BIOLOGIA PLANTARUM (PRAHA)

19 (4) : 300–308, 1977

RNA Synthesis and Polysome Formation in Pollen Tubes

J. TUPÝ

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

Abstract. The formation of polysomes in relation to RNA synthesis was investigated in tobacco (*Nicotiana tabacum* L.) pollen cultivated submersely for a period of 12 h. The percentage of polysomes was estimated by determining the number of ribosomes carrying nascent polypeptides using RNase and 0.8 M KCl treatment of the ribosomal preparation. This approach showed that the proportion of ribosomes "active" in protein synthesis amounts to about 12 % in dry pollen rising to 46 % within 10 min of imbibition and to 66 % during the period 1—4 h of cultivation. The latter increase is accompanied by a rapid incorporation of uridine-^{14}C into polysome-associated RNA and is sensitive to actinomycin D.

The rapidly labelled RNA isolated from the ribosomal preparation sedimented in the range from about 5S to 30S. Longer labelling periods led to a gradual shift of the major peak of this heterogeneous RNA from about 11.5S and 14S to about 7.5S and the development of radioactivity peaks in the position of 18S and 28S RNA. Uridine incorporated both into the active ribosomes and into the subunits of inactive ribosomes at a more or less constant rate during the whole 12-h period of cultivation.

These results present evidence that in addition to the initial combination of the existing ribosomes with the stored mRNA following imbibition, an activation of pollen tube genome and its implication in directing protein synthesis take place during the early phases of pollen tube growth. From the results on kinetics of labelling of ribosomes it appears that in pollen tubes new synthesized ribosomal subunits enter polysomes directly, the entry of 40S subunits being more rapid than that of 60S subunits.

Mature pollen grains like seeds and other metabolically repressed quiescent cells undergo a rapid increase in the rate of protein synthesis under the conditions enabling the onset of their further development. In *Petunia*, polysomes could not be detected in dry pollen and the ribosomal preparation isolated from it failed to direct amino acid incorporation in a cell free system lacking mRNA. During several min of water imbibition pollen monosomes associated into polysomes and coincidently the protein-synthesizing activity of the ribosomal preparation rose rapidly (LINSKENS *et al.* 1970). These observations led the authors to the conclusion that inactive pollen grain is equipped with a store of stable long-lived mRNA species. In *Tradescantia* an important fraction of ribosomes is reported to be prepackaged with mRNA already in dry pollen grain. The proportion of ribosomes present as polysomes rises rapidly during the first 10 min of imbibition, which occurs also in the presence of actinomycin D (MASCARENHAS and BELL 1969).

Received March ?, 1977

* *Address:* Flemingovo nám. 2. 160 00 Praha 6, Czechoslovakia.

The stored mRNA appears to play a greater or lesser part (in dependence on plant species) at least in the rapid initiation of pollen germination and early pollen tube growth (see MASCARENHAS 1975). There are however no indications yet as to the activation of pollen tube genome in terms of its function in directing protein synthesis in place of stored mRNA during the later phases of the development of the male gametophyte. In *Tradescantia* the proportion of ribosomes present as polysomes after 10 min of imbibition did not increase during the further 10 min of incubation (MASCARENHAS and BELL 1969) and the proteins synthesized in pollen tubes were not qualitatively modified in the presence of actinomycin D (MASCARENHAS *et al.* 1974). In *Petunia* the number of polysomes reached its maximum after 60 min of germination and decreased later on (LINSKENS *et al.* 1970). The RNA and protein synthesis inhibitor studies in *Nicotiana alata* suggested the independence of protein synthesis on RNA synthesis and the relative stability of the complex of mRNA with ribosomes in pollen tubes (TUPÝ 1966).

It is reported that rRNA genes are inactive in pollen tubes grown *in vitro* (see MASCARENHAS 1975). In difference to this, the evidence was recently presented that *Nicotiana tabacum* pollen tubes in suspension culture do synthesize rRNA (TUPÝ *et al.* 1977a). This finding suggested further investigation concerning mRNA synthesis in this type of culture and its participation in polysomal formation in the course of pollen germination and pollen tube growth.

Material and Methods

Pollen of *Nicotiana tabacum* L. was cultivated aseptically in 0.3 M sucrose with 0.01 % boric acid under continuous shaking (TUPÝ *et al.* 1977b).

Ribosomes and RNA were isolated by a procedure described previously (TUPÝ *et al.* 1977a).

To determine the proportion of polysomes, the ribosomal preparation was treated with RNase and high salt concentration and fractionated by centrifugation on a sucrose gradient (MARTIN 1973). This treatment enables one to distinguish the complex ribosomes carrying mRNA and peptidyl-tRNA from monoribosomes which, in contrast to the former, are dissociated into two ribosomal subunits. This method permits to estimate even a small amount of polysomes undetectable by direct centrifugation of ribosomal preparations on sucrose gradient and it is not affected by polysome degradation which may occur, to a certain extent, during the process of isolation. The mild treatment with ribonuclease used in this method (5×10^{-4} % pancreatic RNase for 2 min at 0 °C) is sufficient for degradation of polysomes to monosomes in preparations from pollen tubes (Fig. 1) and also from ungerminated pollen. Only a minute proportion of ribosomes remains associated in dimers which are taken together with the peak of 80S particles in calculating the percentage of active ribosomes.

In experiments on RNA synthesis, pollen was cultivated in the presence of uridine-2-^{14}C (0.5 μCi ml^{-1}, s.a. 0.2 mCi mg^{-1}).

The sedimentation analyses on sucrose density gradients were carried out using a Beckman SW 27.1 rotor and the radioactivity of samples from sucrose gradients was determined by liquid scintillation spectrometry.

1 μCi = 37 kBq.

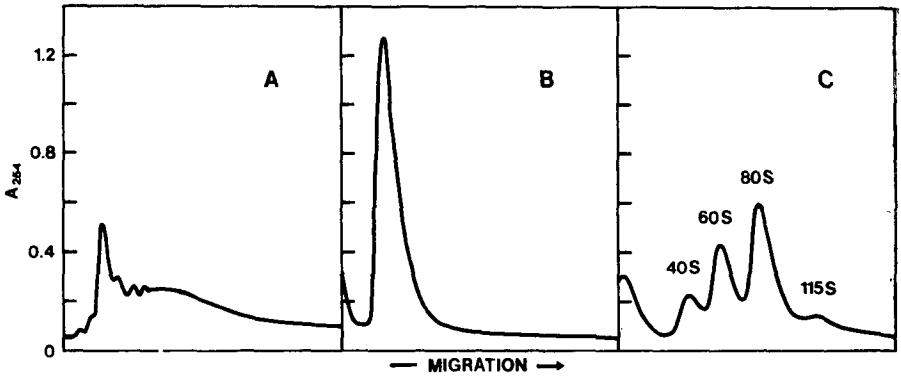

Fig. 1. Sucrose gradient sedimentation analysis of polysomes from 25 mg of pollen after 4 h of cultivation. The suspension of polysomes was analysed directly (A), after treatment with RNase (B) and after treatment with RNase and 0.8 M KCl (C). For further details see Figs. 2 and 4.

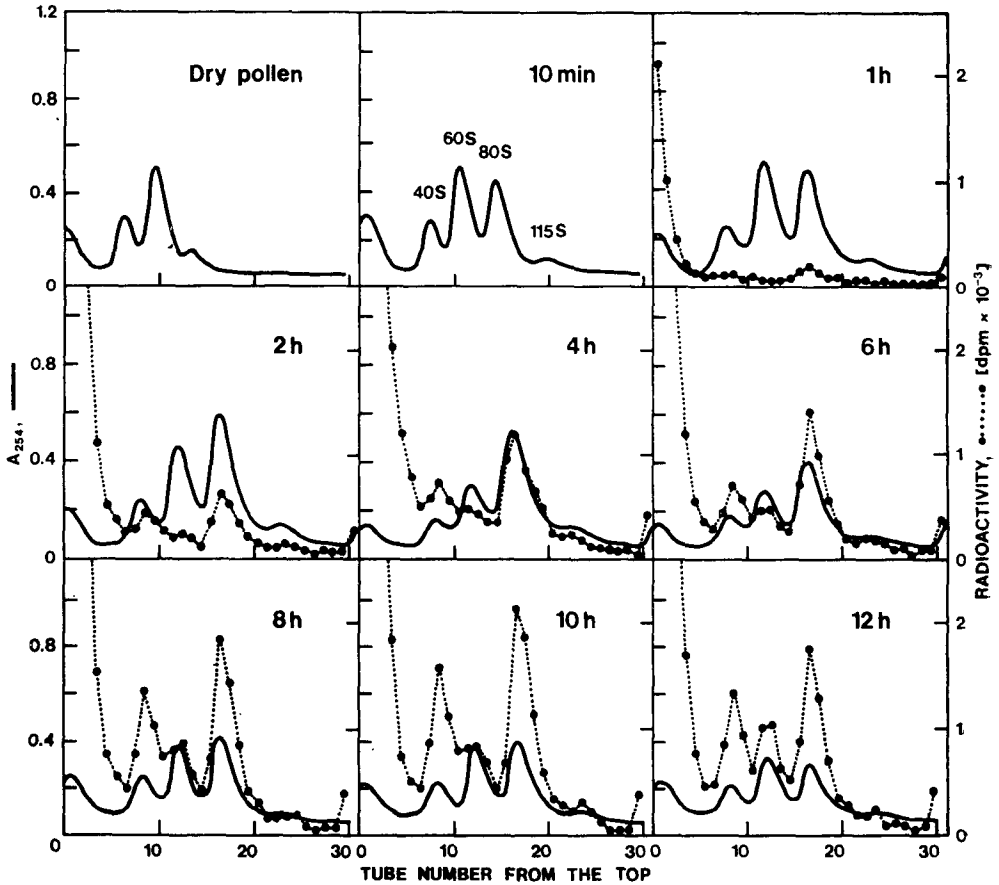

Fig. 2. Profiles of ribosome distribution on 5—20 % linear sucrose gradients in 50 mM Tris-HCl; 15 mM Mg acetate; 800 mM KCl after a 5-h centrifugation at 27 000 rpm, 5 °C. Polysomes were isolated from 25 mg of pollen at different time intervals of cultivation in the presence of uridine-^{14}C. To the cold suspension of polysomes in 0.4 ml of 50 mM Tris-HCl; 15 mM Mg acetate;

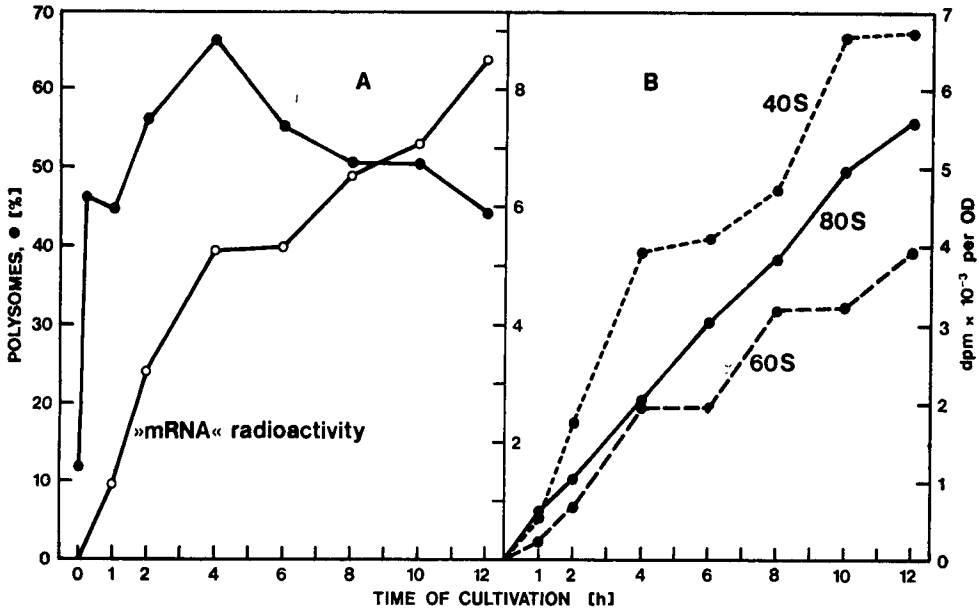

Fig. 3. Time course of polysome formation (A) and uridine-^{14}C incorporation into ribosomal particles (B) during 12 h of pollen cultivation. Evaluation of data from Fig. 2. Part A further includes radioactivity curve for the low-molecular-weight material sedimenting in the region between the top and 40S subunits. As this radioactivity is liberated at least for a greater part from polysomes by RNase treatment, it is called "mRNA" radioactivity. It is expressed as specific radioactivity per 1 OD of ribosomes of the corresponding samples.

Results

The ribosomal preparations isolated from pollen and from pollen tubes at different time of cultivation were treated by RNase and 0.8 M KCl and analysed by sucrose density gradient centrifugation (Fig. 2). The synthetically "active" ribosomes carrying nascent polypeptides sedimented as 80S particles while the ribosomes non-participating in protein synthesis dissociated into 40S and 60S subunits. This approach showed that dry pollen had a significant proportion of active ribosomes amounting to about 12 % of the total ribosomal population. During a 10-min water imbibition the active fraction rose to 46 %; however, no further increase could be observed at 1 h of incubation (Fig. 3A). Later on, the proportion of KCl-resistant ribosomes registered again an important rise and reached 66 % at 4 h, decreasing steadily during the subsequent 8 h of cultivation. Similar time course of the monoribosome to polyribosome transition was found with different pollen batches and also using the direct polysome analysis (see also Fig. 4). Only the total level of polysomes throughout the whole period of cultivation varied somewhat in dependence on the quality of pollen. The decrease in the total amount of isolated ribosomes with longer cultivations seen on Fig. 2 is the

45 mM KCl; pH 7.6, buffer, 10 µl of 0.02 % pancreatic RNase was added and after a 2-min incubation at 0 °C the concentration of KCl was raised to 0.8 M by addition of 2.5 M KCl.

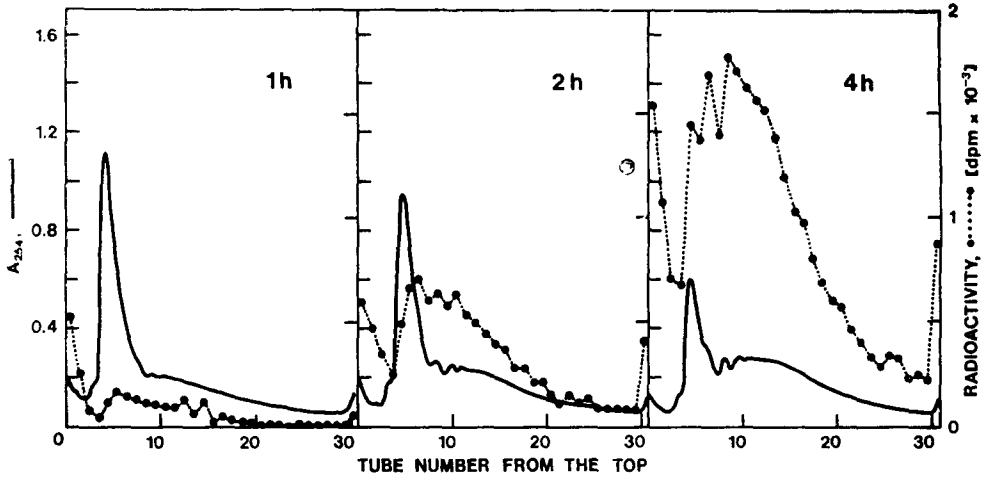

Fig. 4. Incorporation of uridine-^{14}C into polysomes at different time of pollen cultivation. Centrifugation on 18—36 % sucrose in 50 mM Tris-HCl; 20 mM KCl; 5 mM Mg acetate; 5 mM mercaptoethanol; 5 μg ml^{-1} cycloheximide; pH 8.5, buffer for 2 h at 27 000 rpm, 5 °C.

result of a progressing leaching out of rRNA from pollen tubes into the medium (TUPÝ *et al.* 1974).

The results on Figs. 2 and 3 further demonstrate uridine-^{14}C incorporation into the ribosomal particles thus suggesting ribosome formation during pollen tube growth. Some incorporation could be detected already after 1 h especially in the active ribosomes. The specific labelling of all particles is seen to rise more or less continuously throughout the whole 12-h period of cultivation without any apparent depression with the aging of the culture (Fig. 3B). The same is true for the "mRNA" radioactivity liberated from polysomes by RNase treatment (Fig. 3A) and appearing before the 40S subunits on the density gradient profiles (Fig. 2). As to some deviations from the linearity in the course of uridine-^{14}C incorporation, the depression between the 4th and 6th h occurring coincidently in "mRNA" and both subunits may be of some significance. In the same period the number of polysomes shows an important decrease. At all time intervals the specific radioactivity of 40S subunits is seen to be markedly higher than that of 60S subunits, the specific labelling of 80S particles being roughly the average of those of the subunits, except at 1 h of cultivation (Fig. 3B).

The unchanged proportion of active ribosomes at 1 h with respect to a 10-min incubation and the subsequent increase in their relative number suggested that this second delayed rise of polysome formation may be related to pollen tube mRNA synthesis. To test this the synthesis of polysome-bound RNA was examined. The polysomal preparations from pollen incubated in the presence of uridine-^{14}C were directly fractionated by sucrose density centrifugation (Fig. 4). The absorbance profiles obtained confirm that additional polysomes are formed after 1 and 2 h of cultivation. Correspondingly, it is shown that while the polysome-associated radioactivity is very low at 1 h, it increases dramatically later on. The analysis of RNA isolated from polysomal preparations revealed that the greater part of

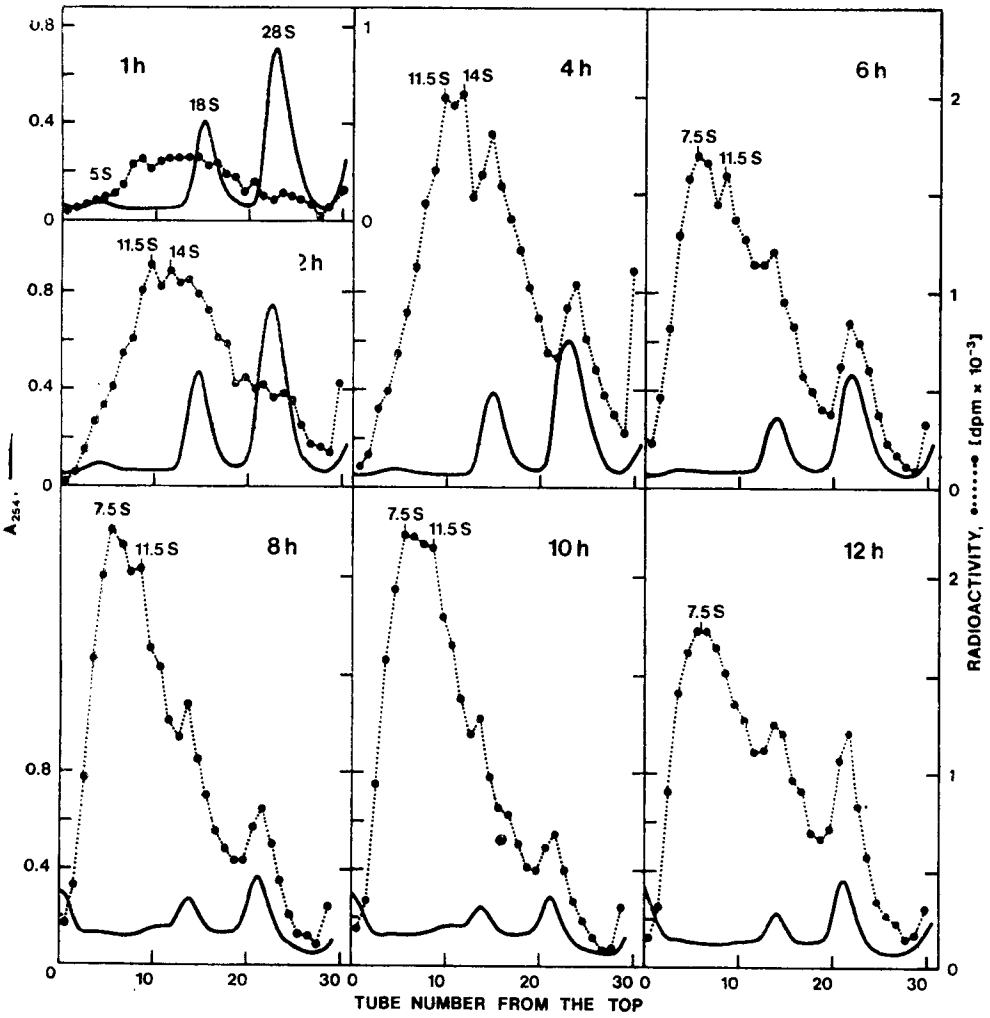

Fig. 5. Sucrose density gradient centrifugation of RNA isolated from polysomal preparations obtained from 25 mg pollen cultivated for different periods in the presence of uridine-^{14}C. RNA was dissolved in 0.5 ml of Tris-HCl, pH 7.4, 10 mM; NaCl, 100 mM; SDS, 0.1 %, layered on a linear gradient of 5 to 20 % sucrose in the same buffer and centrifuged for 16 h at 23 000 rpm, 20 °C.

labelled RNA consists of a heterogeneous population of RNA molecules with sedimentation values ranging from about 5S to 30S (Fig. 5). Longer labelling periods lead to the appearance of radioactivity peaks in the position of 18S and 28S RNA and to a gradual shift of the major peak of the polydisperse RNA from 11.5S and 14S to about 7.5S. The progressive decrease in the total amount of isolated RNA is again due to the increasing release of RNA from pollen tubes into the culture medium (Tupý *et al.* 1974).

The synthesis of RNA in *Nicotiana* pollen tubes has been shown to be fully inhibited by actinomycin D at 30 μg ml^{-1} concentration (Süss and Tupý 1976, Tupý *et al.* 1977a). If the newly synthesized mRNA is implied in

TABLE 1
Effect of actinomycin D on polysome formation during 10 min and 4 h of pollen cultivation

	% active ribosomes 10 min	4 h	Difference of % at 10 min and 4 h	% inhibition
Control	36	62	26	—
Actinomycin D, 30 μg ml^{-1}	36	52	16	38

polysome formation, the monosome association should be decreased by actinomycin D. The corresponding experiment showed that while during a 10-min imbibition the proportion of the active ribosomes was unaffected, the formation of additional KCl-resistant ribosomes was inhibited by about 38 % in the presence of actinomycin D (Table 1).

Discussion

Like in other plant and animal tissues (MARTIN 1973, SPARKUHL *et al.* 1976), the determination of the fraction of ribosomes active in protein synthesis by the KCl dissociation method gave in pollen results consistent with those obtained by direct polysome fractionation. The finding of rapid transition of monosomes to polysomes upon exposure of mature pollen grain to nutrient solution confirms the observations made earlier on *Tradescantia* (MASCARENHAS and BELL 1969) and *Petunia* pollen (LINKENS *et al.* 1970). In analogy to *Tradescantia*, a portion of ribosomes in dry tobacco pollen appears to be combined with mRNA. Moreover, these ribosomes are shown to bear nascent proteins which would suggest a slight protein synthesis in quiescent pollen. This seems not impossible as, though pollen grain represents a system repressed developmentally and metabolically, it exhibits a slight metabolic activity which may depend on several environmental factors such as moisture level, temperature and gas composition of the atmosphere (see STANLEY and LINSKENS 1974). Although protein synthesis is initiated very rapidly at the beginning of imbibition (MASCARENHAS and BELL 1969), it seems unlikely that some ribosomes are activated during the process of homogenization as this was carried out at 0 °C for only about 2 min.

The initial polysome formation resulting from combination of preexisting ribosomes with stored mRNA seems to be completed within 10 min of imbibition, as no further increase in active ribosomes was observed at 1 h of incubation. In contrast to pollen species so far investigated (see MASCARENHAS 1975), it was possible to demonstrate that in tobacco pollen an additional rise in the number of polysomes does occur during the subsequent cultivation. The coincident rapid synthesis of polysome-bound RNA and the sensitivity of ribosome activation during the period 1—4 h to actinomycin D indicate that the formation of polysomes in pollen tubes is dependent on new mRNA synthesis.

The direct sedimentation analysis of ribosomal preparations isolated from pollen tubes labelled with uridine-^{14}C showed that most of the radioactive RNA was associated with the polysomal region, indicating only low con-

tamination of the preparations with RNA from other cell fractions. The results obtained using sucrose density gradient centrifugation revealed that the sedimentation pattern of the heterogeneous population of rapidly labelled polysomal RNA exhibits some changes in the course of 12-h pollen cultivation. It suggests that modifications in the production of new mRNA species may occur as pollen tube growth proceeds, but a possibility of some relation of the observed changes with processing of a precursor RNA cannot be excluded. Recent investigations on seeds brought also evidence that though the dry seeds are equipped with store mRNA, new mRNA synthesis does occur at the earliest time after the onset of germination (SPIEGEL *et al.* 1975).

The results on labelling of polysomal RNA and of ribosomal particles present further evidence of rRNA synthesis in tobacco pollen tubes (TUPÝ *et al.* 1977a). According to the entry of the label from uridine-^{14}C into both active and inactive ribosomes, it appears that the rate of rRNA synthesis is essentially constant throughout 12 h of cultivation.

An interesting result of the labelling experiments are the differences seen in specific activity between 40S, 60S and 80S particles throughout the 12-h period of cultivation. With short labelling times most of the label appears in the polysome-derived ribosomes. As labelling time increases the specific radioactivity of KCl-resistant ribosomes equilibrates approximately with that of the subunit pool of inactive monosomes. This suggests that newly synthesized ribosomal subunits enter polysomes directly and do not form monosomes until they have cycled through the polysomes. In cells of mice ascites tumour (HOGAN and KORNER 1968) and in yeast (PETERSEN *et al.* 1976) newly synthesized subunits were also found in polysomes before they were found in 80S monomers. The observed differences in labelling of the subunits of inactive ribosomes suggest that, similarly as demonstrated in other eucaryotic cells (*e.g.* PETERSEN *et al.* 1976), 40S subunits enter polysomes in tobacco pollen tubes more rapidly than 60S subunits. Alternatively, it can result from different rate in subunit formation which is suggested by a more rapid synthesis of 18S RNA with respect to 28S RNA, observed in higher plant cells (LEAVER and KEY 1970).

The results as a whole show that in tobacco the process of early pollen tube development involves the derepression of pollen genome and the implication of the products of its transcription in directing protein synthesis.

References

HOGAN, B. L. M., KORNER, A.: The role of ribosomal subunits and 80-S monomers in polysome formation in an ascites tumour cell. — Biochim. biophys. Acta **169** : 139—149, 1968.

LEAVER, C. J., KEY, J. L.: Ribosomal synthesis in plants. — J. mol. Biol. **49** : 671—680, 1970.

LINSKENS, H. F., SCHRAUWEN, J. A. M., KONINGS, R. N. H.: Cell-free protein synthesis with polysomes from germinating *Petunia* pollen grains. — Planta **90** : 153—162, 1970.

MARTIN, T. E.: A simple general method to determine the proportion of active ribosomes in eucaryotic cells. — Exp. Cell Res. **80** : 496—498, 1973.

MASCARENHAS, J. P.: The biochemistry of angiosperm pollen development. — Bot. Rev. **41** : 259 to 314, 1975.

MASCARENHAS, J. P., BELL, E.: Protein synthesis during germination of pollen: studies on polyribosome formation. — Biochim. biophys. Acta **179** : 199—203, 1969.

MASCARENHAS, J. P., TERENNA, B., MASCARENHAS, A. F., RUECKERT, L.: Protein synthesis during germination and pollen tube growth in *Tradescantia*. — In: LINSKENS, H. F. (ed.): Fertilization in Higher Plants. Pp. 137—143. North-Holland Publ. Co., Amsterdam 1974.

PETERSEN, N., MCLAUGHLIN, C. S., NIERLICH, D. P.: Ribosomal subunit entry into polysomes in yeast. — Biochim. biophys. Acta **447** : 294—303, 1976.

SPARKUHL, J., GARE, R. L., SETTERFIELD, G.: Metabolism of free and membrane-bound ribosomes during aging of Jerusalem artichoke tuber slices. — Planta **129** : 97—104, 1976.

SPIEGEL, S., OBENDORF, R. L., MARCUS, A.: Transcription of ribosomal and messenger RNAs in early wheat embryo germination. — Plant Physiol. **56** : 502—507, 1975.

STANLEY, R. G., LINSKENS, H. F.: Pollen. Biology, Biochemistry, Management. — Springer-Verlag, Berlin—Heidelberg—New York 1974.

SÜSS, J., TUPÝ, J.: On the nature of RNA synthesized in pollen tubes of *Nicotiana alata*. — Biol. Plant. **18** : 140—146, 1976.

TUPÝ, J.: Synthesis of protein and RNA in pollen tubes stimulated with 2-thiouracil. — Biol. Plant. **8** : 398—410, 1966.

TUPÝ, J., HRABĚTOVÁ, E., BALATKOVÁ, V.: The release of RNA from pollen tubes. — In: LINSKENS, H. F. (ed.): Fertilization in Higher Plants. Pp. 145—160. North-Holland Publ. Co., Amsterdam 1974.

TUPÝ, J., HRABĚTOVÁ, E., BALATKOVÁ, V.: Evidence for ribosomal RNA synthesis in pollen tubes in culture. — Biol. Plant. **19** : 226—230, 1977a.

TUPÝ, J., HRABĚTOVÁ, E., BALATKOVÁ V.: A simple rapid method of determining pollen tube growth in mass culture. — Plant Sci. Lett. **9** : in press, 1977b.

BOOK REVIEWS

MACLEAN, N.: **Control of Gene Expression.** — Academic Press, London—New York—San Francisco 1976, 348 pp., 7.80 £.

The purpose of the book is to provide a brief review of general concepts of the control of gene expression and to describe in greater detail the particular results obtained on this comprehensive problem in a number of diverse eukaryotic systems.

The general chapters describe possible control mechanisms operating on a transcriptional and post-transcriptional level, which is followed by a brief account of our knowledge of gene expression in prokaryotes. The attention is also paid to RNA involvement in gene expression and the concluding chapter discusses the more important concepts of eukaryotic gene regulation.

The greater part of the book is devoted to description and discussion of a number of experimental systems currently used in the study of differential gene function in eukaryotes. According to the complexity they are classified into "systems involving one type of protein", "systems of limited complexity" and "systems not well understood in molecular terms".

Although the book has been written above all for university students and teachers, it will be, no doubt, welcomed by research workers interested in molecular biology as a handy source of information about the results obtained in this field with different biological material.

J. TUPÝ (*Praha*)

VAN DEN ENDE, H.: **Sexual Interactions in Plants.** — Academic Press, London—New York—San Francisco 1976, 186 pp., 6.80 £.

The book appeared as the 9th volume of An International Series of Monographs in Experimental Botany under consulting editorship of J. F. Sutcliffe and P. Mahlberg. It is concerned mainly with biochemistry of cell interactions related to sexual reproduction in lower plants. In ten chapters examples are described in detail, including experimental procedures that were used, the mechanism of sexual differentiation and exchange of information between the mating partners in some genera of fungi, algae and ferns. The last chapter dealing with flowering plants briefly reviews the complex topic of possible interactions between pollen and the tissues of gynaeceum. This volume will be especially useful to those being interested in sexual reproduction in lower plants and in general problem of biochemistry of interaction between plant cells.

J. TUPÝ (*Praha*)