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BRIEF COMMUNICATION

tRNA Synthesis in Germinating Pollen

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Abstract. The synthesis of tRNA was demonstrated in pollen of *Nicotiana tabacum* L. according to the incorporation of labeled uracil, adenosine and guanosine during 4 h of germination. tRNA was extracted from postribosomal supernatant and purified by polyacrylamide gel electrophoresis. The incorporation of guanosine was about 1.68 times higher than that of adenosine. This finding indicates that the whole tRNA chain is formed in pollen tubes.

It is generally accepted that rRNA and tRNA necessary for the growth of pollen tubes are synthesized during pollen grain formation and that during pollen germination no synthesis of these RNA species takes place (see Mascarenhas 1975). Using submersed cultivation and incubation of several hours with labeled uridine it has lately been possible to demonstrate some activity of ribosomal genes in tobacco pollen tubes (Tupý *et al.* 1977a). The synthesis of tRNA has been studied in detail in the pollen of *Tradescantia* by Mascarenhas and Goralnick (1971). On the basis of analysis of small molecular weight RNA synthesized during 90 min germination they have concluded that tRNA in pollen tubes is probably not synthesized. However, the situation in germinating lilium pollen is less clear. In contrast to *Tradescantia* the radioactivity has been demonstrated in 4S RNA after the incubation of pollen with $^{32}PO_4^{3-}$, cytidine-^{3}H or uridine-^{3}H. Short time splitting with snake venom phosphodiesterase has shown that about half of the radioactivity of 4S RNA is incorporated in the end group —CpCpA. Whether the remaining radioactivity belonged to the main chain of tRNA molecules or to the contaminating small molecular weight RNA remained an open question (Steffensen 1971).

The pollen of *Nicotiana tabacum* L. has been collected and cultivated under aseptic conditions (Tupý *et al.* 1977b); 50 mg of pollen germinated 4 h in 10 ml of cultivation solution (0.3M sucrose in 0.01% H_3BO_3) in the presence of 1 μCi ml^{-1} of uracil-2-^{14}C (44.8 mCi mmol^{-1}), adenosine-U-^{14}C (320 mCi mmol^{-1}) or guanosine-U-^{14}C (320 mCi mmol^{-1}). Ribosome sedimentation and extraction were the same as described (Tupý *et al.* 1977a). tRNA was

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separated electrophoretically on 7.5% polyacrylamide gels (LOENING 1967). After registration of absorbancy at 254 nm the gels were cut (MATSUMURA and NODA 1973) into 1.5 mm slices. The slices were incubated in 0.8 ml of 0.3M NH_4OH at 60 °C overnight and 5 ml of a mixture of 0.7% PPO in toluen with Triton X-100 was added for scintillation counting in a liquid scintillation spectrometer.

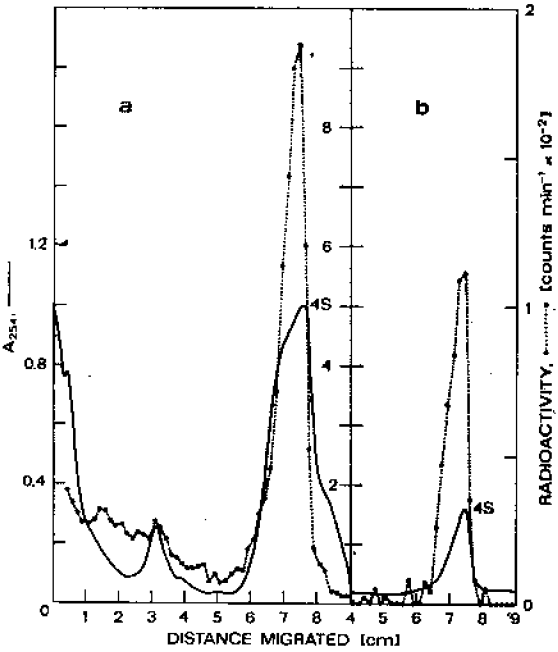

Fig. 1. Electrophoresis of RNA from the postribosomal supernatant obtained from pollen tubes grown in the presence of uracil-2-^{14}C (a). The peak of 4S RNA was eluted with the electrophoretic buffer with SDS and a portion of the extract was fractionated once more on the polyacrylamide gel (b).

In order to minimize the possibility of contamination with small molecular weight mRNA which has been presumed in experiments with lilium pollen by STEFFENSEN (1971), tRNA was extracted from postribosomal supernatant obtained after 1 h centrifugation of postmitochondrial fraction at 60 000 rpm at 4 °C (TUPÝ *et al.* 1977a). The fractionation on polyacrylamide gels has shown that after pollen incubation with uracil-2-^{14}C almost all radioactivity of the extract coincided with the absorption peak of 4S RNA (Fig. 1).

To decide if *de novo* synthesis of the whole tRNA chain or only labeling of the —CCA end group occurs the incorporation of ^{14}C-labeled adenosine and guanosine was compared in a further experiment. We supposed that in the case of a simple change of terminal triplet the incorporation of guanosine would not occur or would be markedly lower than that of adenosine. The electrophoretic fractionation of RNA extracted from postribosomal supernatant showed a 1.68 times higher incorporation of guanosine into 4S RNA as compared with adenosine (Fig. 2). This ratio is similar to the

1 μCi = 37 kBq.

proportion of guanosine and adenosine in the whole cytoplasmic tRNA (*e.g.* Frederiksen *et al.* 1971, Chia *et al.* 1976). This finding can be held as evidence of the activity of tRNA genes in the pollen tubes of tobacco.

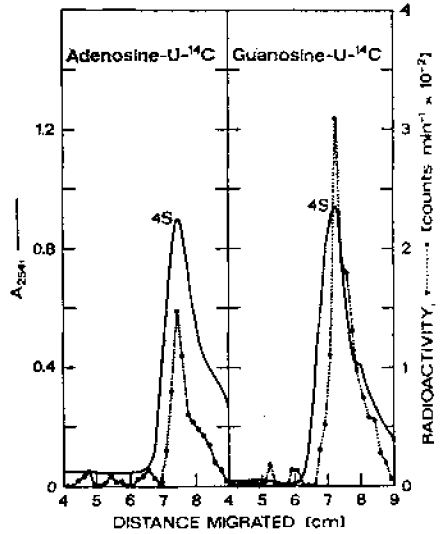

Fig. 2. The comparison of the incorporation of adenosine-U-^{14}C and of guanosine-U-^{14}C into 4S RNA of pollen tubes. Specific radioactivity of 4S RNA synthesized in the presence of guanosine-U-^{14}C is 1.68 times greater than that of 4S RNA formed in the presence of adenosine-U-^{14}C.

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