Structure and Position of a Complex Chromosomal Aberration in Bacteriophage P2

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SUMMARY

The P2 phage mutation vir56, like the previously studied vir22, is the result of an unequal replacement of a chromosome segment with non-homologous DNA. The end positions of the replacements are essentially the same in the two mutants, whereas the lengths of the replacements are quite different. A third chromosomal aberration, del3, has similar structure and position. These results strengthen the suggestion that the left ends of these three aberrations coincide with the point of exchange in integrative recombination.

Recent genetic and electron microscopical studies of the temperate phage P2 (host: Escherichia coli) have suggested that the point of attachment of the P2 prophage to the bacterial chromosome through integrative recombination (Campbell model) coincides with one end of the complex P2 chromosomal aberration vir22, at about 72% of the total chromosome length, measured from the conventional left (references below). We describe here electron microscopical observations on two other complex chromosomal aberrations of phage P2, which give additional support for the above suggestion.

The vir22 mutation is the result of the loss of a DNA segment equal to 5.1% of the P2 chromosome, and its apparent replacement with a non-homologous segment of DNA, about 0.5% in length (Chattoraj & Inman, 1972). The evidence available does not exclude the possibility that the apparent replacement represents in reality only an inversion of a corresponding segment of P2 DNA. Biologically, the vir22 mutant gives clear plaques and is virulent (i.e. unable to establish lysogeny), has lost the specific immunity repressor gene C and part or all of gene int, necessary for prophage integration and excision, and is immunity insensitive in superinfection of P2 lysogens (Bertani, 1975). Furthermore, vir22 chromosomes are not a good substrate for integrative recombination (ibidem).

These facts were interpreted as shown in Fig. 1(a), and the suggestion was made that the left end of the deleted segment coincides with the point where integrative recombination operates. This hypothesis easily explains the complex nature of the vir22 deletion by analogy with the mechanism proposed for the formation of transducing λ phage: a segment of bacterial chromosome contiguous to the prophage attachment site is incorporated in the phage chromosome, and a segment of the latter, at the other prophage end, is lost (Fig. 1b). Upon independent occurrence of such an event, the sizes of the incorporated and of the deleted segments may vary, whereas one end point of the resulting aberrations ought to be invariant, since it corresponds to the point of prophage attachment.

The P2 mutant vir56, like vir22, is virulent, immunity insensitive, and defective for integrative recombination. In contrast to vir22, vir56 particles have higher buoyant density and lower heat stability, hence presumably more DNA, than the wild type (Bertani, 1975). We studied P2 vir56 DNA in the electron microscope after formation of heteroduplexes with P2 del1 del2 DNA: an example of such a heteroduplex is shown in Fig. 2, and its structure is interpreted in Fig. 1(c). The single stranded DNA loop F at one end of the
Fig. 1. (a) Sizes and positions on the P2 chromosome of several deletions according to Chattoraj & Inman (1972) and Chattoraj, Younghusband & Inman (1975). The vir22 deletion is accompanied by a short replacement as indicated in the diagram. Units are percent of wild type P2 DNA length. The positions of four genes and of the ‘early’ operon (Lindahl, 1971; Bertani, 1975) are also indicated. (b) Campbell’s model for the formation of transducing phage from the prophage state, through illegitimate recombination. ▴ ▴ ▴, bacterial chromosome; — — —, prophage; $\gamma$, $\gamma$, ends of phage chromosome (covalently joined in prophage state); $\bullet$, point of exchange in integrative recombination and in normal excision; A, B and C, possible points of exchange in abnormal excision leading to deletion of phage DNA and unequal replacement with bacterial DNA. (c) Structure of the DNA heteroduplexes formed from mixtures of P2 I$^g$ del2 and P2 I$^g$ vir56, in two preparations (I and II). See also Fig. 2. All length measurements were first normalized as percent of total wild type P2 chromosome length, by setting the measured length of segment A equal to 45.5 as determined by Chattoraj et al. (1975). Means and standard deviations of these normalized lengths are given; n is the number of molecules measured. To correct the Ds and DL values for unequal stretching in double and single stranded segments, one could relate the values for del1 and del2 (F and B) to the corresponding lengths (7.2% and 6.1%) given by Chattoraj & Inman (1972) and by Chattoraj et al. (1975), who did take into account differential stretching. Such a correction would be very small, however. The drawing is not to scale.
Fig. 2. A representative heteroduplex DNA molecule formed from P2 lg del2 del2 and P2 lg vir56. Inset: outline of the heteroduplex molecule as interpreted. The phages were prepared as described by Bertani (1975). The heteroduplexes were formed and spread as described by Summers, Brunovskis & Hyman (1973), except that the spreading solution contained 70 % formamide and the hypophase 50 %.
heteroduplex molecule ought to correspond to the del1 deletion, and allows the recognition of the right end of the chromosome. Loop B corresponds then to the del2 deletion, and the two single stranded segments Ds and Dl must reflect the presence of the vir56 aberration. Our measurements (Fig. 1c) show that (a) the sum of the double stranded segments A, C and E, plus the known lengths of del2, del1 and of the small 0.3% right end segment falls short of the 100% value by about 4%, (b) this difference very closely matches the length of Ds (the shorter of the two single stranded DNA segments involved in vir56): the Ds segment must then belong to the del1 del2 component of the heteroduplex, as indicated in the diagram, (c) the left end of the vir56 aberration is at 72.6% to 73.1% from the left (sum of A, C and length of del2, (d) the Dl segment replaces in vir56 the wild type Ds segment, and is longer than the latter by about 3% of wild type chromosome length. The excess DNA expected from the density data, provided that the empirical regression line established for DNA length and particle density (Bertani, 1975) holds also in this range, is 4.1%. We doubt whether the two values are significantly different.

We conclude that within the error of these measurements, the positions of the ends of the vir56 aberration coincide with those of the vir22 aberration, even though the lengths of the non-homologous, replacement segments are quite different in the two cases (more than 6% for vir56 and 0.5% for vir22). These findings are well consistent with the identical biological phenotypes of vir22 and vir56, and with the mechanism proposed for their origin (Fig. 1b). Furthermore, in the vir56 case, the alternative possibility that the presumed replacement be in reality an inverted segment of P2 chromosome obviously cannot be entertained since the total chromosome length in vir56 is greater than wild type.

We have made preliminary observations on another P2 deletion mutant, del3, isolated by L. Elizabeth Bertani (unpublished data), which, like vir22 and vir56, is virulent and int defective, although it is not completely insensitive to immunity. In heteroduplexes to del1 del2 DNA, del3 also appears as a complex aberration with two unequal single stranded segments of lengths 3.7% and 1.6%. These values are averages from measurements of 26 chromosomes, analysed as in Fig. 1(c). The left end point of the del3 aberration is located at 73.6% from the left, which can hardly be said to be significantly different from the position of the left ends of vir22 and vir56.

There is no reason to believe that such unequal replacement aberrations are particularly common in P2: all other chromosomal aberrations examined so far in this phage (Chattoraj & Inman, 1972, 1974; Chattoraj, Younghusband & Inman, 1975) are either simple deletions (del1, del2, del4, vir79), or simple insertions (sig5) or tandem duplications (vir37). All these aberrations are outside what we think is the critical point for integrative recombination at about 72% from the left end of the chromosome. None is accompanied by a replacement. The replacements observed in P2 Hy dis phage (Chattoraj & Inman, 1973) would be an exception to this rule, but they are believed to originate from unequal recombination of P2 with a prophage element carried in strain B of Escherichia coli (Cohen, 1959), and not in strain C, which has been the host for P2 in all experiments relevant to this work.

The interpretation proposed for the origin of the three vir replacements implies that the parent phage, although grown lytically (i.e. through infection of sensitive cells), have occasionally gone through a transient prophage state. If the point of prophage attachment on the bacterial chromosome was in such cases, as one might expect, the preferred site I (Bertani & Six, 1958; Calendar & Lindahl, 1969), our interpretation predicts that the replacement segment (e.g. Dl in Fig. 1(c) for vir56) is bacterial DNA adjacent to site I, counterclockwise from it, that is, in the direction of the closely linked histidine genes.
Short communications

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REFERENCES


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