LOCATION OF SINGLE-STRAND INTERRUPTIONS IN THE DNA OF BACTERIOPHAGE T5+

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Abstract.—The positions of three single-strand interruptions in the DNA of phage T5+ have been located by electron microscopy. All three interruptions were found in the same strand. Uneven base composition along the molecule is indicated by the preferential melting of certain regions. The data suggest a model according to which (1) the first-step-transfer DNA section is separated by a single-strand interruption from the rest of the phage genome, (2) the phage carries only one such section and therefore transfers the asymmetrical DNA molecule always in the same direction into the host cell, and (3) single-strand interruptions are points of preferred breakage.

Bacteriophage T5 has unusual properties not found in most other well-studied coliphages: (1) During infection, the phage transfers its DNA in a two-step process.[1, 2] Initially, a well-defined portion, the so-called first-step-transfer DNA,[3] representing about 8 per cent of the DNA molecule, is transferred to the host cell.[4, 5] Protein synthesis directed by this portion of the DNA is required for subsequent transfer of the rest of the DNA molecule.[6] (2) The nucleotide sequence of the linear, doubled-stranded DNA is unique, not circularly permuted.[7] (3) When exposed to shear in solution, the point of preferred breakage of isolated T5 DNA is acentric.[8, 9] (4) T5 DNA contains single-strand interruptions in defined positions.[10]

It seems pertinent to ask whether properties (1) to (3) above are directly correlated with the occurrence of single-strand interruptions in the DNA molecule. Furthermore, it would be interesting to know whether the DNA possesses a first-step transfer section at each end, and, if so, whether transfer to the host cell can be initiated from either end of the molecule. The knowledge of the exact number and positions of the single-strand interruptions within the DNA molecule might well clarify at least some of these problems.

Based on shearing and heat-denaturation experiments, three alternative models for the distribution of the points of preferred breakage were proposed:[8] (1) one point at 40 and one at 60 per cent of the total molecular length from either end, (2) one point at 40 and another at 80 per cent of the total molecular length when measured from one end, or (3) four points dividing the molecule in five equal segments.

Abelson and Thomas[10] studied the sedimentation of native and denatured DNA in experiments made to determine the number and distribution of single-strand interruptions in the T5 DNA. These studies led to the proposal of 12 alternative models. Common features of all these models were that both DNA strands were interrupted and that the total number of four interruptions existed per DNA molecule. Some of these models can be correlated with those proposed

on the basis of the shearing studies, which suggest that preferred breakage might occur at points of single-strand interruptions.

More recently, the results of experiments with DNA end-group labeling followed by ligase action indicate the presence of two to four single-strand interruptions per molecule.[11] These studies showed also that the interruptions are breaks in the phosphodiester backbone between adjacent nucleotides.

This report describes studies carried out by electron microscopy and analytical ultracentrifugation of purified DNA from phage T5$^+$. The DNA was examined in the native state and after both partial and complete denaturation. Evidence is presented for the occurrence of three single-strand interruptions and for their positions in only one of the two DNA strands. Based on this evidence, a model of the T5$^+$ DNA molecule is here proposed which describes the distribution of the single-strand interruptions and in particular the position of the first-step-transfer DNA within the molecule. Assuming that preferred breakage occurs at the location of single-strand interruptions, we suggest a model that distinguishes between the three alternatives previously proposed to explain the mode of breakage of the molecule.[8] When T5 DNA is exposed to shear or moderate temperature, it denatures irreversibly, but only to a small extent.[12] The model proposed here offers an alternate explanation to the one originally presented for these unusual properties.

Materials and Methods.—Chemicals: All chemicals were reagent grade. Formaldehyde was a 36–38% solution containing 10% methanol. Dimethylsulfoxide was redistilled *in vacuo:* the fraction distilling within a 2°C temperature range was recovered and stored at room temperature. Phenol was distilled in an argon atmosphere under reduced pressure and stored at −20°C.

Stocks: Phage T5$^+$ stock was obtained from Dr. Yvonne Lanni and was grown in strain E. coli F as described earlier.[13]

Preparation of DNA: T5 phage was purified by CsCl density gradient centrifugation. After suspension of the particles in buffer (10^{-2} *M* sodium phosphate, 2×10^{-3} *M* $CaCl_2$, pH 7.5), the DNA was extracted by gentle mixing with buffer-saturated phenol at neutral pH. The phenol was removed from the aqueous phase by dialysis against 10^{-2} *M* sodium phosphate, 10^{-3} *M* EDTA,[3] pH 7.2(S1 buffer), and the DNA was stored at 4°C. DNA from bovine papilloma virus and from phage T3 were prepared as described earlier.[14, 15]

Electron microscopy: DNA was prepared for electron microscopy by the diffusion method in 0.2 *M* ammonium acetate, as described previously.[15] A Siemens Elmiskop IA was used at 80,000 v. Photographs were taken at a magnification of 5,000 or 10,000 × on Kodak electron image plates. The technique developed for quantitative electron microscopy of single-stranded DNA will be described in detail elsewhere.[16] In the present studies, single-stranded DNA was prepared as follows: samples of 8 μg of DNA in S1 buffer were further diluted into 1.5 ml of 0.05 *M* sodium phosphate buffer, pH 7.0, containing 26% dimethylsulfoxide and 5% formaldehyde. For complete denaturation of the DNA, this mixture was kept at 60°C for 20 min. Partial denaturation was achieved by heating the solution at 52–54°C for 2 min, cooling it rapidly in ice, and heating it again at 40°C for 20 min. Dimethylsulfoxide and excess formaldehyde were removed by dialysis against S1 buffer before the mixture was prepared for electron microscopy. Under these conditions, only an insignificant number of breaks are introduced into polynucleotide chains.[16] The contour length of the molecules was measured as described previously.[15]

Analytical ultracentrifugation: A Beckman Model E ultracentrifuge equipped with monochromator and ultraviolet optics was used. The sedimentation of T5$^+$ DNA was studied by band centrifugation.[17] A lamellar volume of 25 μl containing 0.3–2 μg of

DNA in S1 buffer was used to overlay the bulk solution (1 M NaCl, 10^{-2} M sodium phosphate, 10^{-3} M EDTA, pH 7.2) in a 30-mm cell. All experiments were done at 20°C, at a rotor speed of 10,000 or 20,000 rpm. Ultraviolet photographs were traced with a Joyce-Loebl Mark IIIc microdensitometer.

Results.—Molecular weight of T5⁺ DNA: The contour length was found to be 38.3 ± 0.9 μ,[25] corresponding to a molecular weight of 82.5×10^6.[18] In these experiments, DNA from bovine papilloma virus (2.54 μ)[14] and from phage T3 (12.2 μ)[19] were present in the preparation as internal length standards. A molecular weight of 83×10^6 was calculated[20] from the $s^0_{20,w}$ value of 53.3×10^{-13} sec obtained by analytical ultracentrifugation.

Partially denatured T5⁺ DNA: Heating the DNA for a short time at 52.0°C resulted in a change in the structure of the molecules as judged by electron microscopy; i.e., 5–10 per cent of the molecules in the population showed regions of preferential melting. This preferential melting, however, did not cause the appearance of single-stranded "loops," as would be expected if melting occurred in uninterrupted polynucleotide chains. Instead, one single-stranded section was liberated from the DNA duplex which introduced a "single-strand gap" near one end of the partially denatured molecule (region B in Fig. 1*b*). After being heated at slightly higher temperatures (53–54°C), a second region of denaturation appeared in the distal half of the molecule. In some cases, about 60 per cent of the molecular length was converted into the single-stranded form (with the complementary strand missing) while the rest of the molecule remained double-stranded (Figs. 3 and 2*c*). Single-stranded DNA can be distinguished from double-stranded DNA by its lower contrast and the higher density of "kinks" along the molecule. This distinction permits the measurement of the contour length of single- and double-stranded regions and the construction of a map (Fig. 3). It is evident that the T5 DNA molecule can be divided into four distinct regions:

Region A, double-stranded after partial denaturation, representing 7.9 ± 0.9 per cent of the molecule.

Region B, single-stranded after partial denaturation, representing 11.1 ± 1.5 per cent of the molecule.

Region C, double-stranded after partial denaturation, representing 41 ± 4 per cent of the molecule.

Region D, double-stranded after partial denaturation, but separated from C by a short single-stranded region, and representing 40 ± 4 per cent of the molecule.

In three experiments, 5–10 per cent of the molecules in each population (200–300 molecules were examined per experiment) showed denaturation of region B. The majority remained unchanged. A few molecules were completely denatured.

Completely denatured T5⁺ DNA: The mean length of thirty of the longest single-stranded molecules was 24.6 ± 1.5 μ (Fig. 2*c*). Since the linear density of such DNA is 1.66×10^6 daltons/μ,[18] the molecular weight of these molecules is 41×10^6 daltons, or one half of the native molecule. Circular single- and double-stranded DNA from bovine papilloma virus were used in these preparations as internal length standards.

Discussion.—The release of a single-stranded section (region *B* in Figs. 1 and 3) during partial denaturation of the molecule shows that in the T5+ DNA, one single-strand interruption is located at each end of region *B*. Furthermore, the

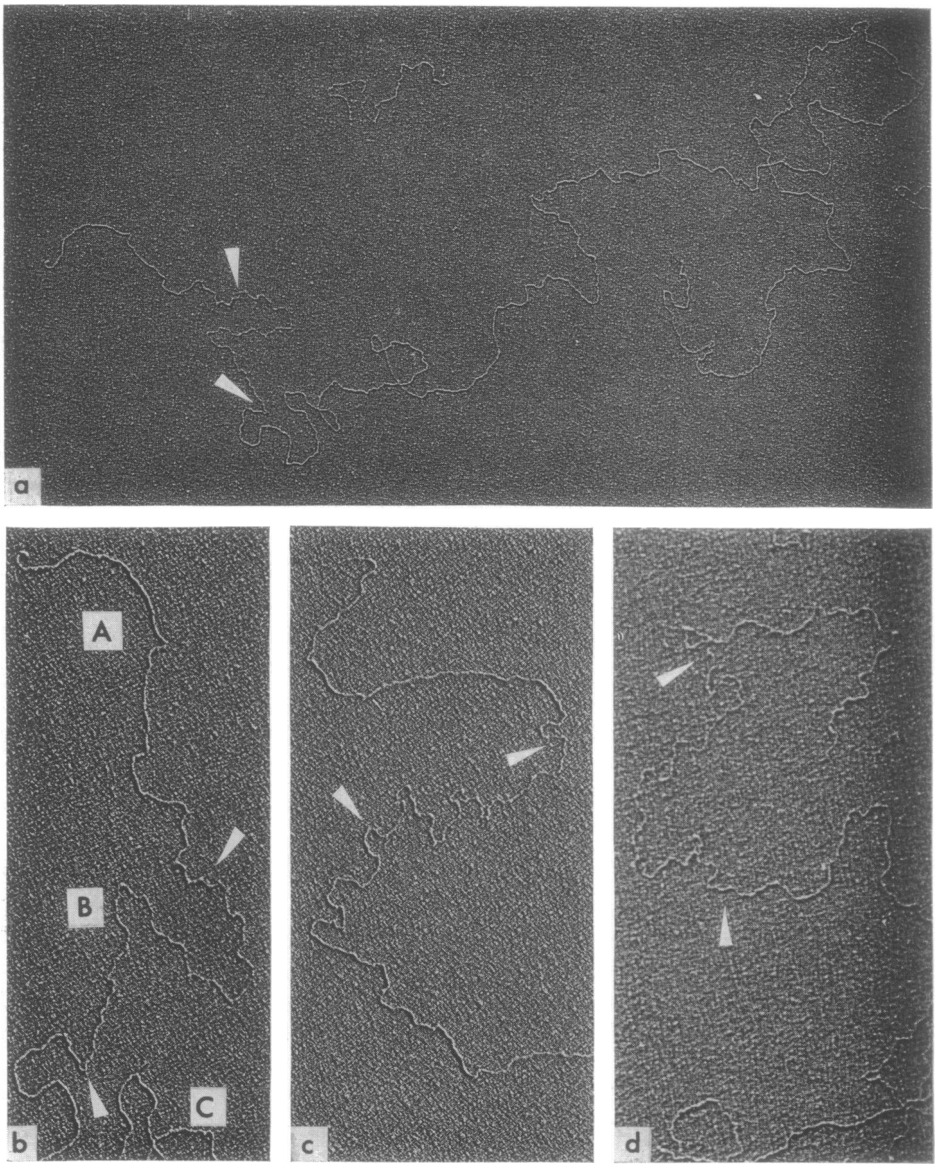

FIG. 1.—Partially denatured T5+ DNA. One of the two strands of region *B* is missing Arrows indicate the transition from single- to double-stranded regions.

(*a*) Full-length T5+ DNA molecule with single-stranded *B* region. The single-stranded piece visible above the large molecule has the length of the *B* region.

(*b*) Enlarged section of (*a*) showing double-stranded region *A*, single-stranded region *B*, and part of double-stranded region *C*.

(*c* and *d*) Enlarged sections of region *A*, *B*, and part of *C* from other molecules. Magnification: (*a*) ×14,000; (*b*) to (*d*) ×28,000.

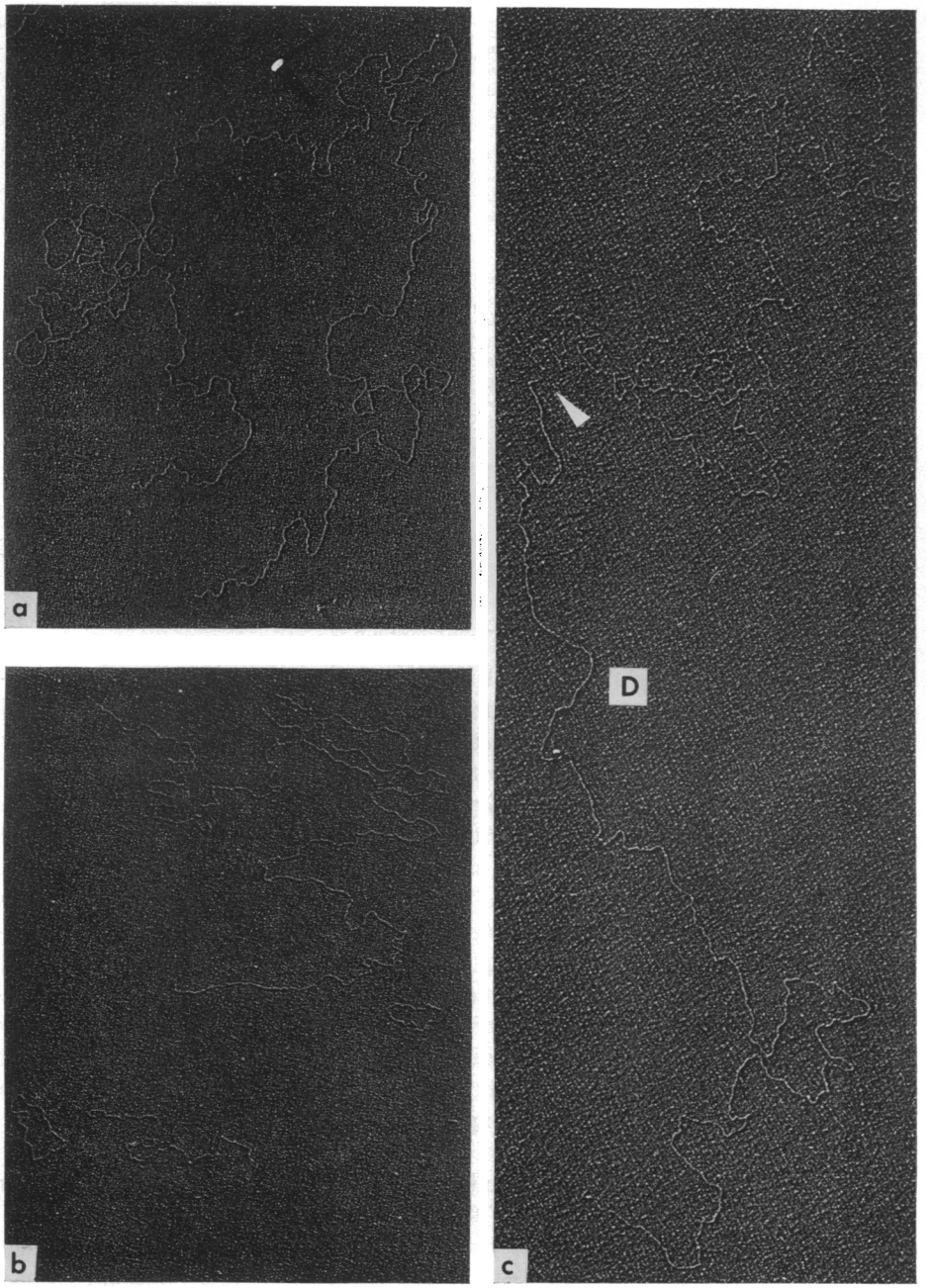

FIG. 2.—(*a*) Native double-stranded T5+ DNA molecule, 38 μ long.

(*b*) Completely denatured T5+ DNA. The largest molecule is 26 μ long, corresponding to half the molecular weight of the native molecule.[18] The circular molecule is bovine papilloma DNA which was used as internal magnification standard.

(*c*) Extensively denatured T5+ molecule. Only region *D* remains double-stranded. The single-stranded region corresponds in length to the sum of regions *A*, *B*, and *C*.

Magnification: (*a* and *b*) ×15,000; (*c*) ×22,000.

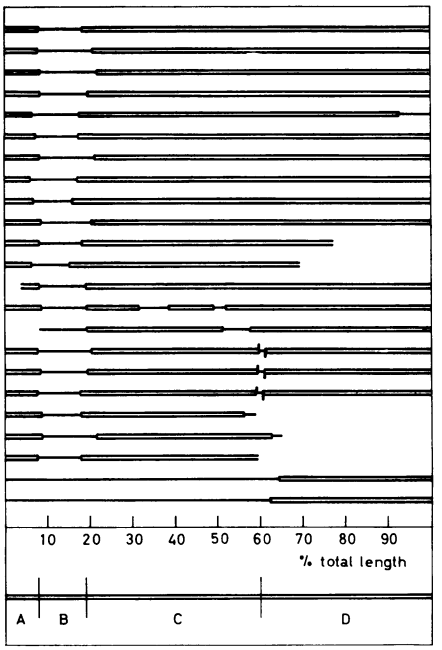

FIG. 3.—Mapping of partially denatured T5+ DNA molecules. Results of one experiment in which three electron-microscope grids (Siemens type, 7 holes) were used. All molecules with the single-stranded region near one end were photographed and measured. The main error in the length determination of the different regions is due to the difficulty in recognizing the exact transition point between single-stranded and double-stranded regions. Double-stranded DNA, ═══; single-stranded DNA, ———; gaps where extruding single-stranded ends were seen ═══⊢⊣═══.

position of a third single-strand break defining regions *C* and *D* (Fig. 3) is indicated since (1) many partially denatured molecules showing the gap in region *B* terminate between *C* and *D*, which suggests great fragility of the molecule at this point; (2) in some molecules, regions *A*, *B*, and *C* are denatured, leaving only region *D* in double-stranded form (Figs. 2*c* and 3); and (3) partial denaturation yields a small single-stranded gap with short extruding single-stranded ends.[21] All three single-strand interruptions must occur in only one of the two DNA strands, since the interruptions at both ends of region *B* cause the release of a single-stranded section and, in samples of completely denatured DNA, single-stranded molecules have been found possessing one half of the molecular weight of the native DNA. There are no indications for a fourth single-strand interruption. The preferential melting of region *B* also suggests a heterogeneous distribution of AT- and GC-rich sequences in the molecule. In fact, during heat denaturation, T5+ DNA exhibits a multistep melting curve, which can be explained in part by the initial melting of region *B*.[22]

These data suggest, then, the model depicted in Figure 4, which describes the number and location of single-strand interruptions in the T5+ DNA. This model is consistent with a number of findings described earlier:

(1) It had been found that when exposed to shear, the DNA of phage T5 (st) breaks into fragments of 40 and 60 per cent of the molecule.[8] Similar results were obtained with DNA from phage T5+.[9] When the larger fragment was isolated and again exposed to shear, it also broke acentrically into fragments representing 20 and 40 per cent of the original molecule. If it is assumed that single-strand interruptions represent points of preferred breakage, this model would be compatible with one of the three alternatives previously proposed,[8] i.e., one preferred point of breakage exists at 40 per cent (between *C* and *D*) and

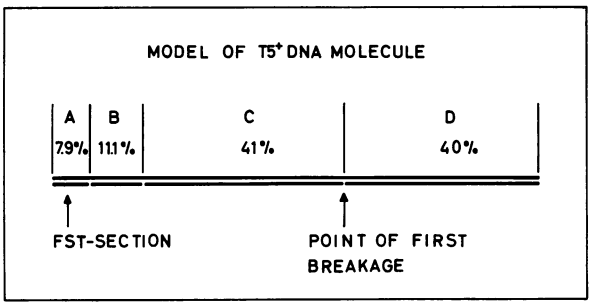

FIG. 4.—Model for the T5+ DNA molecule: Three single-strand interruptions are located in one strand. Region *A* represents the FST-DNA section,[2, 3] which is separated from the rest of the molecule by a single-strand interruption. The single-strand interruption between *C* and *D* may be the first point of preferred breakage, while the interruption between *B* and *C* may be the second point.[8, 9]

another at 80 per cent (between *B* and *C*) of the total molecular length (measured from right to left in Fig. 4).

(2) Under conditions of shear stress or on being heated to just below the melting temperature, T5 DNA undergoes a partial, irreversible denaturation.[12] Although the denaturation was not accompanied by a detectable hyperchromic effect, a surprisingly large portion of the treated DNA was retained on a methylated albumin kieselguhr (MAK) column. However, if the molecule was first broken by shear and then partially denatured, considerably less DNA was retained on the column, which suggested that local denaturation of the unbroken molecules occurred near their centers where the effect of mechanical stress has its maximum. It was difficult to explain, however, the irreversibility of the denaturation. Assuming that under the above conditions region *B* is denatured, the proposed model would predict a high degree of retention on MAK columns if the experiment were performed with unbroken molecules, and much less retention if performed with fragmented molecules. Furthermore, since a single-stranded section of region *B* is physically separated from the molecule, little renaturation should occur.

(3) About 6 per cent of the nucleotides in shear-denatured, unbroken T5 DNA molecules are accessible to exonuclease I,[12] an enzyme which acts on denatured DNA and requires 3′-hydroxyl groups.[23] The section released from region *B* represents 5.5 per cent of the nucleotides and would be a candidate for this enzymatically digestible portion.

(4) First-step-transfer DNA isolated from FST-complexes[3] represents 8.3 ± 1.0 per cent of the whole DNA molecule.[4, 5] Region *A* of the model corresponds to 7.9 ± 0.9 per cent of the total molecular length. This agreement may be coincidental, although it seems reasonable to assume that region *A* represents the first-step transfer section, which would then be separated from the rest of the molecule by a single-strand interruption. It should be noted, however, that in the work presented here T5+ DNA was used, while in most experiments cited from the literature, DNA from heat-stable (st) mutants was used. The DNA of these can be 3 to 6×10^6 daltons smaller in size.[9, 10]

The model proposed, taken together with the results from other laboratories,

makes possible the following deductions: (1) single-strand interruptions are points of preferred breakage when a molecule is exposed to mechanical shear; (2) region *A* of the model represents the first-step-transfer DNA section, suggesting that for productive infection, DNA transfer must occur in one direction only, since the initial transfer of this section of the DNA is essential for the transfer of the rest of the molecule.[2]

Of course, the model does not exclude the possibility that region *A* is redundant at the other end of the molecule, but not separated by a single-strand interruption. In this case, it would seem unlikely that both first-step transfer DNA sequences are functionally identical, and the transfer of DNA might still be initiated at only one end, determined specifically by only one of the two sequences.

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[3] FST–DNA, first-step transfer DNA; FST-complex, bacterial cell into which only the FST-DNA has been transferred.

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[18] The linear density of DNA required for the calculation of molecular weights from contour length measurements on electron micrographs is not precisely known. A value of 2.0–2.2 $\times$ 10^6 daltons/μ best fits the data obtained by sedimentation, viscosity, and diffusion measurements.[24] In the present studies, a value of 2.15 $\times$ 10^6 daltons/μ was used. The linear density of single-stranded DNA is strongly dependent on the ionic strength of the diffusion solution. Under the conditions used, it is 1.66 $\times$ 10^6 daltons/μ. The length of such a single strand is only 0.64 of the length the strand would have in a double-stranded molecule.[16]

[19] A mean value of 12.21 $\pm$ 0.42 (SD) for the length of T3 DNA was obtained from 24 independent experiments performed over a range of four years (D. Lang, personal communication).

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