Localization of Single-Chain Interruptions in Bacteriophage T5 DNA

I. Electron Microscopic Studies¹

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Received for publication 29 December 1976

Bacteriophage T5 DNA was examined in an electron microscope after limited digestion with exonuclease III from *Escherichia coli*. The effect of the exonuclease treatment was to convert each naturally occurring single-chain interruption in T5 DNA into a short segment of single-stranded DNA. The locations of these segments were determined for T5st(+) DNA, T5st(0) DNA, and fragments of T5st(0) DNA generated by *EcoRI* restriction endonuclease. The results indicate that single-chain interruptions occur in a variable, but nonrandom, manner in T5 DNA. T5st(+) DNA has four principal interruptions located at sites approximately 7.9, 18.5, 32.6, and 64.8% from one end of the molecule. Interruptions occur at these sites in 80 to 90% of the population. A large number of additional sites, located primarily at the ends of the DNA, contain interruptions at lower frequencies. The average number of interruptions per genome, as determined by this method, is 8. A similar distribution of breaks occurs in T5st(0) DNA, except that the 32.6% site is missing. At least one of the principal interruptions is reproducibly located within an interval of 0.2% of the entire DNA.

Mature particles of the virulent coliphage T5 contain a single molecule of double-stranded DNA, which has a nonpermuted, terminally repetitious nucleotide sequence (21, 29). T5 DNA is distinguished from most other viral DNA molecules by the presence of a small number of single-chain interruptions (1). These sites can be repaired by DNA ligase and are located within one strand of the duplex (11). Although the interruptions in T5 DNA had been thought to occur at defined positions, several studies have suggested that the exact distribution of these sites is variable. Denaturation of T5 DNA results in the formation of over 40 discrete classes of single chains, which can be resolved by agarose gel electrophoresis (9). The relative abundances of these fragments vary over a wide range. Evidence that interruptions occur at variable positions at one end of T5 DNA has been obtained by electron microscopic studies of circular molecules produced by annealing exonuclease-treated DNA (21).

The purpose of the experiments reported here was to determine the locations of the single-chain interruptions in T5 DNA. This was accomplished by examining T5 DNA in an elec-

tron microscope after limited digestion with exonuclease III of *Escherichia coli*, an enzyme that hydrolyzes both internal and external 3'-termini in duplex DNA (17). Under the appropriate conditions, the short single-stranded segments produced by the exonuclease can be visualized and the positions of the interruptions can be determined. By performing analogous experiments on fragments generated by *EcoRI* endonuclease, we constructed an unambiguous physical map of T5 DNA.

MATERIALS AND METHODS

Purification of exonuclease III. *E. coli* 1100, an endonuclease I-deficient strain (6), was grown at 37°C in 4 liters of M9 medium (2) supplemented (per liter) with 2 g of Casamino Acids, 1 g of tryptone (Difco Laboratories, Detroit, Mich.), and 1 mg of thiamine hydrochloride. At a concentration of 5×10^8 /ml, the cells were chilled to 0°C, harvested by centrifugation at $10,000 \times g$ for 15 min, and suspended in 30 ml of EB (0.02 M Tris-hydrochloride [pH 7.8], 10^{-3} M 2-mercaptoethanol, 10^{-4} M EDTA). All subsequent steps were carried out at 0 to 4°C. The resuspended cells were disrupted by sonic treatment, and the resulting extract was centrifuged for 90 min at 25,000 rpm in a Spinco 40 rotor. After dialysis against 0.01 M phosphate buffer (potassium phosphate [pH 6.8] containing 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA), the supernatant was applied to a column of DEAE-cellulose (100-ml bed volume). Elution was carried out with a 350-ml lin-

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ear gradient formed with 0.01 and 0.30 M phosphate buffer. Exonuclease III activity was recovered in a single peak eluting between 0.075 and 0.140 M phosphate buffer. The active fractions were dialyzed against 0.01 M phosphate buffer and applied to a phosphocellulose column (35 ml). Elution was carried out with a 150-ml linear gradient formed with 0.01 and 0.30 M phosphate buffer. Activity was recovered in a sharp peak at 0.15 M phosphate buffer. This material was dialyzed against EDTA-free 0.01 M phosphate buffer and applied to a column of hydroxyapatite (3.0 ml). Elution was carried out as described above. Exonuclease III was recovered in fractions ranging from 0.075 to 0.115 M phosphate buffer. Contaminating endonuclease activity was found to elute slightly ahead of the exonuclease. The fractions showing the highest ratio of exonuclease/endonuclease activity were pooled and concentrated to 1 ml by pressure dialysis against EB containing 2×10^{-3} M MgCl_2 . This material was applied to a Sephadex G-75 column (1.5 by 30 cm, superfine) equilibrated with the same buffer. Exonuclease III eluted from this column just after the void volume and just ahead of the endonuclease activity. The leading fractions containing exonuclease III were free from detectable endonuclease activity and were used for the experiments reported here (see Fig. 1).

Assays for exonuclease III were routinely performed by measuring the formation of acid-soluble radioactivity from ^{32}P -labeled T5 DNA as described below. At various stages the identity of the exonuclease III activity was verified by the 3'-phosphatase assay of Richardson and Kornberg (22). Endonuclease activity was measured by alkaline sucrose gradient sedimentation.

Digestion of T5 DNA with exonuclease III. Reaction mixtures contained 0.067 M Tris-hydrochloride (pH 8.0), 10^{-3} M MgCl_2 , and 10 μg of ^{32}P -labeled T5 DNA per ml. The concentration of exonuclease III was adjusted so that approximately 4% of the DNA was hydrolyzed within 15 min at 37°C. The formation of acid-soluble radioactivity was measured by thin-layer chromatography on polyethyleneimine-cellulose (12).

Sequential treatment of T5 DNA with exonuclease III and *EcoRI* endonuclease. Exonuclease III incubations were carried out as described above. The reactions were terminated by heating to 65°C for 5 min. The solutions were then made 0.01 M in MgCl_2 and 0.10 M in Tris-hydrochloride (pH 7.6), and sufficient *EcoRI* endonuclease was added to produce a complete digest within 30 min at 37°C. These incubations were terminated by adding 1/5 volume of 0.1 M EDTA. *EcoRI* endonuclease was purified as described by Rhoades (19).

Electron microscopy. DNA was prepared for microscopy by a protein film procedure patterned after that of Westmoreland et al. (30). The hyperphase contained 0.15 M NaCl, 0.5 μg of DNA per ml, 0.01% (wt/vol) cytochrome c, and 30 to 50% (vol/vol) formamide. (Lower formamide concentrations produce clearer junctions between single- and double-stranded DNA. Higher concentrations render the single-stranded regions more visible.) The hyperphase was glass-distilled or deionized water. Specimens

were rotary-shadowed with platinum and examined in a Philips EM 200 or a JEM 100B electron microscope. Tracings of projected molecules were measured with a Numonics graphics calculator. The double-stranded, circular DNAs of bacteriophages ϕX174 and PM2 and the single-stranded DNA of ϕ174 were employed as internal molecular weight standards.

Other methods. The procedures employed for the preparation of radioactively labeled T5 phage and DNA, for sucrose density gradient centrifugation, and for the preparation of ϕX174 DNA have been described previously (21). PM2 DNA was a gift of S. Rogers.

RESULTS

Molecular weight of T5 DNA. A wide range of values has been reported for the molecular weight of T5 DNA. In the case of T5st(+) (wild type), the values vary from 66×10^6 to 68×10^6 as measured by hydrodynamic methods (5, 27) to 83×10^6 as measured by ^{32}P autoradiography (15). The heat-stable deletion mutant T5st(0) has generally been reported to contain 6 to 8% less DNA than the wild type (9).

To obtain a reliable value for this study, the length of T5st(+) DNA was determined, in an electron microscope, relative to the circular, double-stranded forms of ϕX174 DNA (3.4×10^6 ; 28) and PM2 DNA (6.4×10^6 ; 18). All three DNAs were present on the same grid, and at least 5 of each of the reference genomes were included in every field. A sample of 28 molecules yielded a value for T5st(+) DNA of $76.1 \pm 1.6 \times 10^6$ relative to ϕX174 DNA and of $77.0 \pm 1.5 \times 10^6$ relative to PM2 DNA. The average of these two determinations, 76.5×10^6 , is in good agreement with the value of 77.4×10^6 recently determined by electron microscopy for T5st(+) DNA (14). This value also provides a close fit to the empirical relationship between sedimentation coefficient and molecular weight constructed by Freifelder (7) for other coliphage DNA molecules.

The molecular weight of T5st(0) DNA was determined relative to T5st(+) DNA by comparing the fragments derived by cleavage of the two genomes with restriction endonucleases. As previously described (19), *EcoRI* endonuclease digests of T5st(0) DNA lack three fragments found in wild-type digests and contain one new fragment. The molecular weight of this fragment was shown by electron microscopy to be 5.6×10^6 less than the sum of the three missing wild-type fragments. A similar analysis performed with *HpaI* endonuclease gave a value of 5.4×10^6 for the deleted segment. The average of these values represents a 7.2% deletion if T5st(+) DNA has a molecular weight of $76.5 \times$

10^6 . (The details of these experiments will appear in a subsequent publication.)

Digestion of T5 DNA with exonuclease III. *E. coli* exonuclease III catalyzes the stepwise removal of 5'-mononucleotides from internal and external 3'-termini in duplex DNA (17, 23). Since the mode of attack of exonuclease III is random or nonprocessive (16), uniform degradation of all susceptible sites can be achieved with subsaturating levels of enzyme. Limited digestion of T5 DNA with exonuclease III should, therefore, generate short segments of single-stranded DNA at the site of each interruption, as well as at all duplex termini.

The exonuclease III used in this study was extensively purified to remove other nucleases. The principal contaminant during the later stages of purification was an endonuclease, which introduced a limited number of randomly placed single-chain breaks in T5 DNA. This endonuclease can be separated from exonuclease III by gel filtration as described in Materials and Methods. Figure 1 shows the alkaline sucrose gradient profiles of T5 DNA after digestion to 3.3% with purified and partially purified exonuclease III preparations. The sedimentation profile of the DNA treated with purified exonuclease III is virtually unchanged from that of untreated DNA. Treatment with the partially purified preparation, however, resulted in a significant loss of material from the fastest-sedimenting peak, which represents the intact strand of T5 DNA.

The fully purified preparation of exonuclease III has also been analyzed in experiments employing both alkaline sucrose gradient sedimentation and agarose gel electrophoresis (20). Under the conditions described here, T5 DNA can be degraded to at least 7.8% without detectable endonucleolytic cleavage.

Electron microscopy of exonuclease III-treated T5 DNA. Exonuclease III-treated T5 DNA was prepared for electron microscopy by the formamide modification of the basic protein film procedure. As originally described by Westmoreland et al. (30), this technique maintains single-stranded DNA in an extended configuration. Regions of single- and double-stranded DNA can be distinguished, since the former has a thinner and kinkier appearance. Digestion of T5 DNA to 3 to 4% with exonuclease III creates single-stranded segments of 600 to 1,000 nucleotides, which can be recognized without difficulty in an electron microscope. Except where noted, this level of hydrolysis was employed in all of the experiments reported below.

Analysis of *Eco*RI fragments. The positions

FIG. 1. Effect of partial digestion with exonuclease III on the alkaline sedimentation profile of T5 DNA. ^{32}P -labeled T5st(0) DNA was denatured and centrifuged in alkaline sucrose gradients (a) after incubation in a control reaction mixture, (b) after digestion to 3.3% with purified exonuclease III, and (c) after digestion to 3.0% with a partially purified preparation of exonuclease III. The latter preparation was obtained from the gel filtration step described in the text. Untreated ^3H -labeled $\lambda\text{b}2$ DNA was included in each gradient as a sedimentation reference. Centrifugation was carried out for 90 min at 40,000 rpm in a Spinco SW50.1 rotor. The direction of sedimentation is from right to left. Symbols: ●, ^{32}P -labeled T5st(0) DNA; ○, ^3H -labeled $\lambda\text{b}2$ DNA.

of the single-stranded segments generated by exonuclease III were determined for intact molecules of T5st(+) and T5st(0) DNA as well as for *Eco*RI fragments of T5st(0) DNA. It was anticipated that the measurements on intact molecules would provide a satisfactory description of the locations of the single-chain interruptions. The initial results, however, revealed a degree of intermolecular heterogeneity that made it impossible to align individual molecules in an absolute manner. This problem was circumvented by combining the results obtained from intact genomes with those obtained from *Eco*RI fragments.

*Eco*RI digests of T5st(+) DNA contain seven fragments, of which the two largest occur at the ends of the DNA (19). Fragment 1, representing 37% of the genome, defines the right end of the molecule, and fragment 2, a 25% fragment, occurs in the same half of the molecule as the st deletions (26). In the present experiments,

EcoRI digests were prepared after treatment of the DNA with exonuclease III. As a result, fragments 1 and 2 each had one duplex end, generated by *EcoRI* endonuclease, and one single-stranded end, generated by exonuclease III. The orientation of both fragments could thus be unambiguously determined.

The positions of the single-stranded segments in 29 molecules of *EcoRI* fragment 1 and 26 molecules of *EcoRI* fragment 2 of T5st(0) DNA are shown in Fig. 2. The fragments have been aligned as they would occur in wild-type DNA, with the position of each single-strand/double-strand junction plotted to the nearest 0.1% of the total length of T5st(+) DNA. It is apparent that single-stranded segments occur frequently at two sites within *EcoRI* fragment 2, located at approximately 8 and 18%, and at one site in fragment 1, located at 65%. Interruptions occur less frequently at a number of other sites, especially at each end of the DNA, within the terminal repetition.

The nine molecules of *EcoRI* fragment 1 plotted at the top of Fig. 2a possess two duplex termini. These molecules, however, all appear to contain the single-stranded segment at 65% and were aligned on that basis. The probable cause of this will be discussed below.

To determine which end of each single-stranded segment represents the original interruption, it is necessary to know the polarity of the strands in T5 DNA. Several studies (3, 10, 11) have indicated that most, if not all, of the interruptions in T5 DNA are located within one strand of the duplex. The polarity of this strand, which is designated the "interrupted" strand, can be determined by analyzing the effect of λ exonuclease digestion on the single-chain fragments contained in T5 DNA. As originally reported by Rhoades and Rhoades (21), and confirmed in the accompanying paper (20), the 3'-terminus of the interrupted strand occurs at the left end of the molecule. Consequently, the left side of each single-stranded segment in Fig. 2 marks the site of an interruption.

Histograms describing the density of single-chain interruptions along *EcoRI* fragments 1 and 2 are shown in Fig. 3. Each vertical bar represents the frequency of interruptions within a segment equal to 0.2% of T5st(+) DNA. Of particular interest is the site at 65%, which, because of its proximity to the *EcoRI* cut at 62.8%, can be mapped with considerable precision. Over 90% of the interruptions that define this site occur between 64.7 and 64.9%, an interval of approximately 200 base pairs. A his-

FIG. 2. Positions of the single-stranded segments in exonuclease III-treated *EcoRI* fragments 1 (a) and 2 (b) of T5st(0) DNA. T5st(0) DNA was treated with exonuclease III and *EcoRI* endonuclease, and the unfractionated digest was examined in an electron microscope. *EcoRI* fragments 1 and 2 were identified on the basis of size. The lengths of the molecules in the two populations were normalized to 37.2 and 25.0%, respectively, of the wild-type length. The gaps within each molecule represent the duplex length equivalent to each single-stranded region.

FIG. 3. Histograms of the frequency of single-chain interruptions in *EcoRI* fragments 1 (a) and 2 (b) of T5 DNA. The results shown in Fig. 2 were used to determine the frequency of interruptions within segments equal to 0.2% of intact T5st(+) DNA.

togram of the positions of the right end of the single-stranded segments at 65% (not shown) is significantly less sharp. This result would be expected, due to heterogeneity in the extent of exonuclease III digestion, if the polarity of the interrupted strand were as described above.

Electron micrographs of exonuclease III-treated *EcoRI* fragments are shown in Fig. 4 and 5. Figure 4 shows a complete molecule of fragment 1, in which single-stranded segments defining interruptions at 65 and 78% are visible. Figure 5 shows portions of several molecules in which single- and double-stranded ends, as well as internal single-stranded regions, are visible.

Analysis of intact DNA. The positions of the single-chain interruptions in 27 molecules of T5st(+) DNA and 23 molecules of T5st(0) DNA are shown in Fig. 6. These molecules were aligned on the basis of the principal interruptions at 8, 18, and 65% observed in *EcoRI* fragments 1 and 2. For every molecule of T5st(0) DNA and most of those of T5st(+) DNA, one of the two possible orientations gave a better fit at these positions. The pattern of interruptions is sufficiently complex, however, that some of the molecules may be incorrectly aligned. The gap in the molecules of T5st(0) DNA represents the 7.2% deletion that occurs from 26.3 to 33.5% in wild-type DNA. These end points reflect minor revisions of those reported previously (26).

Histograms of the positions of the single-chain interruptions in T5st(+) and T5st(0) DNA are shown in Fig. 7. Each vertical line represents 0.5% of T5st(+) DNA. An electron micrograph of a molecule of T5st(+) DNA containing five single-stranded segments is shown in Fig. 8.

The results in Fig. 3 and 7 reveal that T5st(+) DNA contains four sites, located at 7.9, 18.5, 32.6, and 64.8%, where interruptions occur in most, but not all, molecules. Except for these sites, the internal regions of T5 DNA are relatively free from interruptions. The only other internal sites where interruptions appear to occur in more than 10% of the population are located at 45 and 78%. In contrast, the ends of the DNA contain a relatively high density of interruptions. These terminal sites are largely clustered between 0 and 5% and 91 and 96%. Since the terminal repetition in T5 DNA equals 8.3% of wild-type DNA (20, 21), these clusters occur at analogous positions within the repeated segments. A more detailed analysis of the left end is presented in the next section.

The distribution of interruptions in T5st(0) DNA is similar to that in T5st(+) DNA, except that the interruption at 32.6% lies within the deleted region. The histograms in Fig. 7 suggest that, compared with wild-type DNA, T5st(0) DNA contains fewer interruptions at the right end of the molecule. However, a

FIG. 4. Complete *EcoRI* fragment 1 of T5st(0) DNA after 4% digestion with exonuclease III. The arrows indicate the two ends of the fragment, one of which is single-stranded. Two internal single-stranded segments, specifying interruptions at 64.8 and 77.8%, are also visible. The bar represents a duplex length of 500 base pairs.

larger number of molecules of both types would have to be analyzed to substantiate this conclusion.

The total number of interruptions per molecule is highly variable. The sample of T5st(+)

DNA studied here contained an average of eight interruptions. Some molecules had as few as four, whereas others had as many as 13 interruptions. For T5st(0) DNA, the range was from 3 to 10, with an average of 6 interruptions per molecule.

Analysis of the interval from 0 to 8%. As described in the preceding sections, the interval between 0 and 8% at the left end of T5 DNA contains a high density of single-chain interruptions. This region was investigated in more detail by examining T5st(0) DNA in an electron microscope after degradation to 0.9% with exonuclease III. The single-stranded segments created by this level of hydrolysis (250 nucleotides) were barely large enough to be identified. Only unambiguous single-stranded segments were included in this analysis. An electron micrograph in which several short single-stranded segments are visible is shown in Fig. 9. A histogram of the frequency of single-chain interruptions at the left end of T5 DNA is shown in Fig. 10. This histogram was derived from measurements on 14 intact molecules and 8 molecules whose right ends were either too tangled to measure or obscured by a grid bar.

The results in Fig. 10 indicate that, in addition to the prominent site at 8%, interruptions are clustered at 0.8, 2.4, and 3.4 to 4.0% from the left end of the molecule. Evidence for sites at 2.4 and 3.4 to 4.0% can be seen in Fig. 3. The site at 0.8%, however, is not visible in the more highly degraded molecules. Interruptions appear to occur at these sites in 20 to 30% of the population. The level of interruptions at the right ends of the molecules sampled in this experiment was too low to permit analysis.

DISCUSSION

The results presented above demonstrate that T5 DNA possesses a large number of sites where single-chain interruptions can potentially occur. The frequencies with which interruptions actually occurred at these sites in the mature form of T5 DNA were highly variable. Although a majority of the interruptions were found at four sites and at the two ends of the genome, a significant fraction of the interruptions occurred at sites where the frequency of breaks was less than 0.10. Individual molecules of T5 DNA thus differ with respect to both the number and location of single-chain interruptions. Within the populations of intact T5st(+) and T5st(0) DNA sampled in this study, no two molecules possessed identical structures.

The validity of these conclusions depends largely on the properties of *E. coli* exonuclease III. For an accurate picture of T5 DNA to be

FIG. 5. Portions of several *EcoRI* fragments of *T5st(0)* DNA after 4% digestion with exonuclease III. The arrows indicate single-stranded regions, both internal and external. The molecule centered in (A) is the left half of *EcoRI* fragment 1. The bars represent duplex lengths of 500 base pairs.

obtained, it is essential that exonuclease III initiate hydrolysis at all of the natural interruptions without introducing additional single-chain breaks. The latter requirement appears to have been satisfied, since it was possible to prepare exonuclease III that was free from detectable endonuclease activity. It would be difficult to detect a low level of activity, however, and it is conceivable that a small fraction of the observed interruptions were spurious.

The finding that interruptions do not always occur at each of the principal sites might indicate the occasional resistance of internal 3'-termini to hydrolysis. In fact, Hayward and Smith (9) suggested, on the basis of their electrophoretic analysis of denatured T5 DNA, that the principal interruptions are always present. The electrophoretic studies presented in the accompanying paper (20), however, demonstrate that even the most frequent interruptions are occasionally missing. Moreover, studies on the properties of exonuclease III have indicated that virtually all ligase-repairable single-chain interruptions in a population are susceptible to hydrolysis (17). It is thus unlikely that a significant fraction of the natural interruptions was overlooked by the exonuclease.

A more serious problem concerns the extent of exonuclease III hydrolysis. In most of the experiments reported here, single-stranded segments of 600 to 1,000 nucleotides were created at the site of each interruption. These conditions clearly preclude detection of interruptions located within 1,000 base pairs from an end or from pairs of interruptions separated by less than this distance. This situation can be remedied, in principle, by decreasing the extent of exonuclease III hydrolysis. One experiment in which single-stranded segments of 200 to 300 nucleotides were generated did allow the identification of an additional site, located 0.8% (900 nucleotides) from the left end of the genome. In practice, however, very short single-stranded segments are difficult to identify with certainty (Fig. 9). Furthermore, as demonstrated in the accompanying paper (20), interruptions occur in T5 DNA at a location that would be virtually impossible to detect regardless of the extent of digestion. This site occurs 0.4% (450 base pairs) from the right end of the molecule. Since the 3'-terminus of the intact strand also occurs at the right end of the molecule, the 0.4% segment will be degraded from both directions by exonuclease III. Removal of 225 nucleotides per 3'-

FIG. 6. Positions of single-chain interruptions in intact molecules of *T5st*(+) and *T5st*(0) DNA. After normalization to 100% of the wild-type length, the molecules were aligned to give the best fit at the 7.9, 18.5, and 64.8% sites, where interruptions occur at a high frequency. The vertical bars represent the left end of each internal single-stranded segment. The deleted segment in *T5st*(0) DNA has been left blank so that the maps of the two genomes can be compared directly. (a) *T5st*(+) DNA; (b) *T5st*(0) DNA.

FIG. 7. Histograms of the frequency of single-chain interruptions in *T5st*(+) and *T5st*(0) DNA. The results shown in Fig. 6 were used to determine the frequency of interruptions within segments equal to 0.5% of intact *T5st*(+) DNA. (a) *T5st*(+) DNA; (b) *T5st*(0) DNA.

FIG. 8. Complete molecule of T5st(+) DNA after digestion to 4% with exonuclease III. The left end of the molecule is in (A); the right end is in (B). The symbol "r" indicates a point that is common to both parts of the molecule. The arrows indicate five internal single-stranded regions located at approximately 4, 8, 18.5, 32.5, and 65%. The bars represent duplex lengths of 1,000 base pairs.

FIG. 9. *T5st(0)* DNA after digestion to 0.9% with exonuclease III. The arrows indicate short, internal segments of single-stranded DNA. The bar represents a duplex length of 500 base pairs.

terminus will therefore result in loss of the right (5') end of the interrupted strand. This effect is undoubtedly responsible for the apparent absence of single-stranded material at the right ends of some molecules (Fig. 2).

Many of the structural features of T5 DNA described in this study were first proposed by Hayward and Smith (9, 10), who fractionated denatured T5 DNA by agarose gel electrophoresis into 4 major and over 40 minor single-chain fragments. The major fragments were thought to define primary interruptions that occur in all molecules, whereas the minor fragments were proposed to result from secondary interruptions that occur less frequently within the major fragments. The sizes of both the major and minor fragments were discrete, and the electrophoretic patterns were unaffected by variations in host bacteria and in the method of DNA preparation. The only qualitative difference between the model proposed by Hayward and

Smith (10) and the conclusions obtained in the present study is that, as mentioned earlier, even the most frequent interruptions are occasionally missing. However, because of the assumption that the entire molecule can be reconstituted from major fragments, these authors failed to recognize the segment between 0 and 7.9%. The density of interruptions in this region is too high to yield a major fragment, although a minor fragment that defines this segment can be identified (20).

Several other studies on T5 DNA have mapped single-chain interruptions at sites that agree relatively well with the positions determined for the principal interruptions in the present study. In an electron microscopic examination of partially denatured *T5st(+)* DNA, Bujard (3) identified interruptions at sites located 7.9, 19.0, and 60% from one end of the molecule. Bujard and Hendrickson (4) determined the positions of the major single-stranded fragments of *T5st(+)* DNA, isolated by sucrose gradient sedimentation, by annealing each fragment to the intact strand. Analysis of these structures in an electron microscope gave positions for single-chain interruptions at 3.9, 7.8, 17.8, 30.6, and 65.0% of the wild-type length. Hayward (8) demonstrated that hydrodynamic shearing breaks duplex T5 DNA at single-chain interruptions. The positions of these shear-sensitive points were determined by electrophoretic procedures to occur at 7.4, 16.9, 29.5, and 64.0% of the wild-type length. More recently, Saigo (24, 25) has located interruptions at 8.2, 17.9, 32.6, and 64.0% by examining sheared tail- or ghost-DNA complexes in an electron microscope and at 18.0, 33.1, and

FIG. 10. Histogram of the frequency of single-chain interruptions at the left end of T5 DNA. The positions of the single-stranded segments were determined in 22 molecules of *T5st(0)* DNA that had been digested to 0.9% with exonuclease III. The molecules were aligned as described in the legend to Fig. 6, except that the lengths were not normalized. Instead, the position of each interruption was plotted relative to the left end of the DNA. Each vertical line represents 0.2% of the intact *T5st(+)* DNA.

64.5% by denaturing mapping. Except for the 60% site reported by Bujard (3), all of these values agree reasonably well with the positions determined in the present study.

The T5st(0) deletion mutant has been shown by heteroduplex mapping to lack the segment between 26.3 to 33.5% on the wild-type genome (26; M. Rhoades, unpublished data). Consequently, the prominent interruption located at 32.6% should be missing in T5st(0) DNA. The absence of this site had been previously inferred from the electrophoretic studies of Hayward and Smith (9, 10) and Hayward (8). A different structure for T5st(0) DNA has been proposed by Labedan et al. (13). These authors analyzed the products formed by shearing T5st(0) DNA after attaching one end of the molecule, by phage infection, to the bacterial membrane. Under the assumption that a unique end of the DNA was injected, a model for T5st(0) DNA was proposed, with interruptions located 8, 19, 35, and 60% from the end that initiated transfer. This result indicated that the left end of the genome, as described in the present study, initiated infection. T5st(0) DNA, however, does not contain a detectable level of interruptions in the vicinity of 35% from the left end. The origin of this discrepancy has not been determined.

It was initially anticipated that knowledge of the positions of the single-chain interruptions in T5 DNA would provide some insight into their origin and function. The major contribution of this study, however, has been to demonstrate the complex and heterogeneous nature of T5 DNA. A more detailed description of the locations of single-chain interruptions in T5 DNA is presented in the accompanying paper (20).

ACKNOWLEDGMENTS

We are grateful to Rolf Benzinger and the Biology Department, University of Virginia, Charlottesville, for providing support and facilities for portions of this study. P.S. was supported by Public Health Service training grant 5 T01-GM-5717 from the National Institute of General Medical Sciences. This research was supported by grant GB-20460 from the National Science Foundation.

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