INDICATION OF GENE AMPLIFICATION IN *RHYNCHOSCIARA* BY RNA-DNA HYBRIDIZATION

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The occurrence of gene amplification as a mechanism of cell differentiation was recently demonstrated in the case of ribosomal RNA cistrons of amphibian oocytes (1–3), and it seems that this phenomenon is of widespread occurrence (4). In somatic cells the evidence for amplification thus far is based on cytological observations. The best known cases are those of *Rhynchosciara* and *Sciara*; in the prepupal phase of these insects, it was possible to show "extra" DNA synthesis in certain bands of the polytene chromosomes of the salivary gland cells (5–7).

We now have been able to study gene amplification in *Rhynchosciara* salivary gland cells with the aid of hybridization experiments. For this purpose we used an RNA fraction which we have recently succeeded in obtaining from these cells. This fraction has a high turnover and is mainly of nuclear origin, and thus it was designated as nRNA; its preparation and characteristics are described in a separate paper (8).

In Fig. 1 we present data obtained by hybridizing nRNA-^{3}H from glands at the time of giant puff formation with three different DNA preparations. One of these preparations was obtained from glands of larvae at the same age as the larvae from which the nRNA was obtained, larvae about 52 days old ("puff-DNA"); the other from glands of larvae at an earlier age when no puffs are present, larvae about 40 days old ("before puff-DNA"); and the third was obtained from ovaries of adult virgin flies ("ovary-DNA"). The results show that ovary-DNA and before puff-DNA hybridize equally well with this nRNA; in contrast, puff-DNA hybridizes about twice as much. These same results were obtained in two other separated experiments, and thus we think that they strongly suggest the occurrence of a change in genome composition at the time of appearance of the giant puffs in the salivary glands.

It is plausible to suggest that the above-mentioned change corresponds to amplification of certain genes responsible for the transcription of part of the RNA contained in the nRNA fraction used in the experiments. Assuming this to be the case, it seems worthwhile to comment on three aspects:

(*a*) The fact that the hybridization level is about twice as high for puff-DNA as compared to before puff-DNA does not necessarily mean that we are observing a twofold amplification. This follows because the nRNA fraction that we used is certainly composed of different RNA species, only part of which is transcribed from the amplified genes. Thus it would be possible to obtain hybridization curves similar to those presented in Fig. 1, even with 1000-fold amplification, provided a considerable part of the nRNA would be transcribed from nonamplified genes.

(*b*) The data presented in Fig. 1 indicate that the amplification is an event which is both chronologically and histologically specific in *Rhynchosciara*.

(*c*) Previous results from this Laboratory have shown that, at the time of appearance of giant puffs in *Rhynchosciara* salivary glands, when genome amplification was cytologically observed, there is an inhibition of ribosomal RNA synthesis (10–11). Moreover, the DNA puffs, where amplification occurs, do not contain ribosomal DNA, as shown by *in situ* hybridization experiments (12). Therefore, it would not be plausible to expect that the amplification process would involve ribosomal RNA cistrons. A more direct evidence indicating

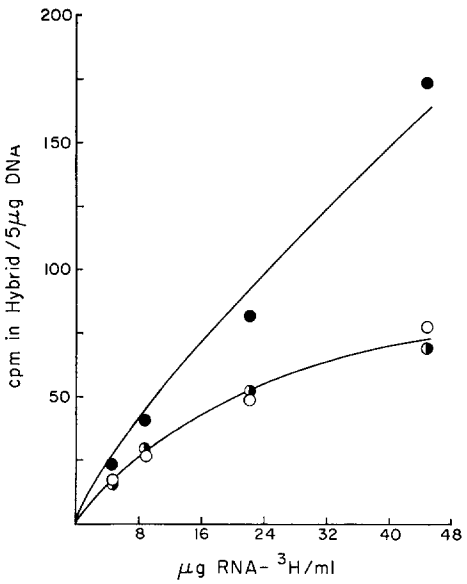

FIGURE 1 DNA/RNA hybridization. Three different DNA preparations of *R. angelae* were hybridized to nRNA-^{3}H (880cpm/μg) obtained from salivary glands at the time of appearance of the giant puffs. Hybridization was performed as described by Gillespie and Spiegelman (9). The method used in DNA preparation will be described elsewhere.[1] The annealing reaction was performed at 62°C for 40 hr, in 0.09 M Na_3 citrate, 0.9 M NaCl, and nRNA-^{3}H in the indicated concentrations. After the radioactivity of each filter was determined, the filters were washed with chloroform, dried, and incubated in HCl at 98°C for 20 min. The amount of DNA retained by each filter was determined by reading the absorbance of the acid solution, at 268 mμ (27.8 units of absorbance corresponding to 1 mg/ml of DNA). A filter without DNA was included in each vial to determine the background. In the case of the more concentrated RNA solution, the total cpm for the blank filter was 35. ●——●, DNA from salivary glands at the time of appearance of giant puffs; ○——○, DNA from salivary glands, about 12 days before the appearance of giant puffs; ◑——◑, DNA from ovary of virgin flies.

that this is the case is presented by the data shown in Figs. 2 *a* and 2 *b*. When nRNA-^{3}H is hybridized to salivary gland DNA fractions obtained by CsCl centrifugation the results are those shown in Fig. 2 *a*. We note that there is a coincidence of the radioactivity profile of the hybrid and the absorbance profile of *Rhynchosciara* DNA. A very different result is found when ribosomal RNA-^{3}H from *Xenopus laevis* is hybridized to *Rhynchosciara* DNA[2] fraction obtained from the CsCl centrifugations (an *Escherichia coli* DNA marker was included in these experiments). In this case, as shown in Fig. 2 *b*, the radioactivity of the hybrid appears in a region between *E. coli* DNA and *Rhynchosciara* DNA, corresponding to a G + C content of approximately 40%. The experiment shown in Fig. 2 *b* was performed with ovary DNA, but the same results were obtained with salivary gland DNA.[1] We conclude that, in *Rhynchosciara*, ribosomal DNA bands at a higher density than the main DNA component, similar to what has been observed for *Rhynchosciara* whole larvae DNA[3] and *Xenopus* DNA (14). These results would be expected in the case of clustering of the ribosomal RNA cistrons, in anal-

TABLE I

Base Composition of Salivary Gland DNA and Ribosomal RNA of Rhynchosciara angelae

Base	Composition		
	DNA	28S RNA	18S RNA
	%	%	%
Guanine	16.6	28.4	27.1
Adenine	34.6	26.7	23.7
Cytosine	16.6	15.0	15.9
Thymine	31.0	—	—
Uracil	—	29.9	33.2

DNA was hydrolyzed in formic acid at 175°C for 1 hr in a sealed tube. After drying, the hydrolyzed material was dissolved in 1 N HCl and applied to No. 1 Whatman filter paper strip for chromatography in isopropanol–HCl–H_2O (170:41:250 v/v) for 26 hr. Spots corresponding to the bases were cut, eluted with 0.1 N HCl, and read at appropriate wavelengths for determination of base composition. 28S and 18S ribosomal RNA were separated by centrifugation in sucrose gradient and hydrolyzed in 0.3 N KOH for 18 hr at 37°C. After the addition of 70% $HClO_4$ to reach pH 4.0, $KClO_4$ was removed by centrifugation and 40 μl of the supernatant was applied to a sheet of Whatman 3MM paper for electrophoresis in 0.02 M sodium citrate, pH 3.5, according to Markham and Smith (15). Spots containing the nucleotides were cut eluted with 0.05 N HCl, and read at appropriate wavelengths for determination of base composition.

[1] R. Meneghini, M. Cordeiro, and F. J. S. Lara. Manuscript in preparation.

[2] R. Meneghini. Unpublished results.

[3] A. G. Gambarini, M. L. Birnstiel, and F. J. S. Lara. Unpublished results.

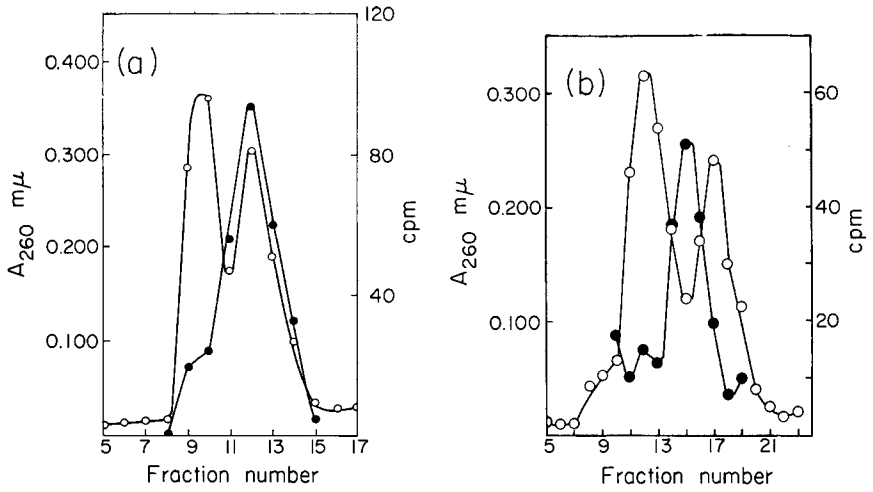

FIGURE 2 Hybridization of nRNA-^{3}H from salivary glands of *Rhynchosciara* and of ribosomal RNA-^{3}H from *Xenopus laevis* with CsCl fractionated DNA from *Rhynchosciara*. (*a*) 50 μg of salivary gland DNA was centrifuged in CsCl together with 50 μg of *E. coli* DNA, which was used as a marker (peak at left). Centrifugation was done in a rotor No. 40 (Spinco Division, Beckman Instruments, Fullerton, Calif.) at 42,000 rpm, for 34 hr at 15°C, according to conditions described by Flamm et al. (13). 22 fractions were collected, and those containing DNA were used for hybridization with 80 μg/ml nRNA-^{3}H (280 cpm/μg), under the conditions described in Fig. 1. (*b*) 30 μg of *Rhynchosciara* ovary DNA was centrifuged in CsCl together with 50 μg of *E. coli* DNA. Centrifugation was done at 38,000 rpm for 46 hr at 15°C in rotor No. 40 (Spinco Division, Beckman Instruments). 25 fractions were collected, and those containing DNA were used for hybridization with 2 μg/ml rRNA-^{3}H of *Xenopus* (20,000 cpm/μg) under the conditions described in Fig. 1, except that the time of annealing was 20 hr. (●——●), radioactivity in the hybrid; (○——○), absorbance at 260 mμ of the fractions.

ogy to what is observed in *Xenopus* (14), since *Rhynchosciara* ribosomal RNA has a G + C content of 43% and its DNA has a G + C content of 33% (Table I). These data taken together with those presented in Fig. 1 indicate that the RNA transcribed from the amplified regions is DNA-like and differs from ribosomal RNA.

It is likely that both our data and the cytological observations for "extra DNA" synthesis in *Rhynchosciara* (5–6) relate to one and the same phenomenon. However, the experiments here described do not permit a conclusion in this connection. This is due to the fact that we do not have a criterion to determine whether the RNA which hybridizes to the amplified DNA is transcribed from the giant chromosomal puffs. It is likely that *in situ* hybridization experiments will clarify this point.

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