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TRANSLATION OF MESSAGES TRANSCRIBED FROM THE "DNA PUFFS" OF RHYNCHOSCIARA

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SUMMARY

"In vitro" translation of RNA extracted from the different segments of the salivary gland of R. americana indicates that the 21,000 M.W. and 29,000 M.W. peptides produced by the gland and which were previously assigned to the B-2 and C-3 "DNA puffs", are coded by [poly(A)$^+$]RNA. Sucrose gradient fractionation of the [poly(A)$^+$]RNA extracted from the proximal segment of the gland, where the B-2 puff is most active, indicates that the 21,000 M.W. peptide is translated from the fractions which contain the RNA transcribed from this puff. The evidence obtained also indicates that the high M.W. peptides synthesized by the gland might be translated from [poly(A)$^-$]RNA.

The so called "DNA puffs" appear in the salivary gland chromosomes of late 4th instar larvae of the flies of the Sciaridae family. These puffs differ significantly from the RNA puffs,which have been studied particularly in Drosophila and Chironomus (1,2,3,4) due to the fact that in them there occurs both transcription and DNA amplification (5,6). One of the aims of the work in our laboratory is to discover the biological significance of these puffs. We have used as experimental animals,larvae of Rhynchosciara americana, which is one the largest members of the Sciaridae.

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The salivary gland of Rhynchosciara can be divided into three segments the proximal (S_1), the median (S_2) and the distal (S_3) and the puff pattern in them is very different (7). For the purpose of interpreting the results we will present,it is important to point out that the B-2 "DNA puff" develops fully only in cells of the proximal segments of the gland during periods IV and V of 4^{th} instar (for detailed definitions of these periods, see Terra et al. (8)). Although this puff can also be found in S_2 and S_3, it does not develop fully in these gland segments and it is found in them only during period IV. There is another large DNA puff, the C-3 puff, which opens fully in S_2 and S_3 during periods V and VI of the 4^{th} instar.

Several reports have indicated that the"DNA puffs" of Rhynchosciara are very likely involved in messenger RNA formation. Thus Okretic et al. (9) have found that a 16S [poly(A)$^+$]RNA was very likely a transcript from the B-2 puff. Recently Bonaldo et al. (10) succeded in hybridizing this RNA to the B-2 locus. Furthermore Winter et al. (11) found very significant qualitative and quantitative changes in the protein synthesis pattern in the salivary gland at the time of appearance of the "DNA puffs". They found that one of the new peptides formed could be assigned as being coded by the B-2 puff and another could be assigned to the C-3 puff.

In this communication we report on the results we have obtained with the translation of the [poly(A)$^+$]RNA isolated from the different segments of the gland, which allow us to conclude that the "DNA puffs" are involved in the production of messenger RNA.

MATERIALS AND METHODS

Animals. The experiments were carried out with R. americana larvae at the period V of 4th instar.

RNA preparation. S_1 or S_2S_3 salivary gland segments were digested in 20 mM Tris-HCl pH 7.4, 1 mg/ml Pronase and 0.5% SDS. After 30 min the RNA was extracted with 1 volume of phenol-chloroform (1:1) and then precipitated with ethanol. The precipitated was recovered and the pellet ressuspended in Tris-HCl pH 7.4, 5 mM $MgCl_2$ and 10 µg/ml DNAse I. After 10 min the solution was treated with phenol-chloroform and the RNA was again precipitated.

Oligo(dT)-cellulose chromatography. For separation of [poly(A)$^+$]RNA the total RNA obtained as described above was submitted to affinity chromatography on oligo(dT)-cellulose as described by Aviv and Leder (12). The RNA that is not retained is designated as [poly(A)$^-$]RNA.

Sucrose gradient analysis. The [poly(A)$^+$]RNA fraction was sedimented in a 15-20% sucrose gradient in Tris-HCl pH 7.4, 100 mM NaCl and 1 mM EDTA, using a SW 65K rotor for 400 min at 45,000 rpm at 5°C. After the run the tube was punctured and 8 drops fractions were collected.

"In vitro" translation. The messenger dependent rabbit reticulocyte lysate system described by Pelham and Jackson (13) was used. As label L-[^{3}H]leu and L-[^{3}H]val both with specific activity 0.6 Ci/mmol were used.

Analysis of the translation products. The proteins were analysed by electrophoresis carried out on a 12.5% SDS-polyacrylamide slab gel at 6V/cm for 4 hours. The gel was submitted to the fluorography procedure (12) and the film was scanned with a Joyce-Loebl microdensitometer.

RESULTS AND DISCUSSION

In Fig. 1, panel C the profile obtained in the translation of the [poly(A)$^+$]RNA isolated from the S_1 segments of the glands in the period V of the 4th instar is presented. One observes that the main translation product is a polypeptide having a M.W. of 21,000. This peptide has the same M.W. as a peptide produced by the S_1 segments of the gland at this developmental period (Fig. 1, panel E) and which Winter et al. (11) have assigned as being coded by the information contained on B-2 puff. We have also analysed the products obtained by the translation of the [poly(A)$^+$]RNA from the

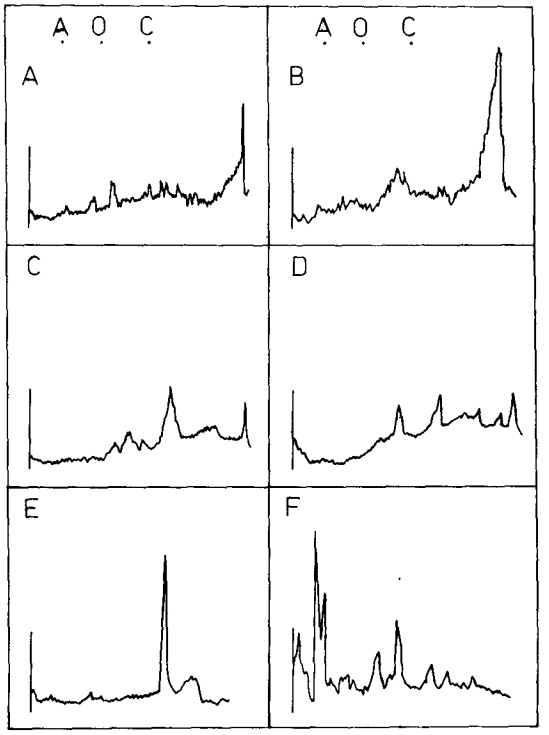

FIGURE 1. Translation of [poly(A)$^+$]RNA. RNA obtained from either S_1 or S_2S_3 segments of salivary glands of R. americana larvae in period V of the 4 instar was dissolved in 500mM KCl, 10mM Tris (pH 7.4) and adsorbed to oligo-dT-cellulose. After elution and precipitation with ethanol, the [poly(A)+]RNA fraction was translated in an mRNA dependent system from rabbit reticulocyte lysate (13), in the presence of L-[^{3}H]leu and L-[^{3}H] val. The salivary gland proteins were obtained by incubating the gland segments from animals in period V in TBGPL medium (16) suplemented with L-[^{3}H]Leu and L-[^{3}H]val according to Winter et al. (11). The electrophoresis was carried out on a 12.5% SDS-polyacrylamide slab gel at 6V/cm for 4 hours. The gel was submitted to the fluorography procedure (14) and the film was scanned with a Joyce-Loebl microdensitometer. Molecular weight markers: A-bovine serum albumin (68,000), O-ovalbumin (43,000), C-chymotrypsinogen (25,700). Control (A). Translation of globin mRNA (B), [poly(A)+]RNA from S_1 (C) and from S_2S_3 (D). Salivary gland proteins from S_1 (E) and from S_2S_3 (F).

S_2 and S_3 segments of the glands of the same period as above and the results are shown in Fig. 1, panel D. Two main peptides were obtained: one has the same mobility as the main product of the [poly(A)$^+$]RNA from S_1 and the other has a mobility corresponding to a M.W. of 29,000. A peptide having this same M.W.

is produced by the gland at this development stage (Fig. 1, panel F), and this is the peptide which Winter et al. (11) have assigned as being coded by the information contained in the C-3 "DNA puff".

It is interesting that among the translation products of the [poly(A)$^+$]RNA fraction from S_2 and S_3, one does not find the high M.W. peptides, which are so evident in the profile of the peptides synthesized by these segments (Fig. 1, panel F), and which are the main components of salivary secretion, during most of the larval life (15). This absence might mean that these peptides are translated from RNA that is not retained by oligo(dT)-cellulose. Alternatively the absence of large peptides could be explained by incomplete translation of messages, but no evidence for an accumulation of small peptides was obtained.

In order to verify if the messages coding for the 21,000 and 29,000 peptides were present or absent in developmental periods previous to the formation of the "DNA puffs", we extracted RNA from the glands of the animals at period III of 4^{th} instar, when these puffs are absent. The [poly(A)$^+$]RNA fraction was translated and the results obtained (not shown) indicate the absence of the sought for messages in the samples tested.

The identification of the 16S [poly(A)$^+$]RNA as the messenger codifying for the 21,000 peptide derives not only from the results presented in Fig. 1, but also from those shown in Fig. 2. Here we present the results obtained with translation of four fractions of [poly(A)$^+$]RNA from S_1 gland segments of animals in period V of 4^{th} instar. The results obtained indicate that there is in the original sample only one messenger, the

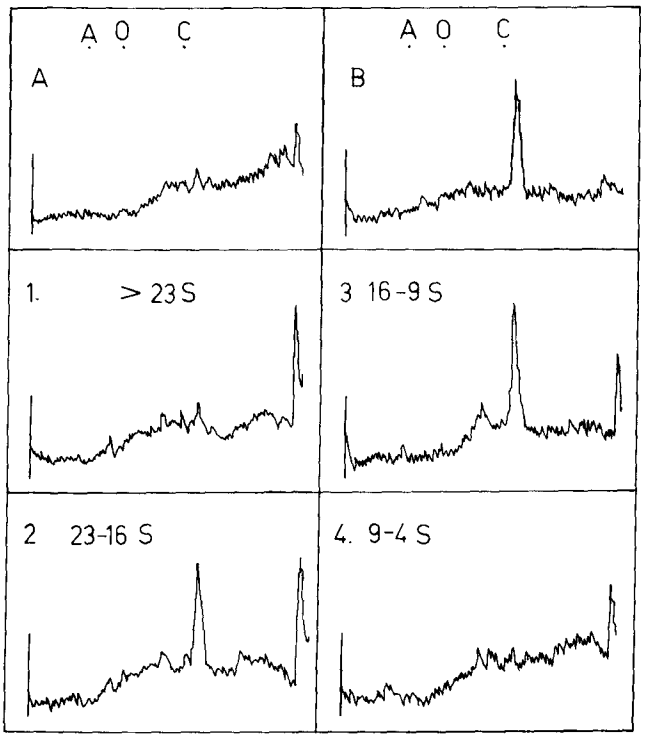

FIGURE 2. Analysis of RNA fractions by "in vitro" translation. RNA from S_1 segments of salivary gland of larvae in period V of 4^{th} instar were adsorbed to oligo(dT)-cellulose and the [poly $(A)^+$]RNA fraction obtained was sedimented in a 15-20% sucrose gradient. The gradient was divided into four fractions, corresponding to the S values indicated in the panels and the RNA was recovered by precipitation with ethanol and then translated as indicated in Fig. 1. The graphs are microdensitometer tracing of the fluorographies as in Fig. 1. Molecular weight markers as in Fig. 1. Control (A). Salivary gland proteins from S_1 (B). Translation of the RNA from the gradient fractions (1,2,3,4).

one giving rise to the 21,000 M.W. peptide. Furthermore this peptide is found only in the 23 S to 9 S fractions of the gradient, as expected from previous results of Okretic et al. (9). Therefore, these results allow us to conclude that at least the B-2 "DNA puff" in involved in messenger RNA production.

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