*CHANGES IN MOLECULAR WEIGHT OF DNA ACCOMPANYING MUTATIONS IN PHAGE**

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Kellenberger *et al.*[1,2] selected mutants of phage lambda characterized by altered buoyant densities in cesium chloride solutions. They obtained direct evidence that one of the mutants contains less DNA per particle than the wild-type phage.[3] In this paper we show that two of the mutants have lost different, non-overlapping segments of the parental DNA molecule.

Materials and Methods.—Four phage lines, obtained from J. Weigle, were studied. All were clear-plaque mutants of the following pertinent genotypes and densities: the parental type b^+ (1.508 gm/cm^3), the mutants *b2* (1.491) and *b5* (1.501), and the double-mutant recombinant *b2b5* (1.483). The density differences were confirmed with our stocks. The phages were grown in a synthetic medium[4] modified to contain 10 times the stated amount of $FeCl_3$ and 0.1 of the stated amounts of KCl and NaCl. An unbuffered medium of the same composition but lacking the tris (hydroxymethyl)aminomethane buffer is also referred to below. Its use caused the pH of the cultures to fall during growth and improved phage yields. *Escherichia coli*, strain W3110 (from J. Weigle), adapted to the buffered medium, was grown to 5×10^8 per ml, sedimented, washed and resuspended at 5×10^9 per ml in 0.01 *M* $MgSO_4$, and infected with 5 phage particles per cell. After 10 min the suspension was diluted 5-fold with 0.01 *M* $MgSO_4$ and recentrifuged. The pellet was resuspended (at time zero) in fresh buffered medium at 5×10^8 per ml, and the suspension was diluted 10 min later with an equal volume of unbuffered medium. Lysis ensued after aeration for two or three hr at 36°C. For preparation of labeled stocks, radiophosphate (1 mc/mg P) was added at time zero, or tritiated uridine (0.015 μM/ml, 3 mc/μM) 10 min later.

The lysate, containing nearly 10^{11} phage particles per ml, was brought to pH 7.2 and treated consecutively with deoxyribonuclease, ribonuclease, and pancreatin. Bacterial debris was removed by centrifugation with the aid of 0.5 mg of analytical grade Celite per ml of lysate, and the supernatant was passed through a 0.45 μ membrane filter supporting a thin layer of additional Celite. The phage particles in the filtrate were then sedimented, washed, and resuspended in dilute, buffered saline containing 0.1 mg/ml of gelatin.

DNA was extracted from the phage particles with phenol and sodium dodecylsulfate.[5] Differently labeled DNA's from phages of two genotypes were extracted from a mixture of the phages, not mixed after extraction. To prevent breakage of DNA when low concentrations (2 μg/ml) were extracted, the tubes were inclined at a low angle and rotated about their long axes at 60 rpm for 15 min, rather than shaken.[6] Extraction at low concentrations proved advantageous to avoid the aggregation to which lambda DNA is prone.[7] Phenol was removed by dialysis against 0.1 *M* NaCl.

DNA labeled with either P^{32} or H^3 showed about 2.5×10^5 disintegrations/μg-min.

Sedimentation of labeled DNA was observed in a density gradient of sucrose.[8] About 0.2 μg of DNA in 0.1 ml of 0.1 *M* saline was layered on 5 ml of the sucrose solution (in 0.1 *M* NaCl, 0.05 *M* PO_4, pH 6.7) and the tubes were spun cold at 28,100 rpm for 6 hr. Two-drop fractions were collected through a hole punctured in the bottom of each tube, and radioactivity was measured in a scintillation spectrometer as described elsewhere.[9]

Results and Discussion.—Phage particles, including those of lambda, are believed to contain single DNA molecules.[10] If the generalization is valid, changes in the

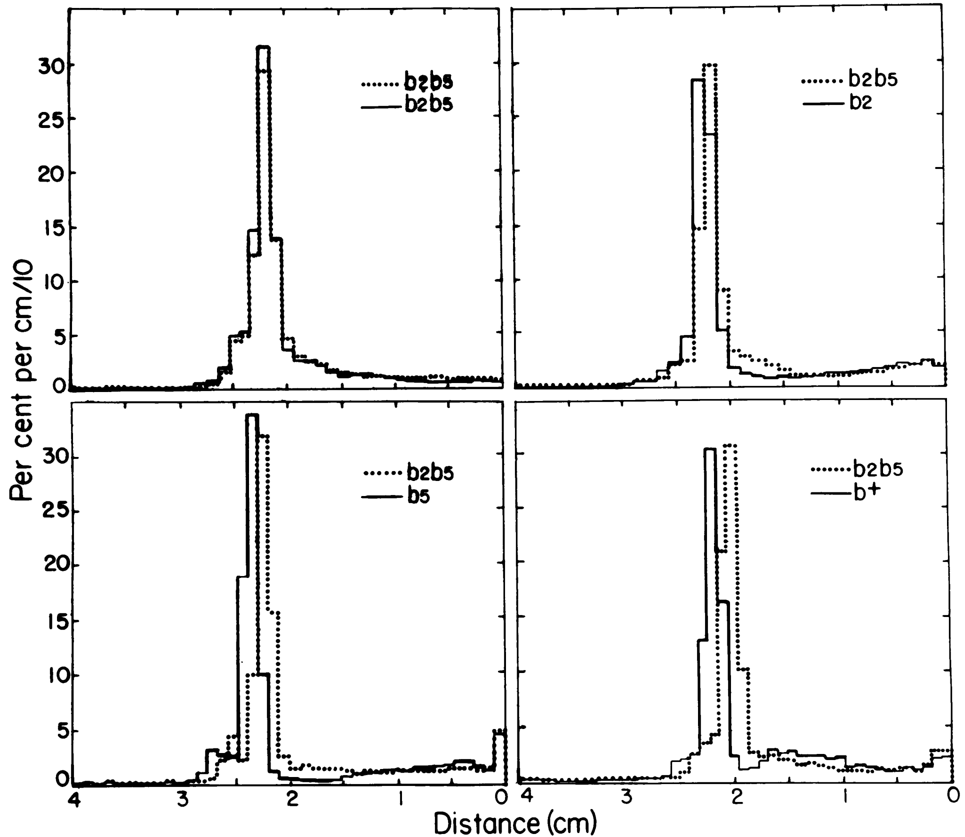

FIG. 1.—Zone sedimentation of differently labeled DNA's from pairs of mutant phages. Solid line, P^{32}; dotted line, H^3. Distance is measured from the meniscus.

DNA content of phage particles should be directly reflected in the molecular weights and sedimentation rates of their extracted DNA's. This expectation is confirmed by the results presented in Figure 1, which show that differently labeled DNA's from phage particles of different genotypes sediment at different rates in the same tube. The individual DNA's sediment as single, narrow bands, an evidence of the molecular homogeneity of each. Their sedimentation velocities increase in the same order as the densities of the phage particles: *b2b5*, *b2*, *b5*, b^+.

If the differences in sedimentation rate reflect differences in molecular length, a faster-sedimenting DNA should be more readily broken under shear than a slower

one.[11] Figure 2 presents the results of an experiment in which a mixture of differently labeled DNA's from phages b^+ and *b2b5* was partly broken by repeated aspiration through the orifice of a pipette. In the figure each DNA shows two sedimenting bands, one corresponding to the original material, the other moving more slowly as a result of breakage. The b^+ DNA has been broken to a greater extent than the *b2b5*. In other experiments we found by analysis of stirred solutions in the optical centrifuge that the unlabeled DNA's show the same difference in fragility. We conclude that at least two and probably all four of the DNA species studied here differ in both mass and length, as indicated by the differences in sedimentation rate.

We now ask whether or not the differences in molecular weight of the DNA's are quantitatively consistent with the differences in density of the phage particles. The relative distances through which two substances sediment in a density gradient of sucrose must be proportional to their relative sedimentation coefficients except as influenced by the increasing density and viscosity of the solution, and by the increasing centrifugal force, encountered by the particles as they move through the solution. Since the field and solvent effects tend to cancel each other, the deviation from proportionality must be slight when the difference in sedimentation rates is small. Accordingly, for structurally homologous DNA's,

$$\frac{D_2}{D_1} = \left(\frac{M_2}{M_1}\right)^k \qquad (1)$$

where D and M are the distances sedimented and the molecular weights of the sedimenting species 1 and 2,[12] and k will be nearly constant for ratios D_2/D_1 in the neighborhood of unity. The exponent k is 0.35 according to the data of Figure 2, which show that unbroken DNA sediments 1.27 times faster than broken DNA consisting of half-length fragments.[11]

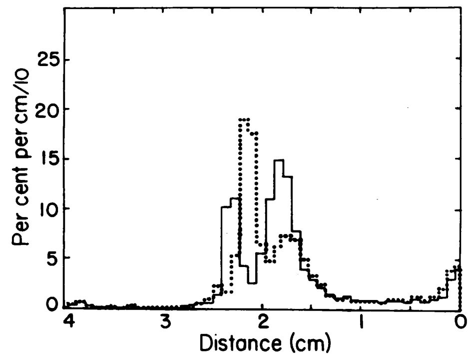

FIG. 2.—Zone sedimentation after partial breakage under shear of mixed DNA's of different sedimentation rate. Solid line, P^{32}-labeled b^+ DNA; dotted line, H^3-labeled *b2b5* DNA.

It will be noted that the estimate of k for equation (1) is lower than the values 0.51 and 0.55 given by Burgi and Hershey.[11] The difference reflects the fact, noticed by those authors, that k depends on the conditions of measurement of sedimentation coefficients for high molecular weight DNA's, a point now being investigated in this laboratory.

Relative molecular weights of DNA computed from the sedimentation rates using equation (1) with $k = 0.35$ are presented in Table 1. The results may be expressed as follows relative to the molecular weight of b^+ DNA taken as unity: *b5*, 0.925; *b2*, 0.849; *b2b5*, 0.793 to 0.810, where the latter range reflects errors of four independent measurements.

Weigle *et al.*[13] suggested a measurement of the DNA content of phage particles based on their buoyant densities in CsCl solutions. In this measurement the particles are assumed to be equivalent to simple mixtures of protein of density 1.30 and DNA of density 1.71. The method yields relative DNA contents per phage particle

TABLE 1

RELATIVE MOLECULAR WEIGHT OF DNA AND DNA CONTENT OF PHAGES OF DIFFERENT GENOTYPES

Phage pair	Relative molecular weight (equation 1) Expt. 1	Expt. 2	Relative DNA content (Weigle *et al.*[13])
b2/b2b5	1.07	...	1.08
b^+/*b5*	...	1.08	1.07
b5/b2	...	1.09	1.10
b5/b2b5	1.16	1.14	1.19
b^+/*b2b5*	1.24	...	1.28

Experiment 1 is shown in Figure 1; experiment 2 was similar except that H^3-labeled *b5* DNA was used.

as follows: for b^+ phage, 1.000; for *b5*, 0.934; for *b2*, 0.847; for *b2b5*, 0.783. The ratios of DNA contents for the phage pairs we studied are compared with the molecular weight ratios of DNA in Table 1. The comparison shows that the molecular weight changes of the DNA's nearly account for the different densities of the phage particles. The small discrepancy can be made to disappear by assigning a density of about 1.28 to DNA-less phage particles, or by assuming that the appropriate value of k in equation (1) is 0.32.

Kellenberger *et al.*[3] found by direct measurement of DNA content per plaque-forming particle that the *b2* mutant contained 18 per cent less DNA than the b^+ phage.

The consistency of these diverse measurements suggests that our molecular weight estimates are not far off, and that the DNA contents of phage mutants of the kind studied here can in fact be measured in terms of the phage particle densities.

Finally, it must be noted that the decreases in molecular weight of DNA attending the *b2* and *b5* mutations are within experimental error additive in the double mutant *b2b5*. We conclude, therefore, that each of the mutations was accompanied by the loss of a different segment of the DNA molecule, segments that do not overlap and that comprise together some 20 per cent of the original length of the molecule.

The mutations themselves occurred in widely separated regions of the phage chromosome.[2] This fact is consistent with the conclusion last stated if the losses of DNA have occurred at the sites of the mutations, an inference for which there is some evidence.[2] At any rate, the losses of segments of DNA molecules recognized here provide a plausible origin for the physiological effects of the mutations, and a model for a class of primary genetic deletions.

Summary.—DNA's from lambda phages of different genotypes sediment at increasing rates in the order *b2b5*, *b2*, *b5*, b^+, which is the same as the order of increasing densities of the phage particles. DNA from b^+ phage is more fragile than that from *b2b5*. The two mutations studied are therefore accompanied by characteristic reductions in the lengths of DNA molecules, reductions that are additive in the recombinant phage carrying both mutations.

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APPARENT CYTOPLASMIC STERILITY IN DROSOPHILA PAULISTORUM

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A hybrid inherits its chromosomes from both parents, but its cytoplasm mainly or exclusively from the mother. This may be expected to result in differences in the outcomes of the reciprocal crosses between a given pair of species or races. Differences between reciprocal crosses are indeed not uncommon,[1] but, at least in animals, most of them cannot be ascribed to influences emanating from the cytoplasm autonomously from the nuclear genes. In the first place, in organisms with separate sexes, the heterogametic sex is chromosomally not identical in the progenies of reciprocal crosses. Furthermore, certain characteristics of a progeny may be due to "maternal effects," i.e., to predetermination of the qualities of the egg cell by the chromosomal complement present in that cell before meiosis.[2] Laven[3] has, however, reported a thoroughly authentic case of hybrid sterility due to cytoplasmic differences in mosquitoes of the *Culex pipiens* species complex. A possible, but not certain, case of cytoplasmic sterility has been described in *Drosophila virilis* × *D. littoralis* hybrids by Patterson.[4] The purpose of the present communication is to report an apparently valid case of cytoplasmic hybrid sterility in *Drosophila paulistorum*.

D. paulistorum is a complex of at least six races or incipient species.[5, 6] Five of the races are difficult to intercross because of sexual isolation; when hybrid progenies are obtained they consist of fertile daughters and sterile sons. The sixth, Transitional race, produces fertile hybrids when crossed to at least one strain of every other race. Hybrids between different strains of the Transitional race are as a rule also fertile, but the case described below is an exception. The strains involved are Santa Marta (referred to below as "S") and Mesitas (referred to as "M"), both from Colombia, South America. The S strain is derived from flies collected in the Sierra Nevada de Santa Marta by Professors H. L. Carson and M. Wasserman; the M strain was collected at Mesitas, west of Bogotá, by Professor Alice Hunter of the Universidad de los Andes, Bogotá, Colombia. The S strain pro-