MUTATION AFFECTING HEAD ANTIGEN OF BACTERIOPHAGE T3*

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A previous report (Eisenstark et al., 1961) described a mutation in phage T3 affecting neutralizable antigen. It was found that antibodies against one of the T3 phages (T3B) are incapable of neutralizing a mutant of this phage (T3C). Anti-T3C sera, however, can inactivate both T3B and T3C. In addition, the mutation to T3C is a morphological one: T3C has a long flexible tail, in contrast to the short tail of T3B. The mutation also results in a striking shift in host range.

It was assumed that all of the observed changes are due to mutation affecting the tail structure of T3B and that there is no change in phage head protein. This assumption was based on the observation that, when anti-T3B serum is mixed with T3C phage of very high titer, a precipitin reaction occurs. Because the major fraction of phage protein resides on the head surface, it was taken for granted that this is probably an agglutination of phage heads. However, certain results in subsequent investigations suggested that mutation of T3B to T3C might affect a change in head antigen as well as neutralizable antigen. Experiments performed substantiated the fact that this mutation, indeed, results in alteration of at least two distinct proteins.

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METHOD

The method of Weigle et al. (1959) was used for centrifugation and collection of fractions. Plaque counts were used when possible but phage were labelled with P^{32} to detect position in tube of phage inactivated by antiserum. Also, the presence of phage often could be observed visually as a blue, milky line in the centrifuge tube. In one experiment, neutralized phage were re-activated with papain to substantiate the fact that labelled fractions consisted, indeed, of phage and not artifactual P^{32}.

Serum (k = ca. 500) was diluted 1/10 in saline. Equal quantities were mixed with phage and incubated at 37°C for 45 minutes before mixing with CsCl. Material was centrifuged at 30,000 RPM, SW39 rotor, for 24 hours.

The results in Table 1 were identical whether T3B and T3C were centrifuged separately or as mixtures.

RESULTS

When either T3B or T3C phage particles are mixed with homologous antiserum, the density of this complex is considerably less than the density of the phage alone. This is as anticipated, since the density of the gamma globulin in the serum is much less than the density of the DNA-protein of the phage, and the union of the two would lower the ratio of the heavier DNA to protein. Thus, when a mixture of phage and homologous antiserum is centrifuged in a density gradient (CsCl density 1.50), the phage are found near the top of the tube, rather than near the midpoint as found when no antiserum is present. Also of interest is the fact that the phage-antiserum union is a firm one; the centrifugation process is not severe enough to separate the two.

The surprising observation, however, was made when either T3B or T3C was mixed with heterologous antiserum. It was anticipated that the antiserum would still "float" the phage, since it was believed that they differed only in tail protein but not head antigen, and

that either anti-T3B or anti-T3C serum would contain antibodies for the common head protein. However, as shown in Table 1, anti-T3B serum fails to alter the density of T3C and the phage remain near the middle of the tube as if no antiserum were present. Thus, it is concluded that the mutation from T3B to T3C affects a change in head protein as well as tail protein. This conclusion was substantiated by complement fixation tests (method of Levine et al., 1958).

TABLE 1

DENSITY ALTERATION OF PARTICLES OF T3 PHAGE ON UNION WITH HOMOLOGOUS ANTISERUM

SAMPLE	POSITION IN DENSITY GRADIENT TUBE AFTER CENTRIFUGATION
T3B alone	19 mm. from bottom*
T3C alone	19 mm. from bottom
T3B + anti T3C serum	19 mm. from bottom
T3C + anti T3B serum	19 mm. from bottom
T3C + anti T3C serum	37 mm. from bottom
T3B + anti T3B serum	37 mm. from bottom

*Total CsCl solution in tube = 42 mm.

The next question is whether the head protein is distinctly different from the tail protein, or whether both structures consist of the same protein. It is always assumed that the two proteins are different, as has been shown with T-even (Lanni and Lanni, 1953) and lambda phage (Soller et al., 1961), but it need not be true of other phages such as T3. However, by examination of data in a previous report (Eisenstark et al., 1961) it may be seen that the head antigen and neutralizable antigen of phage T3 are different. In this report, it was shown that, while T3C is not neutralized by T3B antiserum, it still retains the neutralizable antigen of T3B. This was demonstrated in two different ways: (1) When T3C is injected into rabbits, two kinds of neutralizing antibodies are elicited, one active against T3C plus an additional one active against T3B; (2) When T3C is added to anti-T3B serum in the serum blocking power test, T3C is capable of exhausting neutralizing

antibodies against T3B. If the neutralizable and head antigens of T3C were the same, the mutation would have been such that the above two reactions would not occur.

A parallel, but not identical, situation has been described for phage P22 of Salmonella typhimurium (Yamamoto and Anderson, 1961). In this case, a mutant acquires a new tail structure and antigen, a new host range, and new head antigen. The main difference between this mutant and T3C is that T3C retains an antigen that is also present in T3B, while the mutant of P22 has no antigen in common with the wild type.

The term "mutation" has been used in a broad sense to describe a stable genetic change in T3 bacteriophage. Because this involves a simultaneous change in at least two proteins, this is probably not a point mutation, unless it is a point mutation at a regulatory locus which controls the synthesis of more than one protein. In T3, particularly, as a result of research by Fraser (1957), it is tempting to consider the possibility that the "code" that dictates the synthesis of the two new proteins is provided by the bacterial cell; i.e., this "mutation" is really a product of mating between phage and host genome, or possibly between phage and defective prophage.

SUMMARY

A mutation in phage T3 results in alteration of head antigen as well as neutralizable (tail) antigen. This conclusion is based on the observation that antisera against either wild type or mutant T3 will "float" only homologous phage, and not heterologous phage, when phage-antiserum mixtures are examined by CsCl density gradient centrifugation.

REFERENCES

Eisenstark, A., Maaløe, O., and Birch-Anderson, A., Virology 15, 56-64, (1961).
Fraser, D.K., Virology 3, 527-553, (1957).
Lanni, F. and Lanni, Y.T., Cold Spring Harbor Symp. 18, 159-168, (1953).
Levine, L., Barlow, J.L. and Van Vunakis, J., Virology 6, 702-717, (1958).
Soller, A., Levine, L. and Epstein, H.T., Bacteriol. Proc., p. 164, (1961).
Weigle, J., Meselson, M. and Paigen, K., Brookhaven Sym. in Biol. 12, 125-133, (1959).
Yamamoto, N. and Anderson, T.F., Virology 14, 430-439, (1961).