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Visualization of a novel junction in bacteriophage λ DNA

(electron microscopy/denaturation mapping/genetic recombination)

MANUEL S. VALENZUELA*‡ AND ROSS B. INMAN†

* Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154; and † Biophysics Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisc. 53706

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ABSTRACT **At early times after infection of a *recA* derivative of *Escherichia coli* with λ*b221cI26red270a42* phage, a low but significant proportion of intracellular λ molecules show a novel junction. These junctions are also present, although in reduced numbers, in a lysate obtained at late times after infection of a *recA*$^+$ host with λ*cIIcIII* phage. Fine structure and denaturation mapping analyses showed that these junctions occur at homologous positions and that they are compatible with the occurrence of a cross-strand exchange between λ DNA duplexes similar to the type proposed in most molecular models for genetic recombination. However, the results are also consistent with the structures expected if a replicating growing point undergoes branch migration.**

Although the final result of a recombination event can be rather well predicted on the basis of present genetic evidence, very little is known about how recombination actually occurs. The most popular models involve some type of covalently continuous cross-over between partners during at least one stage of the recombination process (1, 2).

If a recombinational intermediate could be isolated which exhibited a covalently continuous cross-over, then this would afford a means of directly elucidating at least some of the important molecular details of recombination. It has been suggested, on the basis of model building, that such cross-overs could exist without disruption of helical structure in the DNA strands involved (3, 4). If this were true, then the cross-over would not exhibit any characteristic fine structure in the electron microscope, and in fact, the intermediate would probably be indistinguishable from the accidental overlapping of two independent duplex strands. Partial denaturation of the segment involving a completely helical cross-over region would, by opening up the region of cross-over, afford a means of investigating its fine structure.

Broker and Lehman (5) have studied the molecules resulting from multiple infection of *Escherichia coli* with T4 phage defective in both polymerase and ligase and concluded that the resulting branched structures observed in the electron microscope are intermediates of recombination.

In the present study we wish to report the observation of a novel type of junction between duplex strands of λ DNA. Fine-structure and denaturation mapping analyses of these junctions are consistent with the occurrence of a cross-over of DNA from one molecule to the other, and therefore such junctions may be associated with recombination. The junctions could, however, also arise by branch migration of a replicating growing point.

‡ Present address: Biophysics Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisc. 53706.

MATERIALS AND METHODS

Phage and Bacterial Strains. λ*b221cI26red270a42*, a phage harboring an amber mutation in the *redB* gene, involved in general recombination (10), a deletion (*b221*) of the segment between 40.6 and 62.9% (11) and a tandem duplication (*a42*) of the region 82.7–99.2% (7) of the wild-type λ genome, was obtained from Dr. W. S. Emmons via Dr. D. Freifelder. The presence of the *red* mutation was inferred from the poor growth of this phage in an *E. coli* derivative lacking DNA polymerase A (6), and the presence of the deletion and addition mutations was confirmed by heteroduplex and high pH partial denaturation techniques. For simplicity this phage will be called λ100 in the remainder of the *text*. *E. coli* MV1 is a T1 phage resistant derivative of *E. coli* 152 (a *recA* strain). λ100 was grown in MV1, purified through two consecutive CsCl gradients, and stored in 0.01 M Tris·HCl, pH 7.2, 0.01 M $MgSO_4$ in D_2O. ^{3}H-Labeled phage was prepared by growing the phage in the presence of [^{3}H]thymidine; the radioactive phage was pelleted by centrifugation and resuspended in 0.01 M Tris, 0.01 M $MgSO_4$ in D_2O.

Isolation of Intracellular λ DNA. The procedure described by Chattoraj and Inman (8) was followed with minor modifications. (A more detailed description will be given elsewhere; M. Valenzuela, manuscript in preparation.)

Electron Microscopy of DNA, Measuring, and Computation of Data. The methodology described by Schnös and Inman (9) was followed except that the micrographs were traced using a Numonics graphics calculator (Numonics Corp., North Wales, Pa.) interfaced to a Hewlett-Packard 9820 calculator and 9862A plotter.

RESULTS

When λ100 phage infects a *recA* derivative of *E. coli*, we observe, in addition to the normal replicative intermediates [double- and single-branched circles (9)], a low, but significant, proportion of molecules containing an anomalous junction point. These solutions contained 47% simple circles, 50% replicative intermediates, and 3% circular types involving the anomalous junctions.

Of the 14 molecules containing junction points that were analyzed, six were single-branched circles [which in a previous investigation were usually found to be structures involved in unidirectional replication (9)] exhibiting a junction between the circle and the branch (Fig. 1a). Eight were circles involving a junction with linear duplex DNA without a branch point (Fig. 1b). A characteristic fine structure was often observed at the junctions and serves to differentiate

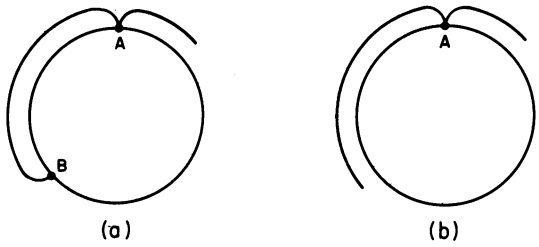

FIG. 1. Diagrammatic representation of structures containing junctions (A). Structure (a) is a single-branched circle with a branch point at (B). Structure (b) is a circle joined [via junction (A)] to a linear duplex segment. The fine structure at the position of the black dot (A) will be discussed in a later section.

them from simple accidental DNA overlaps (this will be described later).

Junctions occur between homologous DNA segments

By means of denaturation mapping (9) it is possible to demonstrate that although the 14 junction points occur at various (and perhaps random) locations along the genome, they *always* involve a union at homologous denaturation map positions of the two DNA segments. Junctions, therefore, occur between identical regions of the λ genomes involved. This fact is demonstrated in Fig. 2b, which shows the denaturation maps of the 14 molecules studied so far. The top line of each pair represents the circle (broken at the mature ends for display purposes) while the lower line shows the other DNA strand involved at the junction. Each junction is shown as a thick vertical arrow. For comparison, representative denaturation maps of mature λ100 DNA are shown in Fig. 2a. It should be noted that the denaturation maps for these molecules are different from λ^+ because of the *b221* deletion and the *a42* tandem addition.

Junctions can occur between two circles

In a separate study involving late rounds of λ*cIIcIII* replication (30 min after infection in D-^{15}N-medium),§ we have also noticed a low frequency of junction points (0.3% of all circular types). However, in these experiments the junction was often between two circles, and again junctions occurred between homologous positions. Fig. 3b shows the 12 molecules that have so far been studied by denaturation mapping. In this small sample, eight junctions were between circles and four between a circle and linear duplex DNA (see 5, 6, 7 and 9 in Fig. 3b). In the case of junctions between circles, one circle was always monomeric while the other partner was either of monomeric, dimeric, or trimeric length.

Fig. 3a shows, for comparison, the expected denaturation maps for λ*cIIcIII* trimers; each map was constructed by artificially adding, in tandem, three mature λ*cIIcIII* denaturation maps.

Fine structure of junctions

Two lines of evidence argue against the junctions' being accidental overlaps. First, as has been established above, *all* junctions so far studied (Fig. 2b and Fig. 3b) occur at homologous base sequence positions between each pair involved in the junction (this statement is true to the precision of the denaturation mapping technique). Second, the junctions do not have the appearance of an accidental overlap of DNA duplex strands. In the clearest examples one can detect four

§ A similar study on early rounds of λ*cIIcIII* replication has not been performed.

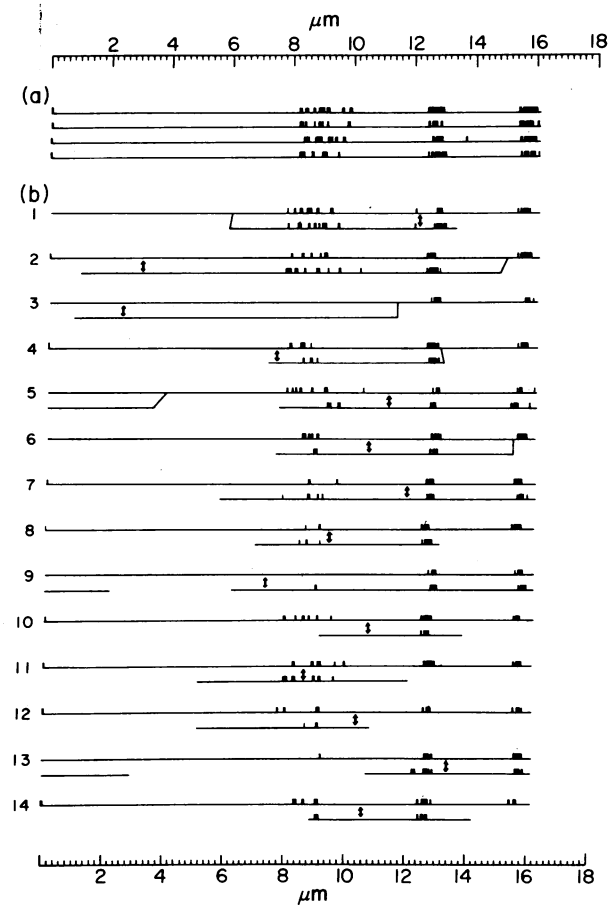

FIG. 2. (a) Denaturation maps of mature λ100. In these molecules the (A+T)-rich region at the center of λ^+ has been deleted and a region at the right end has been duplicated (ref. 7, and M. S. Valenzuela, unpublished). Each black rectangle represents the size and location of a denatured site. (b) Denaturation maps of the 14 molecules exhibiting junctions (↕). Molecules 1 to 6 involve single branched circles (Fig. 1a) and the branch point is indicated by a vertical line. Molecules 7 to 14 correspond to structures of the type shown in Fig. 1b and again the junction is shown by a double arrow. The circular component of each unit is drawn first.

single-stranded regions involved in the junction. Plates 1a, 2a, and 3a show the best examples of the three types of molecules so far discussed. In the worst cases the junctions involve an ill-defined region at the junction point, often simply a small, approximately square area devoid of any significant DNA-like strands. Even in these worst cases the junction "looks" different from a simple overlap. Out of the 14 molecules examined in the λ100 experiment, seven were judged to have a fine structure of the type drawn in Plate 1b or Plate 2b, while the remaining units were ambiguous in this respect. In the λ*cIIcIII* experiment, seven units were judged to have a similar fine structure (Plate 3b). The remaining five molecules exhibited a fine structure consisting of single-stranded material at the junction which often resembled the diagram in Fig. 3b except that various single-stranded segments were either broken or missing or were now present as duplex strands.

Relationship of fine structure to DNA polarity

In the λ100 experiment, *all* molecules exhibiting visible fine structure (seven), and six out of the remaining seven molecules having no well-defined fine structure, were found to have partners arranged with opposite polarity (correspond-

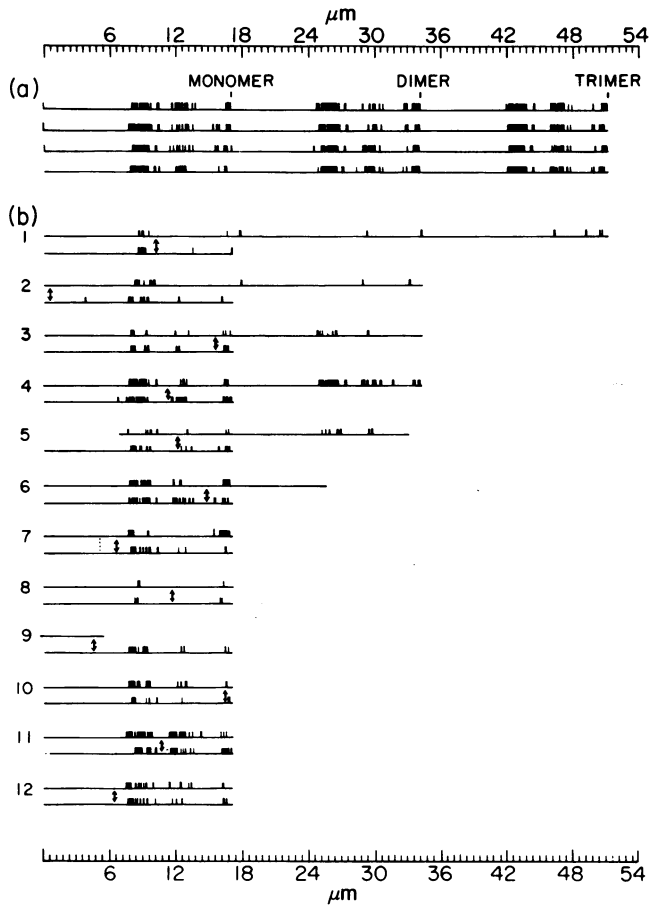

FIG. 3. (a) Denaturation maps of mature λ*cIIcIII* DNA arranged as trimers. Each map was constructed from three monomeric molecules and artificially joined for display purposes. (b) Denaturation maps of 12 junctions between two circles (units 1, 2, 3, 4, 8, 10, 11, and 12) or between a circle and a linear DNA duplex (units 5, 6, 7, and 9). In the former case junctions can involve monomeric, dimeric, or trimeric circles. In the latter case the circular component is drawn last. Broken line in unit 7 indicates the position of another junction; this is the only molecule in which two junctions are present.

ing denatured sites were in *trans* with respect to the junction). Similarly *all* molecules (seven) in the λ*cIIcIII* experiment that had the fine structure shown in Plate 3b were found to be arranged with corresponding denatured sites in *trans*.

Plate 1b, Plate 2b, and Plate 3b give the observed polarities (AB and A_1B_1) deduced from denaturation mapping of the molecules shown in these plates. When these structures are redrawn to produce denatured sites in *cis* about the junction, it appears that the single strands that extend between the circle and the branch (Plate 1a–b), between the circle and the linear segment (Plate 2a–b), and between the two circles (Plate 3a–b) must cross-over (Plate 1c, Plate 2c, and Plate 3c). This fine structure is, therefore, compatible with what would be expected during recombination.

DISCUSSION

The junctions observed in the experiments involving λ100 can in principle be formed in two different ways. First, they could be the result of a recombination event between daughter segments of replicating molecules. Second, they could be produced by branch migration of replicating growing points. A growing point can be wound backwards to produce

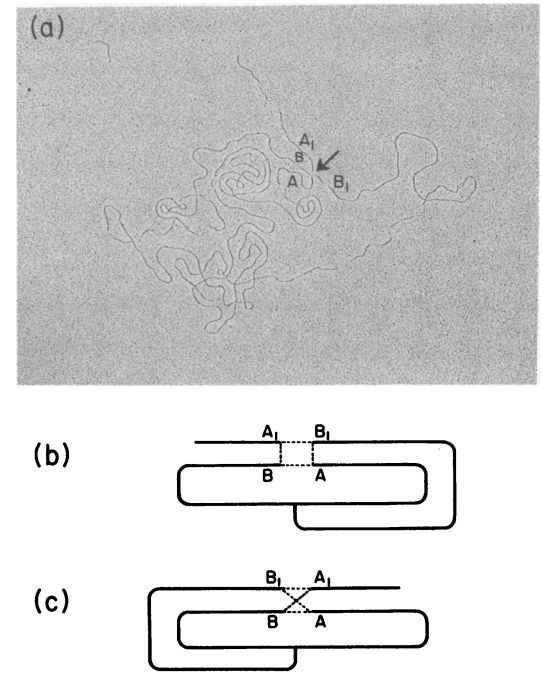

PLATE 1. (a) Electron micrograph of a single branched circle involving a junction. This is the clearest example of junction fine structure (arrow) for this type of molecule. The map and junction positions are shown in Fig. 2 (molecule 1). (b) Diagram of the fine structure observed in many of the junctions of the type shown in (a) above. The observed polarity (as deduced by denaturation mapping) is shown by AB and A_1B_1 for the two partners involved in the junction. (c) Diagram of the fine structure that would result when the corresponding denatured sites are arranged in *cis*.

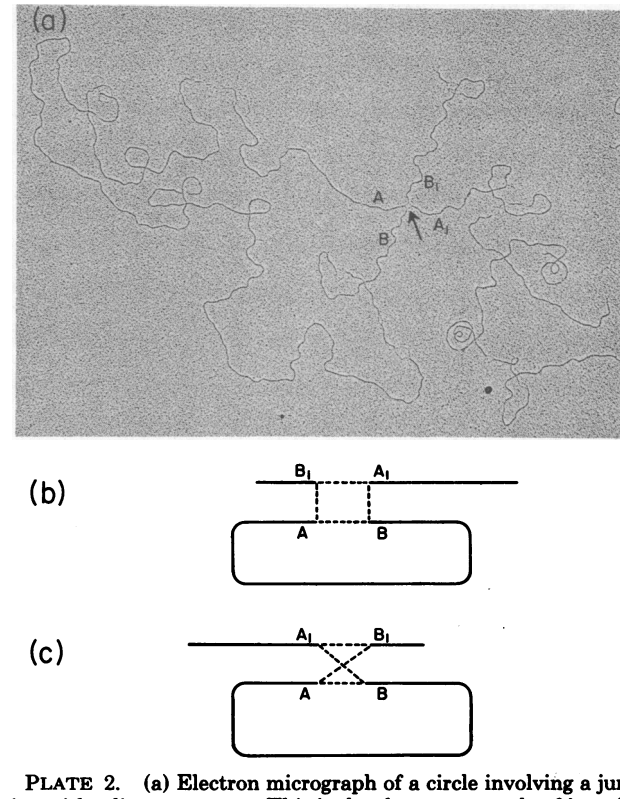

PLATE 2. (a) Electron micrograph of a circle involving a junction with a linear segment. This is the clearest example of junction fine structure (arrow) for this type of molecule. The map and junction position for this molecule are shown in Fig. 2 (molecule 14). (b) and (c) Same as Plate 1b and c.

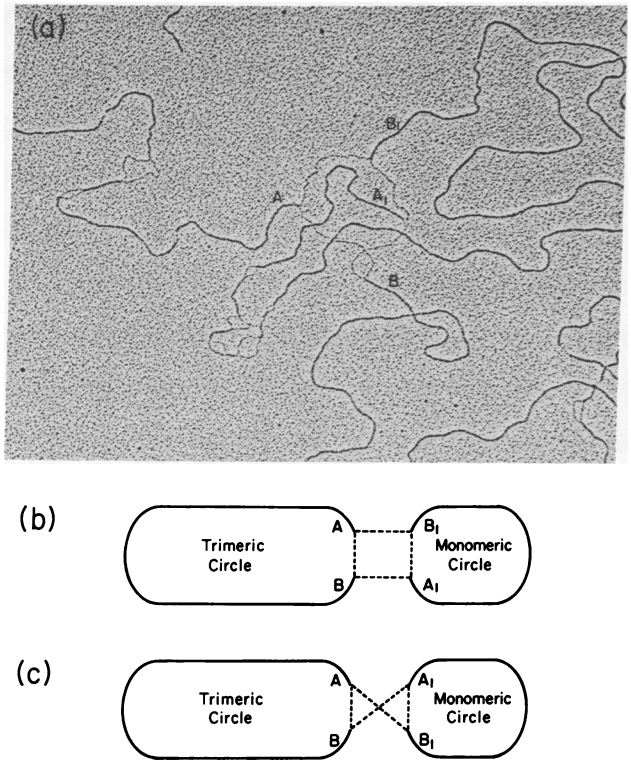

PLATE 3. (a) Electron micrograph of the area around the junction between a trimeric and a monomeric circle. This is the clearest example of junction fine structure for this type of molecule. The map and junction for this molecule are shown in Fig. 3 (molecule 1). (b) Diagrammatic representation of the complete molecule above. (The single-stranded lengths are greatly exaggerated for display purposes.) The polarity of the two circles (as judged by denaturation mapping) is shown by AB and A_1B_1, respectively. (c) Deduced fine structure when corresponding denatured sites are arranged in *cis*.

the original duplex between parental strands and a duplex segment between the newly synthesized strands; the resulting junction will have the properties observed in the present experiments. Such junctions have already been observed and interpreted as migration of growing points (16). Branch migration of a growing point in a double-branched circle and in a single-branched circle (that could arise by breakage of one branch in a double-branched circle) will lead to the structures drawn in Fig. 1a and b, respectively. In the case of the λ*cIIcIII* experiments it is hard to propose a simple explanation for the observed junctions based on migration of growing points and in this case it appears more likely that they arise by recombination.

Most popular models for the recombination event require crossed strand-exchanges of the specific types given by Holliday (12), Broker and Lehman (5), Stahl *et al.* (13), Meselson and Radding (14), and Sobell (15), or the general types listed by Sigal and Alberts (4). The present investigation reports on a structure which appears to have exactly this property. First, the fine structure at the junction is *always* consistent with crossed-strand exchange when molecules are aligned with the same polarity. Second, these junctions *always* occur at homologous positions between each partner involved.

The model-building experiments of Sigal and Alberts (4) and Sobell (3) suggest that crossed-strand exchange could occur without disruption of helical structure. The junctions so far examined have a grossly disrupted helical structure at the cross-over position. Possibly the partial denaturing conditions used in our experiments contributed to this, if in fact a cross-over can occur without disruption of the two duplex strands. In the partially denatured λ100 experiments the length of single strands at the junctions varied from those barely discernible up to a maximum of 600 Å. Junctions of about this size were also observed in undenatured molecules; however, in this case many junctions may have been overlooked if they were very small (in such an experiment there are no denatured sites to allow discrimination between accidental overlaps and very small junctions). The experiment involving λ*cIIcIII* yielded larger junctions with single strands up to 3600 Å long [note particularly that the unusually long single strands involved in the junction shown in Plate 3a occur at the (A+T)-richest region of the λ molecule (Fig. 3b, molecule 1) and that the degree of denaturation is, on an average, higher than in the λ100 experiments (compare Fig. 2b and 3b)].

It is interesting to note that for some unknown reason *all* the unambiguous junctions studied so far showed the partner DNA molecules arranged with opposite polarities with respect to the junction. The result is that the single strands at the junction appear to be open rather than crossed (see Plates 1b, 2b, and 3b).

Sigal and Alberts (4) have suggested that a cross-strand exchange of the Holliday type (12) can actually exist in two forms and Sobell (15) has shown that these forms are interconvertible *via* an open structure similar to that postulated by Broker and Lehman (5). Further investigation is necessary to determine why in our experiments the junctions so far examined always appear to exist in the open configuration.

In the experiments involving λ100 the proportion of circular type molecules with junctions was 3%, whereas in the λ*cIIcIII* experiment the proportion was much lower (0.3%). Possibly the blocks along the recombination pathway caused by the *red* and *recA* mutations present in the former experiment are responsible for the more frequent observation of junctions in this system. However, we cannot be sure that the presence of the tandem duplication in λ100 does not also, in some way, enhance the number of observed cross-overs.

In the experiments involving λ100 a large number of normal replicative intermediates (without junctions) were studied and found to replicate by a predominantly bidirectional mechanism (M. Valenzuela, to be published). Similarly the denaturation maps of molecules with junctions (Fig. 2b) can be used to determine if the positions of branch points and ends of branches (or ends of linear segments) behave as replicative growing points or broken growing points. Although the sample is small the results support the notion that these molecules are also involved in predominantly bidirectional replication. The fact that these molecules were obtained from CsCl fractions of density between LL (light–light) and HL (heavy–light) also suggests that some replication must have taken place. If in fact molecules of the type shown in Fig. 1a–b are replicative intermediates and junctions arise from a recombinational event, then the recombination observed in this experiment is of the incestuous type involving daughter duplex strands.

The occurrence of a growing point and a junction on a single-branched molecule can produce certain novel features in the absence of a swivel. It appears, from examination of simple helical wire models, that the unwinding of parental

DNA at the growing point (a requirement of replication) of a single branched circle (Fig. 1a) will result in migration of the junction towards the growing point.

Although the fine structure observed at junctions is quite consistent with a recombinational event, the results do not help to narrow the choice among various models that give rise to such junctions. Sigal and Alberts (4) have already pointed out that crossed-strand exchanges resulting from cuts in two strands of similar polarity (12) or opposite polarity (5), or those resulting from single cuts in a DNA duplex, all give rise to junctions of the type observed in the present investigation.

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