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*COMPLEMENTARY STRUCTURE OF INTERACTING SITES AT THE ENDS OF LAMBDA DNA MOLECULES**

BY A. D. HERSHEY AND ELIZABETH BURGI

CARNEGIE INSTITUTION OF WASHINGTON, GENETICS RESEARCH UNIT, COLD SPRING HARBOR, NEW YORK

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Molecules of DNA from phage lambda are characterized by terminal cohesive sites that permit end-to-end joining to form rings and chains.[1-3] The joining is reversible, depending on temperature and salt concentration in a manner that suggests denaturation and renaturation of DNA.[1] Joining and disjoining occur at temperatures well below those causing denaturation of the DNA as a whole, which occurs at temperatures appropriate to the over-all base composition. These facts suggest a typical, helical, double-stranded structure modified by short, unpaired ends that are complementary to each other in base sequence. The model predicts specific joining of left to right ends. That prediction is verified by the results of an experiment described below.

Methods.—Lambda DNA labeled with P^{32} was prepared according to Burgi[4] and sheared to half-length fragments by stirring an ice-cold solution containing 10 μg/ml in 0.1 *M* NaCl at 2150 rpm for 30 min in a laboratory stirrer.[5] Molecular weights were measured from the sedimentation rate in sucrose.[6] Equilibrium density-gradient centrifugation in CsCl was carried out in a Spinco model E centrifuge in the usual way except that a rotor temperature of 5°C was maintained to prevent the rejoining of lambda DNA fragments that occurs in CsCl solutions at

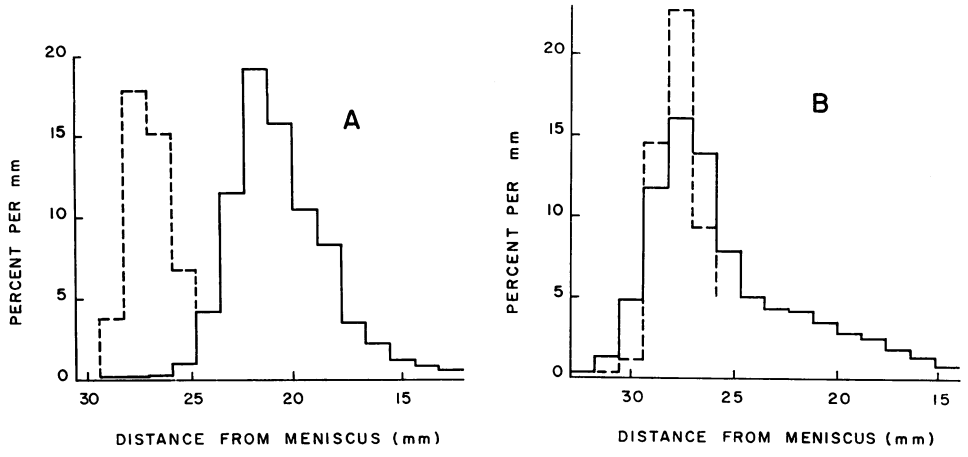

FIG. 1.—(*A*) Sedimentation in a density gradient of sucrose[6] of sheared, P^{32}-labeled lambda DNA (solid line) and unsheared, H^3-labeled marker DNA (broken line). (*B*) Same, after thermal treatment of the P^{32}-labeled DNA to join molecular ends.

higher temperatures. Samples were spun for about 30 hr at 44,770 rpm and the bands formed were analyzed by densitometry of ultraviolet photographs. To ensure that a DNA sample contained only disjoined molecules or fragments, the solution in 0.1 *M* NaCl was heated to 75°C for 1 min and quickly cooled shortly before use, or examined immediately after stirring. To bring about rejoining of fragments, the solution containing 10 μg DNA/ml was made 0.6 *M* in NaCl, heated to 75°C, and allowed to cool slowly (0.11°/min at 65°) in the heating bath with the heater disconnected.[1] Neither of these treatments affects the buoyant density or sedimentation rate of the DNA, except for discrete, reversible changes that reflect joining or disjoining of molecular ends.

Results.—Molecular-weight measurements: Figure 1*A* shows the sedimentation in sucrose of a sample of P^{32}-labeled lambda DNA previously broken by stirring. It sediments as a single band at the rate expected for half-length fragments.

Figure 1*B* shows the sedimentation of an otherwise identical sample heated and slowly cooled after stirring. Most of the DNA sediments at the rate characteristic of unbroken linear molecules. There is some material that sediments more slowly, but none that sediments much faster, than the main component.

The behavior is that expected according to the following argument. Lambda DNA molecules cohere only by their two ends.[1] Half-length fragments cohere by their single remaining natural end and can rejoin only in pairs. A few fragments produced by more than one break per molecule and lacking a natural end do not rejoin at all. (An alternative interpretation of the incomplete rejoining of halves would be that a minority of the original molecules, though possessing complementary ends and able to form rings, differ from the majority of molecules and cannot interact with them.)

Density measurements: Density measurements yield additional information about rejoining because the two halves of the lambda DNA molecule differ in density. For DNA of the wild-type phage used here, the difference is about 0.009 gm/ml,[7] greater than that found in a lambda *dg* DNA.[8] Following Hogness and Simmons,[8] the denser half may be called the left half of the molecule.

Figure 2*A* shows the resolution of right and left halves into two bands when a DNA sample stirred in the manner described is centrifuged in CsCl. The two bands contain roughly equal amounts of DNA. The band corresponding to right halves is narrower than the other because the boundary between denser and less dense DNA lies somewhat to the left of the molecular center, so that variations in length about the mean half length affect mainly the density of left halves.[7]

Figure 2*B* shows the result when the stirred DNA is heated and slowly cooled before centrifugation. One sees a single band lying at the same position on the density scale as that formed by natural molecules of lambda DNA (Fig. 2*C*).

If the two halves could rejoin in random pairs, one would expect only half of the DNA in the band of Figure 2*B* to lie at the position of native molecules. Instead, most of it does, and only small amounts of material, presumably fragments that failed to rejoin (Fig. 1*B*), exhibit the densities of the individual halves. Rejoined pairs therefore consist mainly or entirely of right and left halves.

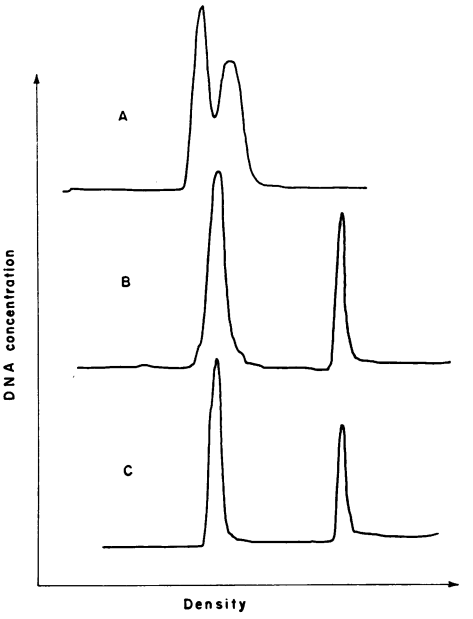

FIG. 2.—(*A*) Band formed in CsCl by half-length fragments of lambda DNA. (*B*) Same, after the fragments are rejoined by thermal treatment. The band on the right contains a density marker of phage SP8 DNA. (*C*) Unsheared DNA.

Discussion.—The conditions under which lambda DNA molecules undergo end-to-end joining and disjoining[1] suggested that the interacting structures might be complementary, unpaired polynucleotide chains. The finding reported here, that the joining occurs between right and left ends, is consistent with that hypothesis. Additional support for the same hypothesis is coming from more specific, but still incomplete, enzymic analysis of structure (Burgi, see ref. 7; A. D. Kaiser, personal communication).

The structure may be thought of as a means for achieving the initial step in formation of the stable molecular rings found in bacteria infected with phage lambda.[9] More generally, structures of the postulated type may play a role in the synaptic phase of molecular-genetic recombination.[10]

Summary.—Half-length fragments of DNA from phage lambda rejoin in pairs when subjected to thermal annealing. The pairs consist mainly or exclusively of right and left halves. The reactive sites at the two ends of the original molecule are therefore different from each other and, at least in a formal sense, complementary in structure. It also follows that the majority of molecular ends of each class are more or less identical, or belong to one of very few types.

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*A DEMONSTRATION OF CODING DEGENERACY FOR LEUCINE IN THE SYNTHESIS OF PROTEIN**

BY BERNARD WEISBLUM,† FABIO GONANO,‡ GUNTER VON EHRENSTEIN, AND SEYMOUR BENZER

DEPARTMENT OF BIOPHYSICS, JOHNS HOPKINS UNIVERSITY MEDICAL SCHOOL, AND DEPARTMENT OF BIOLOGICAL SCIENCES, PURDUE UNIVERSITY

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sRNA acts as an adaptor in the transfer of amino acids into protein.[1–5] Since an organism may contain more than one variety of sRNA for a given amino acid, different coding specificities of the various adaptors would provide a mechanism for degeneracy in the code. In *E. coli* sRNA, several leucine-acceptors have been shown to respond differently to various synthetic polynucleotides.[5, 6] The experiments reported here were undertaken to determine whether the separate leucyl-sRNA's actually contribute leucine to different sites in natural polypeptide chains. Evidence that they do is presented in this paper.

Materials and Methods.—Separation of leucine acceptors: sRNA from *E. coli* strain B prepared by phenol extraction was obtained from General Biochemicals, Chagrin Falls, Ohio. Countercurrent distributions were performed with a 400-tube apparatus according to Holley *et al.* The solvent system was made by dissolving 550 gm K_2HPO_4 and 850 gm $NaH_2PO_4 \cdot H_2O$ in 4 liters of deionized water. To this, 300 ml formamide and 1535 ml isopropanol were added. Four hundred transfers were performed at 28°C. The 400 fractions obtained were combined into 80 sets of 5 tubes each. Assays of acceptor activity were done as previously described,[6] except that a partly purified *E. coli* leucine-activating enzyme preparation[8] was used instead of a crude *E. coli* extract. After incubating the fractions, the sRNA was precipitated with ice-cold 5% trichloroacetic acid (TCA). The precipitate was collected and washed with ice-cold 5% TCA on a membrane filter (Millipore). The filters were glued to planchets, dried, and counted.

Preparation of leucyl-sRNA: Attachment of labeled leucine to sRNA and isolation of C^{14}-leucine- and H^3-leucine-bound sRNA were performed as previously described[5, 6] except that a partly purified *E. coli* leucine-activating enzyme preparation[8] was used. Uniformly labeled C^{14}-leucine, specific activity 240 $\mu c/\mu M$ and 4,5 H^3-leucine, specific activity 5000 $\mu c/\mu M$ were products of the New England Nuclear Corp. Further details are given in the legends to the figures.

Preparation of reticulocyte ribosomes: Ribosomes from rabbit reticulocytes can be used to synthesize hemoglobin *in vitro*. They were prepared by the method described by Schweet *et al.*[9] ex-