Radioactivity of RNA and DNA after ribonuclease treatment.

Incubation	Ribonuclease 80 µg	Total RNA[a] dpm/µg DNA	Percent of control[c]	Phenol extracted RNA[a] 85 °C nuclear RNA fraction dpm/µg RNA	DNA[b] mg/incubated sample	dpm/mg DNA
none[c]	–[c]	99.73	–	123	0.542	43
10 min at 20 °C	–	88.20	88	120	0.540	42
10 min at 20 °C	+	20.76	21	31	0.542	42

[a] Animals were injected with 6-[^{14}C]orotic acid[10]. [b] Animals were injected with methyl-[^{3}H]thymidine[10]. [c] Sample was treated with 0.6 N PCA[11] without incubation and used as control.

RNA fractions were extracted by treatment with phenol at different temperatures[12,13]. The specific radioactivity (dpm/µg RNA) of 85 °C nuclear RNA fraction, that corresponds to hnRNA[12,13], is reported in the table. The reduction of the specific radioactivity to 25% of the control value demonstrated that the rapidly labelled hnRNA is degraded by the RNase treatment of the 1200 g nuclear pellet. Under the same experimental conditions no difference between PCA precipitated control and incubated samples was observed (table) regarding the DNA content and the specific activity (dpm/mg DNA) of thymidine incorporated into DNA.

The present findings show that the reduction of the quantity of perichromatin fibrils (hnRNA) induced a rapid condensation of extranucleolar chromatin and demonstrated that the condensed and dispersed forms of chromatin are due to a low or a high quantity of newly synthesized hnRNA respectively.

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Tissue- and stage-specific RNA patterns in *Drosophila* as revealed by gradient formamide gel electrophoresis

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Summary. The in vitro labeled RNAs in the accessory glands from male adults, and in the salivary glands and fat body from developing larvae of *Drosophila melanogaster* were separated on linear gradient acrylamide gels in formamide. The patterns appear to be tissue- and stage-specific, and several lines of evidence indicate that the visualized radioactive bands include both hnRNA and messages.

We have previously shown that protein synthesis in the lethal mutations *1(3)tr* and *1(2)me* of *Drosophila melanogaster* is greatly reduced compared to that in the wild type at corresponding ages[2,3]. On the other hand, we found that the production of secretion proteins in the accessory glands (paragonia) of male adults is distinctly elevated following copulation[4]. In both cases it would be of interest to know whether corresponding alterations in the synthesis of messages in the fat body or paragonial cells take place. The study of RNAs in differentiated cells suffers from 2 handicaps: 1. In order to have sufficient homogeneous material for biochemical analysis each tissue has to be dissected out individually from the larva or fly. 2. Because of the extremely heterogeneous nuclear RNA (hnRNA) no satisfactory separation of the different RNA species can be achieved by conventional gel electrophoresis. In an effort to overcome these difficulties we have recently worked out a highly sensitive microelectrophoretic procedure by using formamide to eliminate the conformational effect of the RNA molecules[5], which are then separated on a linear gradient acrylamide gel.

Materials and methods. The wild type (Sevelen) of *Drosophila melanogaster* was cultured on a standard diet containing maize, agar, sugar and yeast at 25 °C. Accessory glands from male adults and salivary glands or fat body from larvae of the desired age were dissected out in a drop of cold Mops buffer solution and incubated for 60 min with tritiated uridine, cytidine, adenosine and guanosine (The Radiochemical Centre, Amersham; 6.4–52 Ci/mM, 10 µCi/µl). The in vitro labeled RNAs were extracted with 0.1 M acetate buffer (pH 5) and 80% phenol at 65 °C and precipitated by adding 2.5 volumes of ethanol. Following centrifugation the RNA precipitate was taken up in 98% formamide (Fluka) and heated at 100 °C for 4 min.

Stock solutions described previously by Mitchell et al.[6] were used for preparation of slab gels (0.18 × 10 × 11 cm). Monomer solutions containing 3.5% and 5% acrylamide in formamide were pumped between 2 glass plates with a

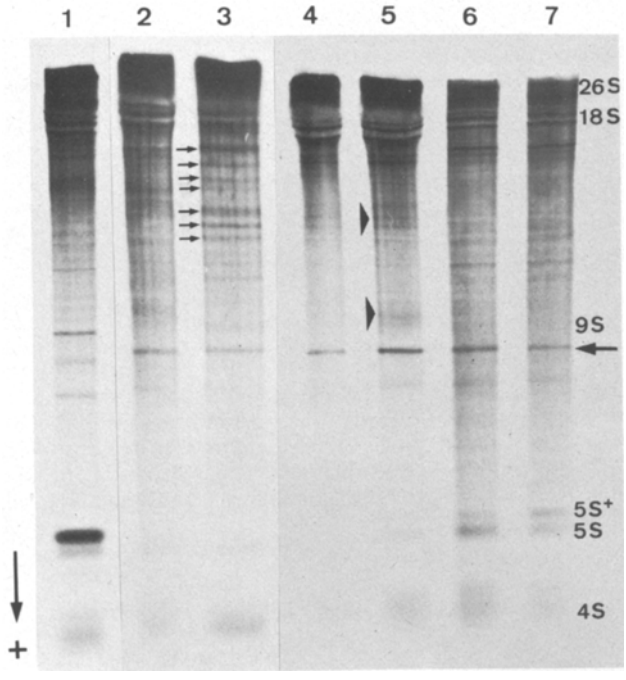

Fig. 1. Electrophoretic analysis of in vitro labeled RNA from heat-shocked (1) and normal (2–7) *Drosophila* tissues on gradient acrylamide gels in formamide. Lane 1: RNA from male accessory glands labeled at 37 °C. Notice the accumulation of the 5 S^+ precursor and the appearance of new radioactive bands. Lane 2: RNA from fat body of 4-day-old larvae. This and the following RNA samples were all labeled at 25 °C. Lane 3: RNA from fat body of 3-day-old larvae. The arrows indicate the most prominent stage-specific messages. Lane 4: RNA from salivary glands of 5-day-old larvae. Lane 5: RNA from salivary glands of 4-day-old larvae. The arrows show the stage-specific accumulation of messages at 2 broad gel positions. Lane 6: RNA from accessory glands of mated male adults. Lane 7: RNA from accessory glands of unmated male adults. The arrow indicates the mitochondrial mRNA. Positions of RNA markers are given on the right side of the fluorograph. The total radioactivity of each sample varied from 1.8×10^4 to 1.7×10^5 cpm, and the exposure time from 2 to 5 days.

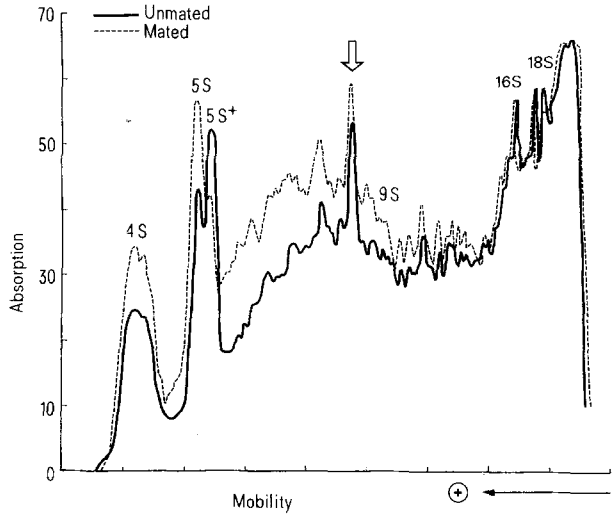

Fig. 2. Densitometrical tracings of the fluorographs of in vitro labeled paragonial RNAs from mated (– – –) and unmated (——) male adults. The arrow indicates the mitochondrial message. The mobilities of several RNA markers are given. The reciprocal changes in the ratio 5 S/2 S^+ before and after mating are evident.

peristaltic pump from a linear gradient-making device over a period of 20 min. Following polymerization a stacking gel with a constant concentration of 3% acrylamide was again poured. The electrophoretic run was performed at first for 30 min at 8 mA and then for 4–5 h at 16 mA. The radioactive RNA bands were visualized by fluorography[7]. Densitometrical tracing of the fluorograph was carried out by a DD2 densitometer and a DB5 recorder (Kipp and Zonen). More detailed information about the electrophoretic system is given in Chen[8].

Results and discussion. Patterns of the in vitro pulse labeled RNAs from the accessory glands of adult males as well as from the salivary glands and fat body of developing larvae are illustrated in figure 1. There is a very high radioactivity in the high-molecular-weight gel region corresponding to 26 S RNA. But this can not be due to ribosomal RNA because following denaturation with formamide the 26 S rRNA in *Drosophila* is known to dissociate into 2 18 S RNA species. Our chase experiments indicated that there is a rapid turnover of this labeled material most of which represents, apparently, hnRNA. Slightly in front of it at the gel position of 18 S RNA 2 closely located radioactive bands are visible. As just mentioned, these must be derived from ribosomal RNA. At about the middle part of the gel there is a sharp, heavily labeled band (indicated by arrow in figure 1, lane 7). Its occurrence in all tissues and the inhibition of its synthesis in the presence of ethidium bromide suggest that it may have its origin in mitochondria. In the low-molecular-weight gel section the location of 5 S and 4 S RNA is clear. The band indicated as 5 S^+ is probably a precursor of 5 S RNA, as it disappeared when the labeled tissue was chased for 4 h.

As shown by the fluorographs in figure 1, the remaining gel regions between the bands described above are occupied by a large number of RNA species. Evidence from affinity chromatography with poly(U)-sepharose confirmed that many of these are poly(A)-containing RNA. Furthermore, drastic alterations of the patterns occurred when the tissues were heat-shocked at 37 °C (figure 1, lane 1). In RNA samples prepared from the fat body of 3-day-old larvae at least 7 well defined radioactive bands can be detected in the 11 S to 16 S gel region, but not from the same tissue of 4-day-old larvae (see lanes 2 and 3 in figure 1). Previous studies by Roberts and colleagues[9] and in our own laboratory[2,3] demonstrated that the synthesis of hemolymph proteins increases most rapidly in the larvae aged 3 days, and the observed bands are most likely the corresponding messages. The same is true for the salivary glands. 2 broad radioactive gel regions at positions 9 S and 12 S were observed in the sample from 4-day-old larvae, but not in that from 5-day-old animals (lanes 4 and 5 in figure 1). Again the occurrence of the 9 S and 12 S bands corresponds to the period of active synthesis of glue proteins which according to Korge[10] and Beckendorf and Kafatos[11] takes place at about 90–106 h after oviposition.

In the accessory glands the RNA patterns were found to be the same between mated and unmated male flies, but copulation resulted in an elevated synthesis of virtually all RNA species (see lanes 6 and 7 in figure 1 and the densitometrical tracings in figure 2). It appears that the coordinated synthesis of individual proteins in the depleted glands following copulation is correlated with a coordinated synthesis of the corresponding messages. A remarkable enhancement of the processing of 5 S RNA from its 5 S^+ precursor after mating is also evident.

In conclusion, the RNA patterns revealed by the electrophoretic procedure described here are tissue- and stage-specific. Most of the radioactive bands represent no doubt mRNAs. A previous purification of the RNA sample prior

to electrophoresis is not necessary. The method has a high sensitivity and is applicable to studies dealing with µg quantities of tissues.

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Role of fungal staling growth products in inter-specific competition among phylloplane fungi

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Summary. The effect of fungal staling growth products on leaf-inhabiting microfungi, with special reference to a leaf spot pathogen *Pestalotiopsis funerea* Desm. of *Eucalyptus globulus* Labill. was studied. Results depict that antibiotics produced by competing microfungi caused the phenomenon of mycostasis on the leaf surfaces.

Leaf-inhabiting microfungi may inhibit the development of invading pathogens by creating a nutrient shortage or by producing inhibitory metabolic substances in an antagonistic manner[3-5]. As yet, few studies have been undertaken on staling growth products leading to the phenomenon of mycostasis among leaf-inhabiting microfungi. In the present investigation, staling growth products of various leaf-inhabiting microfungi of *Eucalyptus globulus* Labill. were studied for their effect on microfungal colonization of leaf discs with special reference to *Pestalotiopsis funerea* Desm., a leaf spot pathogen.

Materials and methods. Leaf-inhabiting microfungi of *E. globulus* were isolated by different techniques described by Dickinson[6], and maintained in pure cultures on Czapek-Dox+0.05% yeast extract agar. For the preparation of staling growth products of these fungi, 250 ml Erlenmeyer flasks containing 100 ml liquid nutrient Czapek-Dox+0.05% yeast extract were taken, and each was inoculated with 2 6-mm mycelial discs of the respective fungus. The flasks were incubated for 120 h at 25±1 °C on a reciprocating shaker (100 shakes per min with a 10-cm coverage) and cultures were filtered through Whatman filter paper No. 42 and finally through a Seitz filter. The pH of all the staling growth products was adjusted to 5.8 before treatment. The effect of staling growth products with reference to their antibiotic action on leaf colonization was studied by an immersion method. 40 leaf discs (10-mm size) were soaked in 30 ml of a particular fungal culture filtrate for 48 h and then blotted dry in folds of sterile filter papers. 4 such treated discs were placed on solidified Czapek-Dox+0.05% yeast extract agar in each plate. 10 replicates for each treatment were prepared and a suitable control was maintained by inoculating leaf discs treated only with liquid Czapek-Dox+0.05% yeast extract. They were incubated at 25±1 °C and examined after 7 and 14 days for developing fungal colonies.

Results and discussion. When leaf discs were treated with different fungal staling growth products, a significant decrease in the number of fungal species was observed. 21 species (17 Deuteromycetes and 4 sterile mycelia) appeared in the control, whereas there were not more than 12 species in any of the treated groups (figure).

Alternaria alternata, A. humicola, Aspergillus flavus, A. niger, Aureobasidium pullulans, Cladosporium cladosporioides, Penicillium oxalicum and *Pestalotiopsis funerea* colonized the untreated (control) leaf discs frequently; however, only *A. flavus, A. niger, Penicillium chrysogenum* and *P. oxalicum* were frequent on the treated leaf discs, thereby showing their good tolerance capacity for the staling growth products of other fungi. *Aspergillus candidus, Cephalosporium roseum, Cladosporium herbarum, Nigrospora sphaerica* and *Phoma hibernica* seemed to be highly susceptible, since they occurred rarely on the treated discs. Overall, staling growth products of *A. candidus, A. chevalieri, A. terreus, C. roseum, Penicillium* spp. and *Trichoderma viride* were highly toxic to the phylloplane fungi colonizing treated leaf discs, whereas *A. alternata, Curvularia lunata, Epicoccum nigrum, P. hibernica* and *P. funerea* were less effective.

P. funerea was found to be intolerant of (most susceptible to) the staling growth products of *A. chevalieri, A. candidus, A. terreus, Fusarium oxysporum, F. chlamydosporum, N. sphaerica, P. oxalicum, P. chrysogenum* and *T. viride,* as they did not allow it to appear on any of the treated discs. *P. funerea* was not very susceptible to the staling growth products of *A. alternata, C. lunata, C. roseum, F. chlamydosporum* and *P. hibernica* as it colonized treated discs to some extent. It was found to be most tolerant to the liquid metabolites of *A. humicola, A. flavus* and *C. herbarum,* in which its percentage occurrence was more than in the control. Likewise, *A. humicola, A. flavus, A. niger, A. pullulans, Papulospora* sp, *P. chrysogenum* and *P. oxalicum* exhibited an increase in percentage occurrence on the treated discs with certain metabolites. Speculation about the reason for this observation is that these fungi possessed a better capacity of tolerance to the staling growth substances as compared with the others. Furthermore, some growth substances present in the metabolites[7] might be a possible