THE PROPORTION OF DNA COMPLEMENTARY TO RIBOSOMAL RNA IN DROSOPHILA MELANOGASTER

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Received February 15, 1965

Molecular hybridization experiments have indicated that about 0.3 per cent of the DNA in E. coli (Yankofsky and Spiegelman, 1962a, 1962b), B. megaterium (Yankofsky and Spiegelman, 1963) and Pisum sativum (Chipchase and Birnstiel, 1963) is reserved for the specification of ribosomal RNA. Evidence will be reported here that a similar proportion of the DNA in Drosophila melanogaster can be hybridized with r-RNA.

Materials and Methods

DNA preparation. DNA was prepared from adult Oregon-R wild-type flies by the method of Mead (1960). It was then diluted to 200 µg/ml in SSC (.15 M NaCl-.015 M Na citrate) and precipitated with 1 volume of cold isopropanol. The ppt. was dissolved in 0.15 M NaCl-0.01 M Tris, pH 7.3, with 10 µg/ml of ribonuclease (XII-A, sigma), then boiled 5 minutes and fast cooled to room temperature. Within 30 minutes of denaturation, pycnographic fractionation was begun in CsCl according to the protocol of Hall and Spiegelman (1961). Fractions in the density range 1.716-1.732 were dialyzed against TMS (0.3 M NaCl-0.01 M Tris, pH 7.3-0.001 $MgCl_2$), to give the final preparation to be used in the experiments.

RNA preparation. Flies were allowed to oviposit on corn meal-molasses medium containing 1mc/ml H^3-uridine. After an average of 5 days of growth at room temperature on this medium, larvae of mixed ages up to

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early third instar were removed to nonradioactive medium for 15 hours, then homogenized in cold TMS with added heparin, 0.1 mgm/ml. The homogenate was deproteinized by the phenol method of Gieren and Schramm (1956). Crude r-RNA was then isolated by sucrose gradient centrifugation. The two rapidly sedimenting peaks were isolated, brought to pH 7-0, boiled for 5 minutes, fast-cooled and filtered through nitrocellulose filters (Bac-T-Flex, type B6) to remove traces of DNA. The RNA was then adsorbed to MAK (methylated albumin-Kieselguhr) from 0.6 M NaCl and eluted in 3 ml of 0.9 M NaCl. The specific activity of the r-RNA was 10,200 cpm/µg. The preparation was frozen and stored at -10°C in TMS.

r-RNA-DNA hybridization. Hybridization was carried out under the conditions used by Yankofsky and Spiegelman (1962a). The hybrid was isolated by the salt-filter method of Nygaard and Hall (1963) followed by RNase treatment to remove unhybridized RNA. Thirty µg of denatured DNA and varying amounts of the H^3-r-RNA were added to tubes and made up to 1.25 ml in 0.5 M NaCl-0.001 M $MgCl_2$-0.01 M Tris, pH 7.3. These were heated for 3 hours at 55°C and allowed to cool to room temperature over a period of 5 hours. Five ml of distilled water were added to each and the contents made to 10 µg RNase/ml. After incubation at 30°C for 30 minutes, the samples were brought to 0.5 M with 2 M NaCl and filtered. The filters were air-dried and the radioactivity counted in a Packard Tri-Carb Scintillation Counter.

Results

Figure 1 shows a pycnographic fractionation of denatured drosophila DNA used for hybridization. The peak is at density 1.721 corresponding to a G+C content of about 42 per cent. A DNase-treated duplicate showed no banding. Mead (1964) obtained 39 per cent G+C by chromatography of the nucleotides. Figure 2 is a sedimentation profile of the H^3-RNA; fractions 12 through 19 were used. Figure 3 is the NaCl gradi-

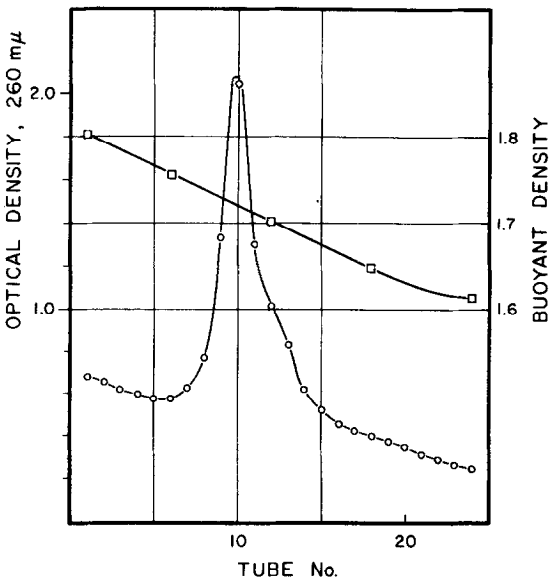

Figure 1. Distribution of denatured drosophila DNA in CsCl gradient after 72 hours at 30,000 rpm in SW-39 rotor at 21°C. Squares are bouyant density from refractive index; circles, optical density 260 mμ of 6:1 dilution in H_2O of each fraction.

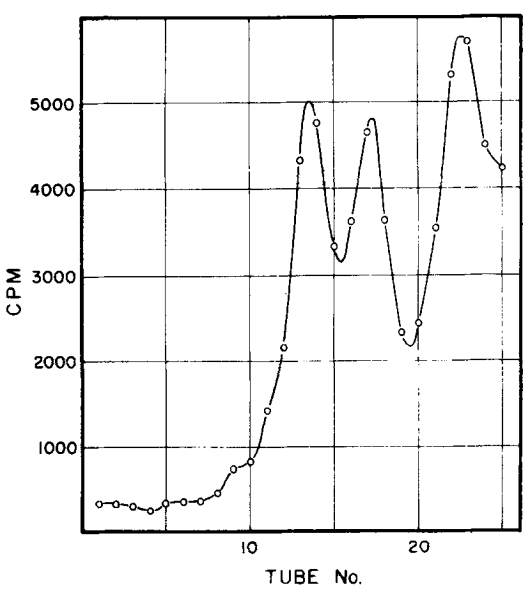

Figure 2. Four-hour sedimentation profile of drosophila H^3-RNA in 5%-20% sucrose gradient. One ml of H_2O added to each fraction and 1/20 ml counted.

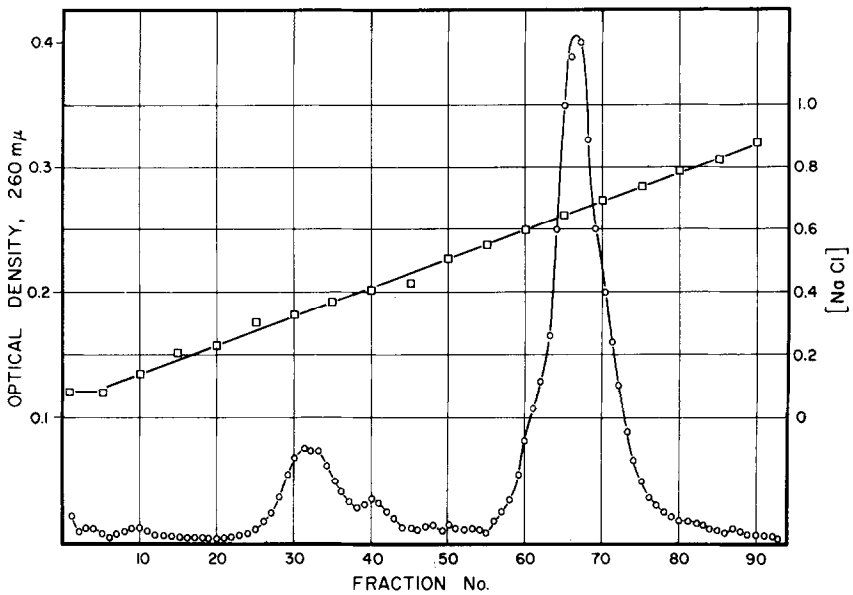

Figure 3. Salt gradient elution of drosophila RNA from MAK column, five ml fractions. Squares, NaCl concentration from refractive index; circles, o.d. 260 mμ.

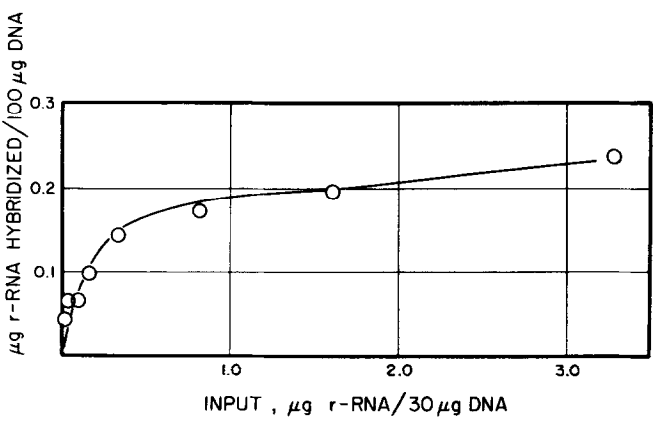

Figure 4. Proportion of drosophila DNA hybridized vs. H^3r-RNA input.

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ent elution profile of drosophila total RNA from MAK column, showing that the r-RNA is eluted between 0.55 M and 0.8 M NaCl. The two sizes of drosophila r-RNA do not separate upon gradient elution from a MAK column.

The amount of enzyme-resistant hybrid bound to filters is plotted vs. the amount of r-RNA added in figure 4. The DNA appears to be saturated when about 0.25 per cent of its weight in RNA has been hybridized.

Discussion

An approximately constant proportion of the DNA is complementary to r-RNA in genomes of different size; two bacterial species, a higher plant and a higher animal. This may be interpreted to mean that the maximum required rate of r-RNA synthesis is proportional to the size of the genome, and that this proportionality is maintained in evolution by adjustment of the number of templates. Such genetic mechanisms as unequal crossing-over and intrachromosomal exchange (Peterson and Laughnan, 1963) could very rapidly change the number of cistrons in a tandemly repeated group. These mechanisms could operate in asexual cell clones as well as in meiosis.

An exception to the foregoing proportionality is reported by McConkey and Hopkins (1964). They find a saturation plateau for 28S r-RNA-DNA from HeLa cells to be about 1/33 of the corresponding figure for peas and for E. coli and B. megaterium (with 23S r-RNA), and 1/50 of the proportion of DNA hybridized with total r-RNA in drosophila and the bacteria. A technical difficulty can explain part of the discrepancy: denaturation of DNA may be incomplete when carried out in 2 X SSC or higher salt concentrations (Attardi, unpublished, cited by McConkey and Hopkins in a note added in proof). It remains to be seen whether the DNA of HeLa cells is representative of the human genome, or whether the number of r-RNA cistrons has decreased during the history of the cell strain.

Suggestive evidence that the ca. 10 r-RNA cistrons in E. coli are contiguous is given by Yankovsky and Spiegelman (1962b). The mol. wt. of the DNA in their experiments was only a small fraction of the bacterial genome, yet the density of the hybrid was such that each DNA must be paired with more than one r-RNA molecule. If the haploid genome of droso-

phila contains about 10^{-12}g of DNA (Rudkin and Schultz, unpublished, cited by Steffenson, 1963), then it contains several hundred r-RNA cistrons. Ritossa and Spiegelman (in press) have convincing evidence that all of the r-RNA cistrons in drosophila for both 18S and 28S RNA are located in a small chromosomal segment at or near the nucleolus organizer. Their saturation value for DNA from flies having one such segment per haploid genome is similar to ours, although obtained by a different procedure.

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