DIFFERENT STRUCTURES ASSOCIATED WITH RIBOSOMAL AND NONRIBOSOMAL DNAS IN DROSOPHILA MELANOGASTER

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ABSTRACT

DNA from adult flies was found to be supercoiled as evidenced by its sedimentation behaviour in sucrose gradients containing varying concentrations of ethidium bromide. These structures survive treatment with proteases and ribonuclease A. The DNA containing the ribosomal genes is also supercoiled but appears to have a structure different from that of the bulk of the DNA.

INTRODUCTION

We have previously reported the isolation of high molecular weight DNA from adults and larval tissues of Drosophila melanogaster and the presence in certain genotypes of ribosomal DNA which we interpreted as not being integrated into the DNA of the chromosome (1-4). In this paper we report that the DNA which we isolate from wild-type female adult flies contains supercoiled structures, which survive treatment with proteases and RNase A. We also present evidence that in the wild type fly we have studied the DNA containing the ribosomal genes has a structure different from that of the bulk of the DNA. An understanding of these structures may provide insight into the mechanisms responsible for the unusual patterns of inheritance of these genes (5,6).

RESULTS AND DISCUSSION

Fig. 1A shows the sucrose gradient sedimentation profile of [^{14}C]-labeled DNA prepared from adult Oregon R females by our usual procedure, which involves digestion with pronase after treatment with detergent (1). We have previously presented evidence that this DNA has a molecular weight of at least 3×10^9 (4) and is not an aggregate of low molecular weight DNA (1,4). Fig. 1 and 2 show the effect of adding increasing concentrations of ethidium bromide to the

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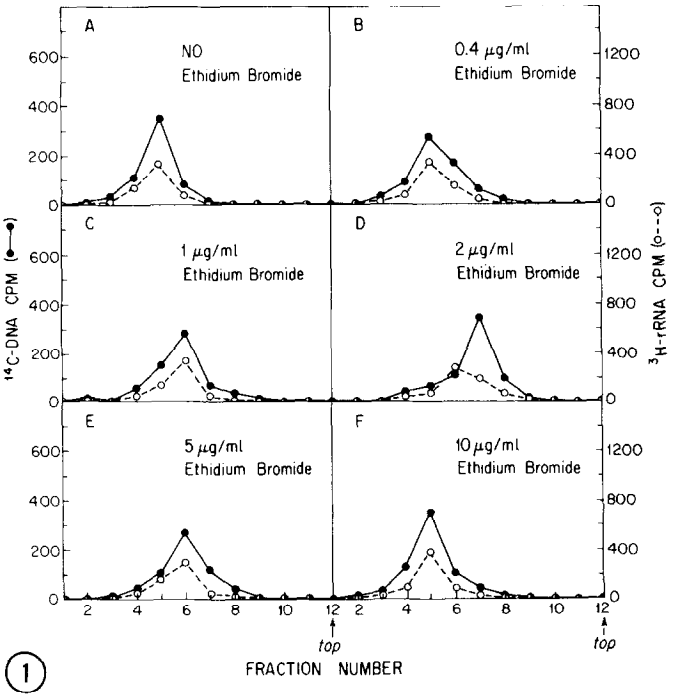

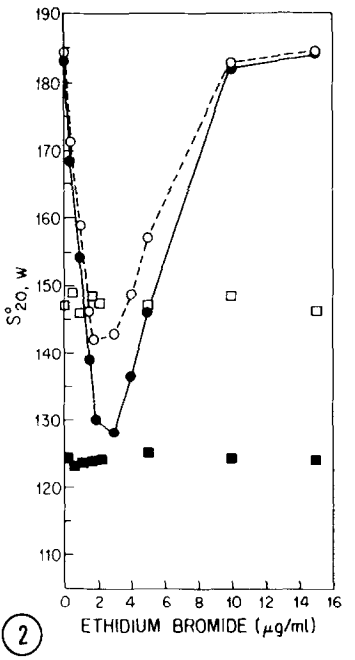

Fig. 1. Sedimentation of [^{14}C] DNA from Oregon R female adults in sucrose gradients containing varying concentrations of ethidium bromide. Shown are a typical set of gradients in HS buffer. Sedimentation is from right to left. Gradient fractions were hybridized to saturating amounts of [^{3}H]-rRNA from Drosophila tissue culture cells as previously described (1).

Fig. 2. Weight average S values ($S_{20,w}$) as a function of ethidium bromide concentration with and without a layer of 5 x 10^{-9} μg/ml of DNase (see Materials and Methods). Each point represents an average of the weight averages obtained from three gradients except for points at 3 and 4 μg/ml where only two gradients were run. For a given concentration of ethidium bromide the weight averages of all gradients were within 4 S of one another. Gradients with ethidium bromide alone contained HS buffer. Gradients containing 5 x 10^{-9} μg/ml of DNase were constructed in the manner described for DNase gradients in Materials and Methods using HSM. Some gradients with ethidium bromide alone (2 μg/ml and 10 μg/ml, 3 gradients for each concentration) were also run in HSM to verify that the results were the same as with HS, but these latter gradients were not used to calculate the S values shown. ●——● [^{14}C]DNA; ■ ■ [^{14}C]DNA in the presence of DNase; o----o [^{3}H]-rRNA hybridization; □ □ [^{3}H]-rRNA hybridization in the presence of DNase.

gradient. The S value decreases until a concentration of 2 or 3 μg/ml is reached, at which point it begins to increase again. The increase at the higher concentrations rules out the possibility that the effect is due to contamination of the ethidium bromide with nucleases. The observed behaviour

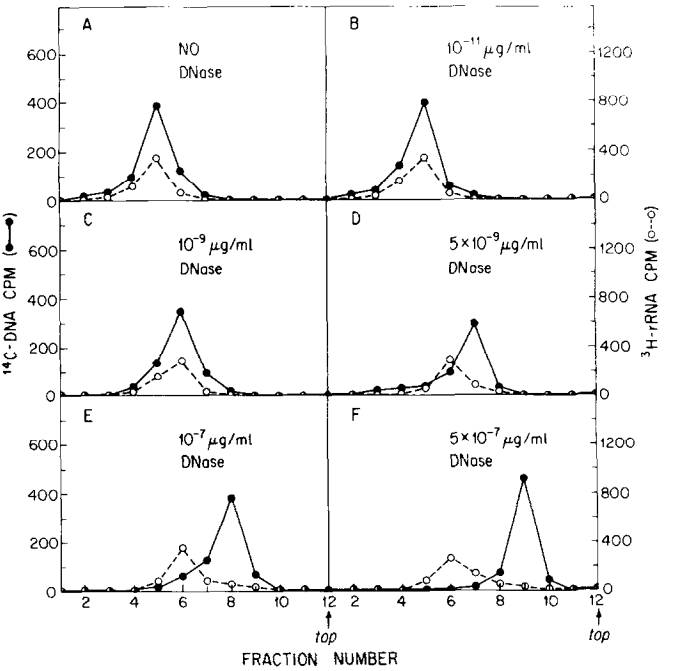

Fig. 3. Sedimentation of [^{14}C] DNA from Oregon R female adults in sucrose gradients containing a layer of DNase (see Materials and Methods). Sedimentation is from right to left. Gradient fractions were hybridized to saturating amounts of [^{3}H] ribosomal RNA from Drosophila tissue culture cells as previously described (1). Shown are typical gradients from a total of at least five for each concentration with the exception of 5 x 10^{-9} µg/ml and 5 x 10^{-7} µg/ml, where three and two gradients respectively were run.

is that expected for supercoiled DNA (7). The effect is completely eliminated by sedimenting the DNA through a layer of pancreatic DNase I (5 x 10^{-9} µg/ml) as seen in Fig. 2. This is the result expected since nicking relaxes supercoiled DNA (7).

We can also progressively lower the sedimentation coefficient with increasing concentrations of DNase alone (Fig. 3). From the pattern of change in sedimentation with increasing DNase amounts, we would argue that at the lower concentrations (5 x 10^{-9} µg/ml or lower) the change in S is due to conformational changes due to nicking of the DNA rather than changes in molecular weight resulting from double-strand breaks. The argument can be made as follows: 10^{-9} µg/ml of DNase moves the peak from fraction 5 to

fraction 6 leaving no more than 20%, and probably less, of the DNA unaffected. If we assume that a minimum of one double-strand break is necessary to change the sedimentation coefficient, then from the Poisson distribution it is apparent that in order for 80% of the molecules to have at least one break, the average number of breaks per molecule must 1.6. Since the number of such breaks should be proportional to the square of the enzyme concentration, we would predict that 10^{-7} µg/ml should produce at least 1.6×10^{4} breaks and 5×10^{-7} µg/m at least 4×10^{5} breaks, yielding final average molecular weights of no more than 2.5×10^{6} and 1.0×10^{5} respectively, assuming an initial molecular weight of 4×10^{10}, which is the estimated size of the largest chromosome (8). It seems more plausible, then, that the effect of low amounts of DNase is to introduce nicks, which allow the supercoiled DNA to relax. 10^{-9} µg/ml of DNase produces an intermediate state of relaxation. This can be seen both in ethidium bromide gradients (data not shown) and in Fig. 3. From this result it would appear that each molecule contains more than one independent region of supercoiling, and thus we are probably not dealing with single closed circles.

The finding that the DNA is supercoiled provides at least a partial explanation of why the DNA we isolate sediments at about 180 S and does not show the substantial rotor speed effect expected for linear DNA (9).

Hybridization of the gradient fractions with [^{3}H]-labeled RNA shows that the DNA containing the ribosomal genes behaves differently from the bulk of the DNA (Fig. 1-3). Upon removal of supercoils with ethidium bromide, the DNA containing the ribosomal genes sediments somewhat more rapidly than the bulk of the DNA (Fig. 2). A more striking effect is seen in the gradients containing DNase, where the sedimentation of the ribosomal DNA is much less sensitive to DNase than is that of the bulk of the DNA. Noteworthy is the almost complete separation of the ribosomal genes from the rest of the DNA at 5×10^{-7} µg/ml of DNase. The considerable purification of these genes obtained should be useful in their study. From these results it is apparent that the DNA molecules containing the ribosomal genes have a different structure from that of

the bulk the DNA. The very broad specificity of pancreatic DNase I (10) makes it unlikely that the differential effect of the enzyme is due to a lower density of potential cleavage sites in the DNA containing the ribosomal genes. At least a 100-fold difference in frequency of sensitive sites would be necessary to explain our results.

Other workers have isolated supercoiled structures containing DNA, RNA, protein, and in some cases membrane fragments (11-17). The relationship between these structures and ours is unclear.

Also unclear is the nature of the rotational constraints which maintain the supercoiling. However, it is noteworthy that our procedure includes exhaustive digestion with pronase (1). In addition, we have found no effect on the sedimentation of adding proteinase K (2 mg/ml) to the 15% sucrose layer of the gradient (see Materials and Methods). To test the possibility that some proteins might be inaccessible to the protease because of the compact nature of a supercoile structure, the DNA was sedimented through proteinase K in the presence of 2 μg/ml of ethidium bromide to relax the structure. The DNA was then gently removed from the gradient, dialyzed, and rerun in the absence of the dye. The normal sedimentation pattern of untreated DNA was seen. Similar results were obtained with 40 μg/ml of RNase A.

Variations in compactness along chromosomes have long impressed cytologists. We are encouraged to think that other sequence specific differences in chromosome structure might be revealed by the techniques used here. Further work will test whether the satellite sequences, which are the primary constituents of heterochromatin in Drosophila melanogaster (18), differ in their structural properties from the bulk of the DNA, which is derived from euchromatin.

MATERIALS AND METHODS

All flies were adult females from an Oregon R stock obtained originally from the Bowling Green Stock Center (stock no. a 15). The procedures for growth and labeling of flies, preparation of DNA, and hybridization of sucrose gradient fractions with [^{3}H]-labeled ribosomal RNA (rRNA) from Drosophila tissue culture cells have been described (1).

For the ethidium bromide experiments sucrose gradients (15-30 % w/v) were constructed as previously described (1). Ethidium bromide was present at a

uniform concentration throughout the gradient. Pancreatic deoxyribonuclease I (Worthington) was placed in only one portion of the gradient. These gradients were constructed by consecutively layering 2.5 ml of 30% sucrose, 2.5 ml of 25%, 2.5 ml of 20%, 2.5 ml of 15% containing the DNase, and 1.0 ml of 10%. Four buffers were used in the sucrose gradients. LS (low salt): 0.01 M NaCl, 0.01 M tris-HCl, 1 mM EDTA, pH 7.0; HS (high salt): 0.9 M NaCl, 0.01 M tris-HCl, 1 mM EDTA, pH 7.0; LSM: LS + 0.1 M $MgCl_2$; HSM: HS + 0.1M $MgCl_2$. All gradients were prepared in polyallomer tubes (Beckman) treated for at least 30 min. with Silicad (VWR Scientific) and spun at 11,000 rpm in a Spinco SW 41 Ti rotor for 16 hr. at 18°C.

In experiments involving proteinase K or RNase, gradients were constructed in the same manner as the DNase gradients except that in place of the DNase was substituted either 2 mg/ml of proteinase K (Beckman) or 40 µg/ml of RNase A (Sigma). Gradients were run in both LS and HS with and without ethidium bromide. The activities of both enzymes were checked at 18°C. in the HS buffer containing the appropriate concentration of sucrose either with or without 2 µg/ml of ethidium bromide. 2 mg/ml of proteinase K degraded more than 80% of 1 mg/ml of added casein (Difco; pH matched to that of the proteinase K soltuion) in 5 min. as assessed by visual inspection of precipitated casein using the precipitation reagent described by Nomoto and Narahashi (19). In 1 hr. or less 40 µg/ml of RNase A liberated from 1 mg/ml of yeast RNA (Sigma; pH adjusted to 7.0) acid-soluble material equivalent to an $O.D._{260}$ = 15 (calculated with respect to the undiluted enzyme reaction mixture). Acid-soluble material was measured as described by Kalnitsky et al. (20).

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