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Virus Strains of Identical Phenotype but Different Genotype

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Virus Strains of Identical Phenotype but Different Genotype

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Delbruck and Bailey (*1*) noticed an anomaly in the lysate of bacteria which was obtained by mixedly infecting the B strain of coli with the bacterial viruses T2 and T4. Subsequently, Luria (*2*) found this anomaly to be even more pronounced when he repeated Delbruck's experiment—using, however, virus T2 that had been exposed to ultraviolet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were led to the following result: If we infect a culture of the B strain of coli mixedly with the bacterial viruses T2 and T4 and incubate to permit lysis of the bacteria, there are present in the lysate 3 easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T2 and T4, i.e., one of them behaves like T2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T2) but is able to grow in the mutant strain B/4 (which is sensitive to T2); the other behaves like T4, being unable to attack B/4 (which is resistant to T4) but is able to grow in B/2 (which is sensitive to T4). The third type of virus present is phenotypically like T4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T4), but it is genotypically like T2 inasmuch as, after one passage in the strain B/2, it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T2).

The presence of this third type of virus, which may be called "latent T2," can be demonstrated in the following manner: We add to a culture of the B strain of coli viruses T2 and T4 in ratios corresponding to 10 T2 and 10 T4 virus particles per bacterium, incubate to permit lysis of the bacteria, and then filter the lysate.

If we plate a sample of this lysate on agar that is inoculated with the strain B/4 (which is sensitive to T2 but resistant to T4), those virus particles contained in the lysate which have the phenotype T2 will show up as plaques on these plates. T4 virus particles will not give plaques on this plate because B/4 is resistant to T4. The number of plaques is thus a measure of the number of T2 particles in the lysate.

Using a sample of the lysate, we determine in this manner the number of plaques obtained on an agar plate inoculated with the strain B/4. When we repeat this experiment—with the difference that before plating on the B/4 plate we add to the sample of our lysate a certain quantity of the strain B/2, allow 5 min for absorption, dilute with broth, and incubate for 1 hr to permit lysis of the bacteria—then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "latent T2") which is capable of multiplying in B/2 and subsequently forming plaques on B/4. In order to account for our observation, the concentration of the "latent T2" in the lysate would have to be about 10% of the concentration of T2. We were not able to obtain, after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "latent T2." Before drawing the conclusion that the presence of a "latent T2" is in fact responsible for our phenomenon, it is necessary to exclude alternative explanations.

As an alternative explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T2 and a T4 particle that stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T4 component) and, once inside, both virus particles T2 and T4 might then be able to multiply, and thus to produce the observed phenomenon. We were able to rule out this possibility, however, by performing the following experiment.

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow 5 min for absorption and plate on an agar plate that has been inoculated with both B/2 and B/4. If there are present any B/2 bacteria into which has entered an aggregate of virus particles composed of T2 and T4, and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T2 and T4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T4 or the B/4 is lysed by T2). This rules out the alternative explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2, so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T2 and T4.

We are thus led to conclude that the phenomenon described is due to virus particles that have the phenotype of T4, but the genotype of T2. The properties of this "latent T2" virus would seem to merit investigation.

References

1. DELBRUCK, M., and BAILEY, W. T., JR. *Cold Spring Harbor Symposia*, **11**, 33 (1946).
2. LURIA, S. Private communication (1947).

Proposed note to SCIENCE - Rough draft - Not for release

August 11, 1950

Virus strains which are of identical Phenotype but different Genotype.

By A. Novick and Leo Szilard

Delbruck and Bailey (1) had first noticed an anomaly in the lysate of bacteria which was obtained by mixedly infecting the B strain of coli with the bacterial viruses T_2 and T_4. Subsequently, Luria (2) found this anomaly to be even more pronounced when he repeated Delbruck's experiment, using however virus T_2 which had been exposed to ultra-violet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were led to the following result: If we infect a culture of the B strain of coli mixedly with the bacterial viruses T_2 and T_4 and incubate to permit lysis of the bacteria, there are present in the lysate three easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T_2 and T_4, i.e., one of them behaves like T_2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T_2) but is able to grow in the mutant strain B/4 (which is sensitive to T_2) and the other one behaves like T_4, being unable to attack B/4 (which is resistant to T_4) but able to grow in B/2 (which is sensitive to T_4). The third type of virus present is phenotypically like T_4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T_4) but it is genotypically like T_2 inasmuch as after one passage in the strain B/2, it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T_2).

The presence of this third type of virus, which may be called "incipient T_2", can be demonstrated in the following manner: We add to a culture of the B strain of coli viruses T_2 and T_4 in ratios corresponding to 10 T_2 and 10 T_4 virus particles per bacterium, incubate to permit lysis of the bacteria and then

filter the lysate.

If we plate a sample of this lysate on agar that is innoculated with the strain B/4 (which is sensitive to T_2 but resistant to T_4), those virus particles contained in the lysate which have the phenotype T_2 will show up as plaques on these plates. T_4 virus particles will not give plaques on this plate because B/4 is resistant to T_4. The number of plaques is thus a measure of the number of T_2 particles in the lysate.

Using a sample of the lysate, we determine in this manner the number of plaques obtained on an agar plate innoculated with the strain B/4, and then we repeat this experiment - with the difference that before plating on the B/4 plate we add to the sample of our lysate a certain quantity of the strain B/2, allow five minutes for absorption, dilute with broth and incubate for an hour to permit lysis of the bacteria - then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "incipient T_2") which is capable of multiplying in B/2 and subsequently to form plaques on B/4. The concentration of the "incipient T_2" in the lysate would have to be about ten per cent of the concentration of T_2 in the lysate in order to account for our observation. We were not able to obtain, after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "incipient T_2". Before drawing the conclusion that the presence of an "incipient T_2" is in fact responsible for our phenomenon, it is necessary to exclude other alternate explanations.

As an alternate explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T_2 and a T_4 particle which stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T_4 component) and once inside, both virus particles T_2 and T_4 might then be able to multiply, and thus to produce the phenomenon we observed. We were able to rule out this possibility, however, by performing the following experiment:

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow five minutes for absorption and plate on an agar plate which has been innoculated with both B/2 and B/4. If there are present any bacteria B/2 into which has entered an aggregate of virus particles composed of T_2 and T_4 and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T_2 and T_4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T_4 or the B/4 is lysed by T_2). This rules out the alternate explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T_2 and T_4.

Thus, we are led to conclude that the phenomenon described is due to virus particles which have the phenotype of T_4, but the genotype of T_2. The properties of this "incipient T_2" virus would seem to merit investigation.

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(1) Ms Delbruck and Bailey, Cold Spring Harbour Symposia Vol II p. 33. 1946

(2) S. Luria, Private communication, 1947.

Proposed note to SCIENCE - Rough draft - Not for release

August 10, 1950

Virus strains which are of identical Phenotype but different Genotype

By A. Novick and Leo Szilard

M. Delbruck had first noticed an anomaly(1) in the lysate of bacteria which were obtained by mixedly infecting the B strain of coli with the bacterial viruses T_2 and T_4. Subsequently, Luria found(2) this anomaly to be even more pronounced when he repeated Delbruck's experiment, using a bacterial virus T_2 which had been exposed to ultra-violet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were lead to the following result: If we infect a culture of B strain of coli mixedly with the bacterial viruses T_2 and T_4 and incubate to permit lysis of the bacteria, there are present in the lysate three easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T_2 and T_4, i.e., one of them behaves like T_2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T_2) but is able to grow in the mutant strain B/4 (which is sensitive to T_2) and the other one behaves like T_4, being unable to attack B/4 (which is resistant to T_4) but able to grow in B/2 (which is sensitive to T_4).

The third type of virus present is phenotypically like T_4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T_4) but it is genotypically like T_2 inasmuch as after one passage in the strain B/2 it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T_2).

The presence of this third type of virus, which may be called "incipient T_2", can be demonstrated in the following manner: We add to a bacterial culture of the B strain of coli

viruses T_2 and T_4 in ratios corresponding to 10 T_2 and 10 T_4 virus particles per bacterium, incubate to permit lysis of the bacteria and then filter the lysate.

If we plate a sample of this lysate on agar, that is innoculated with the strain B/4 (which is sensitive to T_2 but resistant to T_4), the virus particles contained in the lysate which have the phenotype T_2 will show up as plaques on these plates, because B/4 is resistant to T_4. The number of plaques is thus a measure of the number of T_2 particles in the lysate.

Using a sample of the lysate, we have determined in this manner the number of plaques obtained on an agar plate innoculated with the strain B/4 and when we have repeated this experiment, with the difference that before plating on the B/4 plate, we add to the sample of our lysate a certain quantity of the strain B/2, allow five minutes for absorption, dilute with broth and incubate for an hour to permit lysis with B/2, then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "incipient T_2") which is capable of multiplying in B/2 and subsequently to form plaques on B/4. The concentration of the "incipient T_2" would have to be about ten per cent of the concentration of T_2 in the lysate in order to account for our observation. We were not able to obtain after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "incipient T_2". Before drawing the conclusion that the presence of "incipient T_2" is in fact responsible for our phenomenon, it is necessary to exclude other alternate explanations.

As an alternate explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T_2 and a T_4 particle which stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T_4 component) and once inside, both virus particles T_2 and T_4 might then be able to multiply, and thus produce the phenomenon we observed. We were able to rule out

this possibility, however, by performing the following experiment:

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow five minutes for absorption and plate on an agar plate which has been innoculated with both B/2 and B/4. If there are present any bacteria B/2 into which has entered an aggregate of virus particles composed of T_2 and T_4 and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T_2 and T_4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T_4 or the B/4 is lysed by T_2). This rules out the alternate explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T_2 and T_4.

Thus, we are led to conclude that the phenomenon described is due to a single virus particle which bears the phenotype of T_4, but the genotype of T_2. The properties of this "incipient T_2" virus would seem to merit investigation.

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(1) M. Delbruck .....

(2) S. Luria, Private Communication, 1947.

May 19/50

Virus Strains Which Are of Identical Genotype but Different Phenotype

Max Delbruck had first noticed an anomaly in the lysate of bacteria which were obtained by mixedly infecting coli with the bacterial viruses T_2 and T_4. Subsequently Luria found this anomaly even more pronounced when he repeated Delbruck's experiment using the bacterial virus T_2 which had been exposed to ultra-violet irradiation.

When we undertook certain experiments in an attempt to understand these anomalies, we were led to the following result: In a lysate which is obtained by infecting a culture of the B strain of coli, simultaneously with the bacterial viruses T_2 and T_4 and incubating to permit lysis, there are present three easily distinguishable types of bacterial viruses. Two of these if exposed behave like the original parent strains T_2 and T_4, inasmuch as one of them is unable to attack the bacterial strain B/2 which is resistant to T_2 but able to grow in B/4 which is sensitive to T_2, and the other one behaves like T_4 being unable to attack B/4 but able to grow in B/2, which is sensitive to T_4.

There is, however, present also a third type of virus which behaves phenotypically like T_4 inasmuch as it is capable of infecting B/2 and to grow in it, but it is genotypically like T_2 inasmuch as after one passage in B/2 it is no longer capable of growing in B/2 but is capable of growing in B/4.

In our experiments we mixed to the bacterial culture that virus particles T_2 and T_4 in ratios corresponding to 10 T_2 and 10 T_4 virus particles per bacterium, and used a filtrate obtained after the lyses of the bacteria. If we add B/2 to a certain quantity of this filtrate allowing five minutes for absorbtion, dilute with broth, plate before lyses on the strain B/4 and incubate the plates the vrius particles in the lysates which behave like T_2, (i.e., do not grow in B/2 but will grow in B/4) will show up on the plate as plaques.

The number of the plaques is a measure of the number of the T_2 particles in the lysate. T_4 particles in the lysate will not form plaques on this plate

because B/4 is resistant to T_4.

We found, however, that if we added to the filtrate B/2 and allowed five minutes for absorbtion and dilute with broth--as before--but then instead of plating on B/4 first incubated for one and a quarter hours to permit lyses, and then only plated on B/4, we obtained about 10 to 25 times higher number of plaques suggesting that there was present in the filtrate a virus which was capable of growing in B/2 and after the lyses of the B/2 was capable of forming plaques on B/4. Such a virus would be phenotypically like T2 but genotypically like T4 and might be called „imitation of T2". —

finding is consistent with the assumption