Characterization of ribosomal RNA from insect eggs (*Euscelis plebejus*, Cicadina; *Smittia spec.*, Chironomidae, Diptera)[1]

H. Jäckle[2] and O. Schmidt[3]

Biologisches Institut I (Zoologie) der Universität Freiburg, Albertstrasse 21a, D-78 Freiburg (Federal Republic of Germany), 5 April 1978

Summary. The molecular weights of ribosomal RNAs from eggs of 2 different insect species and of their mitochondrial rRNA were determined. By denaturating conditions, the 28 S rRNA from both insect eggs is converted to 18 S products.

Extraction of ribosomal RNA from insect tissue with hot phenol[4] yields only RNA with a sedimentation coefficient of 18 S[5]. In contrast, extraction at room temperature yields 2 species of rRNA, the 28 S and 18 S components. A dissociation of the 28 S rRNA from diverse insect tissues into 18 S products has been observed earlier[5-9]. We have performed similar studies of thermal and chemical dissociation on rRNA from eggs of 2 different insect species, the leaf hopper *Euscelis plebejus* and a chironomid midge, *Smittia spec.*

Materials and methods. RNA of the eggs from both insect species was labelled with ^{3}H-uridine[10,11]. RNA was extracted as described previously[11] and resuspended in 0.1 M Tris HCl (3% SDS; pH 7.4). RNA samples were incubated for 10 min at different temperatures or with denaturating agents (90% formamide, 90% dimethyl sulfoxide). Mitochondria from *Smittia* eggs were prepared by differential centrifugation[12], and RNA was extracted[11]. RNA extracts were electrophorized on 1.5% agarose gels[13] or 3% polyacrylamide gels[14,15]. Gels were fixed and stained with methylene blue[16]. Radioactivity was measured after cutting the gels into 1.2-mm slices[11].

Results. The apparent mol. weights of rRNA from labelled eggs of *Smittia* and *Euscelis* were determined, using *E. coli* rRNA as mol. wt markers (figure 1). In *Smittia* eggs, about 2% of the total radioactivity was found incorporated into the RNA of the mitochondria. We determined the mol. wt of mitochondrial rRNA as 0.66×10^6 dalton, and 0.31×10^6 dalton, respectively (figure 1), which were calculated as 17 S and 13 S rRNA components.

The influence of temperature and chemical denaturing agents on the 28 S rRNA of eggs was tested. With both species increasing temperature converted the 28 S rRNA into 18 S products (figure 2, a-f). With *Euscelis* eggs, virtually the same results were obtained (figure 2, e, f). By treatment with formamide or DMSO the 28 S rRNA could also be dissociated; but the conversion into 18 S products was not completed within the reaction period (figure 2, d).

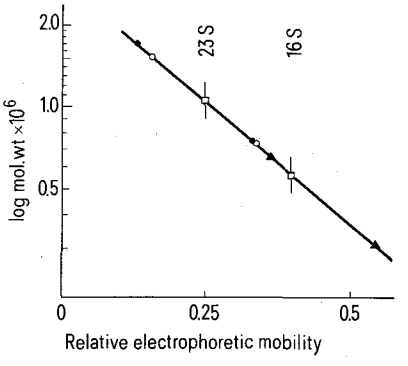

Fig. 1. Molecular weight determination of ribosomal RNA. The apparent mol. wt of 28 S and 18 S rRNA from *Euscelis* (○) and *Smittia* eggs (●), and mitochondrial rRNA (▲) were determined on a 3% polyacrylamide gel according to the method of Loening[17]. As reference, 23 S and 16 S RNA from *E. coli* (Serva) were used.

To ensure that thermal treatment converted the 28 S rRNA exclusively to 18 S products, we took 1 slice from an agarose gel which only contained 28 S rRNA. This slice was heated to 60 °C, and its RNA was analysed on a 2nd agarose gel. The resulting 18 S products (from a selected slice) were heated up to 100 °C for 10 min. Even after this heat treatment, no further dissociation products were found.

In the case that thermal conversion of the 28 S rRNA into the 18 S products were dependent on a conformational change in the secondary structure, it should be possible to renaturate some 28 S rRNA from the 18 S products.

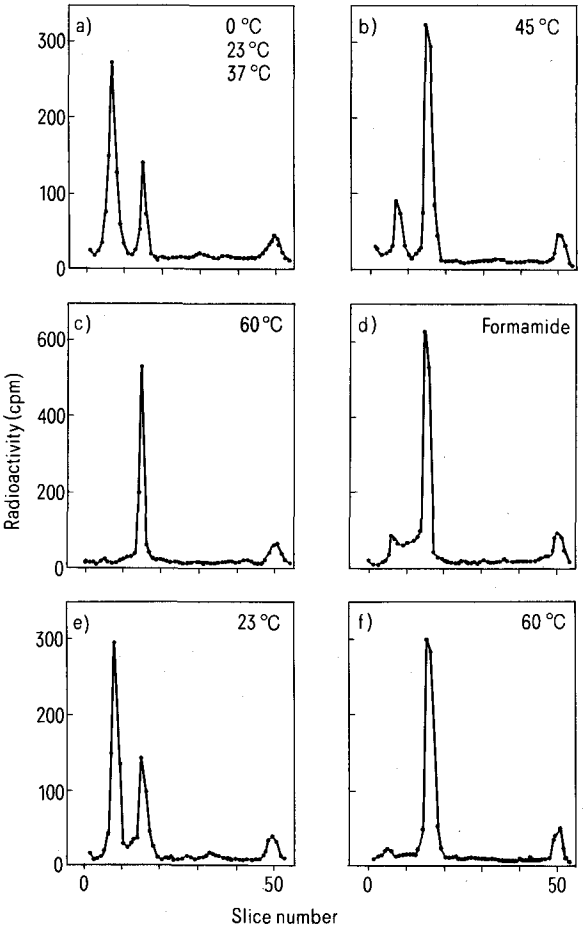

Fig. 2. Electrophoretic analysis of rRNA after denaturating treatments. ^{3}H-uridine labelled RNA from eggs was separated on 3% polyacrylamide gels together with unlabelled RNA of *Smittia* eggs and 10 µg tRNA of *E. coli* (Serva,), respectively. Arrows indicate the positions of the 28 S, 18 S and 4 S RNA. *a-c* RNA from *Smittia* eggs, incubated at different temperatures. *d* RNA from *Smittia* eggs after denaturating treatment with 90% formamide. Identical results were obtained after treatment in 90% DMSO on RNA of *Smittia* and *Euscelis* eggs. *e, f* RNA from *Euscelis*, incubated at different temperatures.

However, after heating the RNA in 1×SC buffer (0.15 M NaCl, 0.015 M Na_3 citrate, pH 7.0) followed by rapid or slow recooling to 4 °C, no renaturated 28 S rRNA could be observed. Therefore, the conversion of the 28 S rRNA seems to be an irreversible dissociation process under the conditions employed in our experiments.

Discussion. For deuterostomians, a correlation between the evolutionary level reached by a species and the mol. wt of its 28 S rRNA has been shown[17]. If this were so in protostomians also, we should expect from the higher evolved dipterans a higher mol. wt of the 28 S rRNA than from the leaf hopper. Furthermore, the values for 28 S rRNA from different dipteran species should be close together. But in contrast to this, the mol. wt of 28 S rRNA from different dipterans differ significantly (1.40×10^6 dalton for *Drosophila*[17], and 1.73×10^6 dalton for *Smittia*), while the mol. wt of 28 S rRNA from leaf hopper (1.50×10^6dalton) and *Drosophila* were found to be similar. We therefore think that the relation between evolutionary stage and the mol. wt of the 28 S rRNA does not hold true for protostomians.

In both insect species under investigation, a comparable similar lability of the 28 S rRNA was observed. Under the conditions employed (thermal treatment, denaturating agents), the 28 S rRNA was converted to 18 S products. The results reported here, and by several other authors[5,18,19], suggest a dissociation of the 28 S rRNA. Its mol. wt seems to be large enough to generate 2 18-S molecules from 1 28-S rRNA molecule. The conclusion from this might be that both, *Smittia* and *Euscelis* 28 S rRNA, consists of 2 rRNA chains of similar mol. wt held together by hydrogen bounds which can be dissociated by denaturating conditions.

Our results are in agreement with the suggestion of Ishikawa[19] that thermal lability of the 28 S rRNA might be common to all protostomians. In contrast, heat treatment of 28 S rRNA from deuterostomians results only in a slight increase of the electrophoretic mobility[19].

1 This study was supported by the Deutsche Forschungsgemeinschaft (SFB 46). We wish to thank Prof. K. Sander and Prof. K. Kalthoff for critical discussion, Dr C. Nüsslein-Volhard and Dr. O. Vogel for critically reading, and Mrs. M. Scherer for typing this manuscript.
2 Present address and reprint requests: Department of Zoology, University of Texas, Austin, Texas 78712, USA.
3 Present address: Yale University, New Haven, Connecticut 06520, USA.
4 K. Scherrer and J.E. Darnell, Biochem. biophys. Res. Commun. *7*, 486 (1962).
5 S.W. Applebaum, R.P. Ebstein and G.R. Wyatt, J. molec. Biol. *21*, 29 (1966).
6 N. Balazs and N. Agosin, Comp. Biochem. Physiol. *27*, 227 (1968).
7 J.R. Greenberg, J. molec. Biol. *46*, 85 (1969).
8 H. Ishikawa and R.W. Newburg, Biochem. biophys. Res. Commun. *40*, 654 (1970).
9 H. Ishikawa and R.W. Newburg, Biochim. biophys. Acta *232*, 661 (1971).
10 H. Jäckle and K. Kalthoff, Photochem. Photobiol., in press (1978).
11 O. Schmidt and H. Jäckle, Wilh. Roux Arch. devl. Biol., in press (1978).
12 H. Winter, Wilh. Roux Arch. devl. Biol. *175*, 103 (1974).
13 J.-E. Edström and R. Tanguay, J. molec. Biol. *84*, 569 (1974).
14 D.H.L. Bishop, J.R. Claybrook and S. Spiegelman, J. molec. Biol. *26*, 373 (1967).
15 F.W. Studier, J. molec. Biol. *79*, 237 (1973).
16 A.C. Peacock and C.W. Dingman, Biochemistry *6*, 1818 (1967).
17 U.E. Loening, J. molec. Biol. *38*, 355 (1968).
18 J. Shine and L. Dalgarno, J. molec. Biol. *75*, 57 (1973).
19 H. Ishikawa, Comp. Biochem. Physiol. *56B*, 229 (1977).

Biosynthèse in vitro d'octopamine par le système nerveux et le cœur du mollusque gastéropode *Helix pomatia*
In vitro biosynthesis of octopamine by the nervous system and the heart of the mollusc gastropode *Helix pomatia*

J. Cardot

Laboratoire de Physiologie animale, Faculté des Sciences et des Techniques, Place Leclerc, F-25030 Besançon Cédex (France), 22 mars 1978

Summary. The tyrosine is decarboxylated in vitro by the central nervous tissue and by the intracardiac nervous tissue of *Helix pomatia* by aromatic amino-acid decarboxylase. The tyramine obtained is then partially transformed into octopamine by tyramine-β-hydroxylase. The inhibition of monoamine oxidase favours apparition of the 2 amines. The monoamine oxidase seems able to regulate their synthese and to assure their inactivation.

L'octopamine (p-hydroxyphényléthanolamine) a été identifiée pour la première fois dans le produit se sécrétion des glandes salivaires postérieures des céphalopodes octopodes, d'où son nom[1,2]. Chez les mammifères elle est présente dans le système nerveux central et dans le système orthosympathique. Ses rôles physiologiques possibles ont été récemment passés en revue[3]. Chez les invertébrés de nombreux arguments permettent d'envisager avec fermeté ses fonctions de neurotransmetteur[3,4]. Chez *Helix* elle existe dans le système nerveux et dans le cœur[5,6]. Notre étude a pour but de démontrer, chez *Helix pomatia,* la formation de tyramine par le système nerveux et son hydroxylation conduisant à l'octopamine grâce à la dopamine-β-hydroxylase[7].

Méthodes. Première série d'essais. Des homogénats de tissu nerveux (colliers œsophagiens et nerfs) et de cœurs correspondant à 500 mg d'organes sont incubés pendant 3 h, à 27 °C, en tampon phosphate pH = 7,2, en présence de 0,1 µmole de DL-3-^{14}C-tyrosine d'activité spécifique = 50 mCi/mM (volume final: 5 ml), selon les modalités suivantes:

Tissu nerveux + tyrosine + PLP (50 µg).
Tissu nerveux + tyrosine + parnate (4×10^{-3} M) + PLP.
Tissu nerveux + tyrosine + parnate + DL-α-méthylDOPA ($2{,}5\times10^{-3}$ M) + PLP.
Tissu nerveux + tyrosine + parnate + RO 4-4602 ($2{,}5\times10^{-3}$M) + PLP.
Tissu nerveux + tyrosine + parnate + FLA-63 (2×10^{-3} M) + PLP.
Cœurs + tyrosine + parnate + PLP.

Le PLP (phosphate de pyridoxal) est la coenzyme de la décarboxylase; il est ajouté par routine aux milieux réactionnels. L'α-méthylDOPA et le RO 4-4602 sont des inhibiteurs de la décarboxylase. Le parnate (tranylcypromine) est un IMAO (inhibiteur de la monoamine-oxydase ou MAO) et le FLA-63 est un inhibiteur de la dopamine-β-hydroxylase.

Après incubation et addition d'entraîneurs froids les amines