visible even through the *r* phenotype, thus demonstrating the efficacy of the mutagenic treatment. These stocks were subjected to the selection procedure described above.

At intervals during the selection process, 20 plaques were picked from the K(λ) lysates and spot-tested for B activity.

Analysis of activated deletions. The *r*II region of activated deletions was first examined by spot tests for recombination between the mutant and a series of *r*II mutants. This series consisted of several point mutations contained within the deleted segment of the original mutant, to confirm its presence; and of several deletions encompassing altogether most of the *r*II region outside of the activated deletion, to determine the location of any newly-arisen *r*II mutations (Figure 1). Activated deletions always contained the complete original deletion, and in addition a point mutation close to one end of the deletion (suppressor mutation); other mutations were not observed.

The activated deletion mutant was then backcrossed to wild type to separate the deletion from its suppressor. B cells were infected with five particles of the activated deletion plus ten particles of T4B per cell. After ten minutes at 37°, 100 phage lethal hits of ultraviolet irradiation were delivered to the complexes to stimulate recombination. From the lysates, many *r* plaques were picked with sterile paper strips into 1 ml of broth. These were spotted directly onto plates

seeded with two drops of stationary phase K(λ) cells containing two percent of stationary phase B cells plus 4×10^7 particles of *r*164, a nonreverting mutant lying about midway under each of the test deletions (Figure 1). Mutants not identical to the original deletion gave a positive response of about a dozen plaques. All such *r* mutants were purified by replating and repicking, and were spot tested against the activated deletion to eliminate new *r* mutants introduced by the irradiation.

Mapping experiments. About eight particles of each parental phage were adsorbed to broth-grown log phase B cells for six minutes at 37°. The complexes were then diluted, and unadsorbed phages were measured by the chloroform method; adsorption exceeded 98 percent. Lysis was completed after 30 minutes with chloroform. The lysates were assayed on K(λ) and B cells. The assay ratio on K and B, corrected for the plating efficiency of T4B on K, measured the frequency of wild-type recombinants. Total recombinants were obtained by multiplying this number by two. No further corrections were made.

RESULTS

Activation of B function. The map positions of *r*II mutants used in this work are shown in Figure 1, constructed from the data of BENZER (1961), CHAMPE and BENZER (1962), and DRAKE (1963).

Selection for activated derivatives of *r*1231, *r*NB7006, and *r*1605 was initiated with proflavin-treated stocks. K(λ) cells were simultaneously infected with the mutant and with *r*638, a mutant with a deletion of the entire B cistron. Most of the phage yield arose from cells infected with a mutant containing an activated B cistron and also with *r*638 containing the complementary A cistron. After two such passages, derivatives of *r*1231 exhibiting B cistron activity were detected. Three additional passages failed to yield activated derivatives of *r*NB7006 or *r*1605. These two mutants were treated with proflavin and 5-bromouracil simultaneously, and selection was again initiated. Six passages failed to yield activated derivatives.

B function was measured by simultaneously infecting K(λ) cells with *r*638 (which supplies an active A cistron) and with a mutant which may or may not supply an active B cistron. A normal yield of phage indicates the presence of an active B cistron. Burst sizes with activated *r*1231 derivatives, as well as with *r*1589 (which possessed B function naturally), were normal (Table 1).

Genetic composition of activated deletions. Isolates of activated *r*1231 were backcrossed to wild type T4B, and suppressors of *r*1231 were isolated, which were themselves *r*II mutants. The suppressor mutants which were isolated mapped in one of three sites, each closely linked to the left end of *r*1231 in the A cistron, and lying in the interval defined by the left ends of the mapping deletions *r*J3 and *r*H88 (BENZER 1961). Figure 2 shows the linkage relationships among these mutants. The nonadditivity of the map distances probably reflects both the high negative interference characteristic of crosses with very closely linked sites (see HERSHEY 1958), and distorted chromosome pairing in the region of deletions.

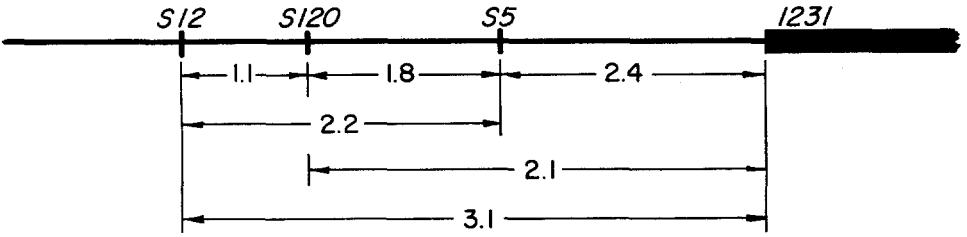

FIGURE 2.—The A cistron in the region of the left end of *r*1231, showing the suppressors of this mutant. Distances (in units of 10^{-3}) are the frequencies of wild-type recombinants, corrected for the efficiency of plating of T4B on K cells, and multiplied by two to take into account the undetected double–*r* recombinants.

TABLE 1

B cistron activity in rII mutants

Infection with single mutant		Infection with two mutants	
Mutant*	Burst size	Mutant*	Burst size
*r*638	<0.1	*r*638 × *r*1589	294
*r*1589	<0.1	*r*638 × *r*1589 + *r*FC125	<0.1
*r*1589 + *r*FC125	<0.1	*r*638 × *r*1231	<0.1
*r*1231	<0.1	*r*638 × *r*1231 + *r*S5	321
*r*1231 + *r*S5	<0.1	*r*638 × *r*1231 + *r*S12	325
*r*1231 + *r*S12	<0.1	*r*638 × *r*1231 + *r*S120	300
*r*1231 + *r*S120	<0.1	*r*638 × *r*$^+$	324
r$^+$	325		

* The + symbol indicates a double mutant, with both sites on the same chromosome. The × symbol indicates that the *r*638 site is on the chromosome of one infecting bacteriophage, while the other sites are on the chromosome of a co-infecting bacteriophage particle. Mutants with the S prefix are suppressors of *r*1231; *r*FC125 is a point mutant closely linked to the B-cistron end of *r*1589.

TABLE 2

Induced reversion of r1231 suppressors

	K/B × 10^8*			
Suppressor	Spontaneous	5-bromouracil	2-aminopurine	Proflavin
S5	3.0	15	0.6	320
S12	29	52	35	2600
S120	7.6	6.2	5.5	830

* Ratio of phage titer on K(λ) cells (revertants) to titer on B cells (total phages).

The suppressor mutations were examined for their susceptibilities to induced reversion by proflavin and by base analogues (Table 2). Each was induced to revert by proflavin, but not by 5–bromouracil or by 2–aminopurine.

DISCUSSION

Among the four deletion mutants of the T4*r*II region extending into both the A and B cistrons, the deletion extending least far into the B cistron was already

functional (CHAMPE and BENZER 1962). The next largest deletion of the B cistron could be activated by proflavin-induced suppressor mutations. These suppressor mutations were typical of the acridine type (CRICK *et al.* 1961): they were induced to revert by proflavin but not by base analogues, and they suppressed a mutant (*r*1231) consisting of an alteration in the number of DNA base pairs in the phage chromosome.

The remaining two deletion mutants could not be activated, either by proflavin or by 5–bromouracil, despite the use of powerful mutagenic and selective procedures. They may have escaped activation because their deleted portions extend too far into the B cistron. An alternative explanation would be that, while only the extreme right end of the B cistron is in fact required for activity, this portion is active only when attached to certain portions of the A cistron, coding polypeptides which assume suitable configurations.

The minimum portion of the B cistron which is dispensable may be estimated from the number of sites in the B cistron. Of the 121 B cistron sites listed by BENZER (1961) and DRAKE (1962), 21 (17.3 percent) are covered by *r*1598, and 23 (19.0 percent) by *r*1231. About 32 percent of the B cistron sites are covered by *r*NB7006 (TESSMAN 1962), which could not be activated. Cistron intervals measured by their content of sites are approximately proportional to map distances measured by recombination (BENZER 1961), so that the above figures are also approximations to map distances. At least one-fifth to one-third of the left end of the B cistron is therefore unnecessary for growth in K(λ), although this region might still be required for other (unknown) functions of the B cistron.

This portion of the B cistron may be compared to the dispensable portions of several biologically functional polypeptides. Of the 180 amino acid residues in papain, 58 percent are dispensable (HILL and SMITH 1960). Yeast enolase may be sheared of approximately 100 residues from either its carboxy-terminal or amino-terminal end without loss of activity (MALMSTRÖM 1958). Autodigestion of pepsin yields a dialyzable fraction, as yet uncharacterized, with slight activity towards hemoglobin and with high activity towards a small synthetic substrate (PERLMAN 1954). Other examples, including ACTH, growth hormone, trypsin, and ribonuclease, were discussed by ANFINSEN and REDFIELD (1956). Experiments are in progress to isolate the gene product of the B cistron and to measure its biological activity. The product, if a protein, can then be tested for activity after the removal of considerable numbers of amino acids from either of its ends.

SUMMARY

Four deletion mutations of the *r*II region of phage T4, which extend into both the A and B cistrons but which cover neither cistron completely, were tested for their ability to perform the function of the B cistron. One, as reported previously (CHAMPE and BENZER 1962), was already active. Another could be activated by suppressor mutations of the acridine type falling within the A cistron. Despite the application of powerful mutagenic and selective procedures, the other two could not be activated. A minimum of one-third to one-fifth of the B cistron was

dispensable for its functioning. This result is analogous to the shearing of considerable numbers of amino acid residues from certain enzymes, without the destruction of their activities.

ACKNOWLEDGMENTS

This work was initiated during a visit to the laboratories of DR. SIDNEY BRENNER and DR. FRANCIS CRICK, and their provision of facilities and many helpful suggestions are appreciated. It is a pleasure to thank MRS. ROBERTA NEUMAN for her excellent assistance.

LITERATURE CITED

ANFINSEN, C. B., and R. R. REDFIELD, 1956 Protein structure in relation to function and biosynthesis. Advances Protein Chem. **11**: 1–100.

BAGLIONI, C., 1962 The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. Proc. Natl. Acad. Sci. U.S. **48**: 1880–1886.

BENZER, S., 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403–415.

BENZER, S., and S. P. CHAMPE, 1961 Ambivalent *r*II mutants of phage T4. Proc. Natl. Acad. Sci. U.S. **47**: 1025–1038.

CHAMPE, S. P., and S. BENZER, 1962 An active cistron fragment. J. Mol. Biol. **4**: 288–292.

CRICK, F. H. C., L. BARNETT, S. BRENNER, and R. J. WATTS-TOBIN, 1961 General nature of the genetic code for proteins. Nature **192**: 1227–1232.

DRAKE, J. W., 1963 Properties of ultraviolet-induced *r*II mutants of bacteriophage T4. J. Mol. Biol., (in press).

FREESE, E., 1959 The difference between spontaneous and base-analogue induced mutations of phage T4. Proc. Natl. Acad. Sci. U.S. **45**: 622–633.

HERSHEY, A. D., 1958 The production of recombinants in phage crosses. Cold Spring Harbor Symp. Quant. Biol. **23**: 19–46.

HILL, R. L., and E. L. SMITH, 1960 Isolation and characterization of an enzymatically active fragment of papain. J. Biol. Chem. **235**: 2332–2339.

MALMSTRÖM, B. G., 1958 Some observations on the structural basis of enolase activity. pp. 338–340. *Symposium on Protein Structure.* Edited by A. NEUBERGER. John Wiley and Sons, Inc. New York.

NOMURA, M., and S. BENZER, 1961 The nature of the "deletion" mutants in the *r*II region of phage T4. J. Mol. Biol. **3**: 684–692.

PERLMANN, G. E., 1954 Formation of enzymatically active, dialysable fragments during autodigestion of pepsin. Nature **173**: 406.

TESSMAN, I., 1962 The induction of large deletions by nitrous acid. J. Mol. Biol. **5**: 442–445.