

## Ribonucleic acids and ribosomal proteins synthesis during germination of unripe and aged wheat caryopses

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### Abstract

Caryopses collected at milk, wax and full ripeness as well as aged ones stored at air humidity of 30, 60 and 80 per cent were subjected to germination. The more unripe were the caryopses the lower was their germination capacity and dry mass increment of the sprouts, the same was true for fully ripe but aged caryopses. After 48 h of germination in the presence of radioactive precursors the embryos were removed from the caryopses and used for isolation of the total ribosomal fraction and RNA. Distinct synthesis of three basic total RNA fractions and of the polyribosomal fraction was observed in all samples and in embryos from grain which had completely lost its germination capacity. High radioactivity was recorded in preparations of total RNA and polyribosomal ones from 48-h sprouts growing from caryopses collected at milk ripeness and in those of aged caryopses stored under air humidity of 30 and 60 per cent. According to the authors, the depressed amount of latent forms of RNA (preformed mRNA, tRNA and ribosomes) is the cause of intensive  $^3\text{H}$ -uridine incorporation into RNA in embryos from unripe and aged caryopses. For germination of grains with a reduced amount of latent forms the biosynthesis mechanisms must be based to a much higher extent on newly synthesised molecules of ribonucleic acids.

### INTRODUCTION

There exist many theories of ageing. None of them, however, gives a comprehensive explanation of this phenomenon. According to Orgel (1963, 1973) who advanced a hypothesis called "the catastrophe of errors", these errors occurring at all stages of protein synthesis are the cause of formation of changed protein molecules.

The problem of germination of seeds of various ripeness has long been an object of interest of agriculturists. This is due to the necessity

of collecting seeds from plants of unequal ripeness on the same field, or from plants on which seeds do not ripen uniformly in the inflorescence. Thus, unripe seeds are present in nearly all grain batches and the physiological-biochemical properties of not completely ripe seed are an important problem.

A knowledge of the germination mechanisms in unripe and aged caryopses has many practical aspects. In both these cases germination has a different course, deviating markedly from the normal one. In the present investigations particular attention was devoted to two essential elements in protein biosynthesis: the synthesis of total RNA and synthesis and formation of polyribosomes.

#### MATERIAL AND METHODS

Winter wheat of the Grana variety was cultivated on experimental plots of the Institute of Plant Biology of the Agricultural-Technical Academy in Olsztyn in 1980. Sampling was done three times:

- at the time of milk ripeness with 71 per cent of water in the caryopses (sample A),
- at the time of wax ripeness with 41 per cent of water in the caryopses (sample B),
- at full morphological ripeness with 19 per cent of water in the caryopses (sample C — control).

The wheat caryopses harvested at full ripeness in 1976 were subjected to accelerated ageing. After mild drying three batches of grain were stored under various air humidity conditions for 24 or 36 months:

- in a hygostat under relative air humidity conditions of 25-30 per cent for a period of 36 months (sample D),
  - uncovered, in the laboratory at relative humidity of about 60 per cent for 36 months (sample E),
  - in a hygostat under relative humidity of 80 per cent (sample F).
- After 24 months of storage the wheat grain completely lost its germination capacity.

In order to restrict the development of microflora, the caryopses subjected to accelerated ageing were dried and stored in tight jars until used for analysis.

#### STERILIZATION AND GERMINATION OF THE GRAIN

Whole wheat caryopses were washed with tap water and placed in a 2 per cent sodium hypochloride solution for 3 min (Rejman and Buchowicz 1971). They were then thoroughly washed with sterile water, dried on sterile cheesecloth and placed in sterile Petri dishes.

To check whether the experiment in its particular steps was run under sterile conditions, the solutions (including isotope solutions after germination) were plated on maximal medium used for bacterial cultures and incubated at 37°C for 48 h. No development of bacterial colonies was noted after incubation.

The seed was germinated for 48 h at 22°C in a 5-<sup>3</sup>H-uridine solution (0.01 mCi/ml; 29 Ci/mM). In the studies of ribosomes a <sup>14</sup>C amino acids mixture with 0.01 mCi/ml activity and specific activity 35 mCi/mA <sup>14</sup>C was used simultaneously. The seeds germinated on Petri dishes in darkness under constant humidity. The embryos isolated from the caryopses were washed and stored in closed vessels with liquid nitrogen.

#### ANALYSIS OF RIBONUCLEIC ACIDS AND POLYRIBOSOMES

Isolation and fractionation of total RNA was done after Tanifuji et al. (1970) with the thermal modification applied by Wasilewska and Kleczkowski (1974).

The purified RNA preparations  $\left( \text{value } \frac{E_{280}}{E_{260}} < 0,5 \right)$  were dissolved in 2-3 ml of 25 mM Tris-HCl (pH 7.4) containing 5 mM NaCl and 5 mM EDTA.

The RNA solution (1 mg in a 1 ml buffer volume) was carefully placed on the surface of the linear sucrose density gradient of 5-20 per cent concentration. The samples were centrifuged for 5 h at 4°C at 196 000  $\times g$  in a Beckman L-3-40 ultracentrifuge with an SW-41 rotor. After centrifugation the contents of the test tubes (13 ml) were separated into about 40 fractions, made up with water to 3 ml and extinction (at 260 nm) as well as radioactivity were measured.

The total ribosomal fraction of wheat embryos, consisting of monosomes and polyribosomes was isolated and separated in a sucrose gradient by the method of Davies et al. (1972).

Radioactivity was measured with a scintillation counter (Beckman type FL-100 C) with addition to 1 ml of the sample of 10 ml tritosol as scintillator (yield for <sup>3</sup>H 47 per cent and for <sup>14</sup>C 87 per cent, Fricke 1973).

#### RESULTS

Caryopses collected in 1980 at full ripeness showed a 100 per cent germination capacity. The less ripe and the more aged was the grain the lower was its germination capacity (Table 1). A similar relation was observed in the dry mass content in 48-h sprouts growing from unripe and aged caryopses.

Table 1

Germination capacity and dry mass of 48-h sprouts growing from wheat caryopses of various ripeness and those aged to various degrees

Wheat caryopse samples of various ripeness (A, B, C) and variously aged (D, E, F)	Dry mass of 100 sprouts, g	Germination capacity of caryopses, %
A — at milk ripeness	0.0592	54
B — at wax ripeness	0.1319	86
C — at full ripeness (control)	0.1760	100
D — at full ripeness after storage in air of 30% humidity	0.1606	90
E — at full ripeness after storage in air of 60% humidity	0.1232	44
F — at full ripeness after storage in air of 80% humidity	0.0664	0

Sedimentation in the sucrose gradient of total RNA from 48-h wheat sprouts from grain of various ripeness and from grain aged to a different extent is shown in Fig. 1. Total RNA was isolated from sprouts after 48-h germination in a  $^3\text{H}$ -uridine solution. Purified RNA preparations were separated by ultracentrifugation into three fractions of 25S, 18S and 4S RNA. The profile of the radioactive RNA was distinctly correlated with that of RNA optical density, this means that maximum radioactivity was present in the fractions exhibiting maximal optical density. The intensity of  $^3\text{H}$ -uridine incorporation into both rRNA classes (18S and 25S) and tRNA depended on the degree of ripeness or of ageing of the germinating seed.

High radioactivity was found in the RNA preparations from caryopses of milk ripeness (sample A) and in those stored under air humidity of 30 and 60 per cent (samples D and E, Fig. 1). Synthesis of RNA (evaluated in terms of  $^3\text{H}$ -uridine incorporation) as compared with that in the control (sample C) was recorded in sprouts from caryopses collected at wax ripeness. Lowest incorporation of the radioactive precursor was observed in sample F. The caryopses in this sample had completely lost their germination capacity (Table 1). In the latter samples the per cent of both ribosomal RNA fractions (18S + 25S) in total RNA (41%) was low, whereas the proportion of the low molecular weight fraction 4-5S RNA was high (Fig. 1). In the remaining caryopse samples

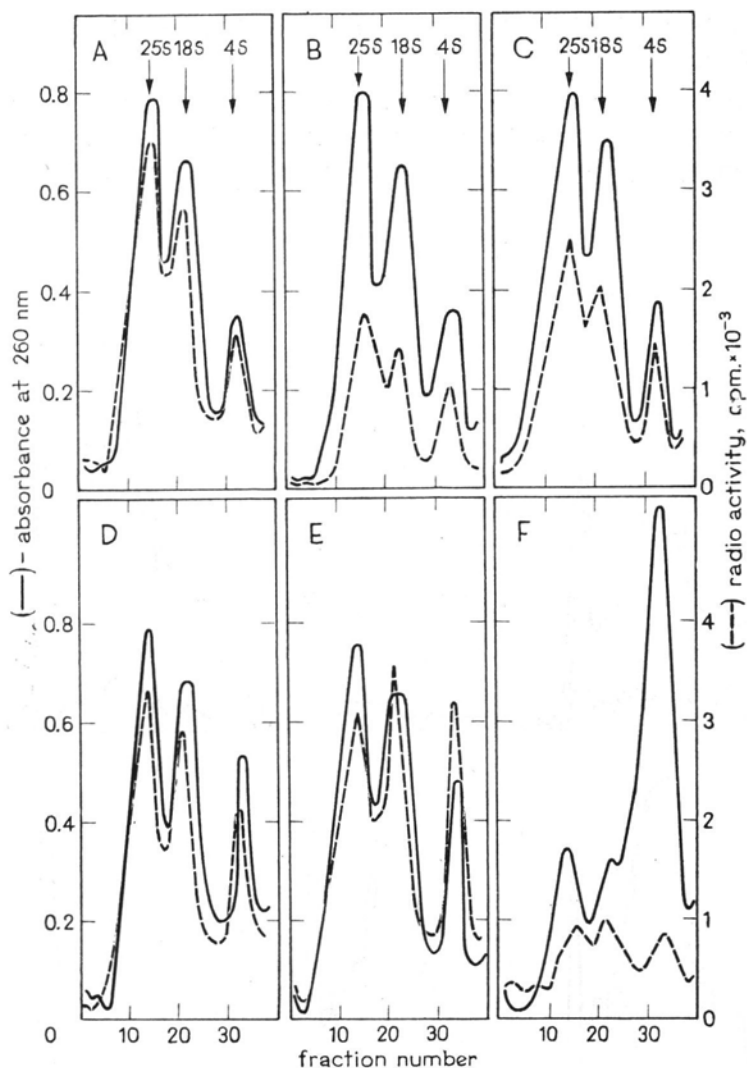


Fig. 1. Sedimentation profiles of total RNA (in 5-20% sucrose gradient) isolated from 48-h wheat sprouts growing from caryopses of various ripeness and those aged to different degrees. The caryopses were germinated in the presence of  $^3\text{H}$ -uridine (5  $\mu\text{Ci}/\text{ml}$ ; 29  $\text{Ci}/\text{mM}$ ). Caryopses harvested at milk ripeness — A, at wax ripeness — B, at full ripeness — C (control); D, E, F — fully ripe caryopses stored at air humidity of 30, 60 and 80 per cent, respectively

the percentual proportion of ribosomal RNA in total RNA was similar amounting to about 83-86 per cent.

The fact that in all the samples, notwithstanding their germination capacity, the radioactivity profile is correlated with optical density indicates that the caryopses synthesise in this period all the basic kinds of cellular RNA. Noteworthy is the finding of distinct rRNA synthesis

(18S + 25S) and 4-5S RNA in the embryos of the most aged grain deprived of germination capacity (Fig. 1F).

The ribosomal fraction obtained from 48-h sprouts of caryopses of various ripeness and stored under various conditions was represented, beside sample F, in 85-90 per cent by polyribosomes. Monoribosomes

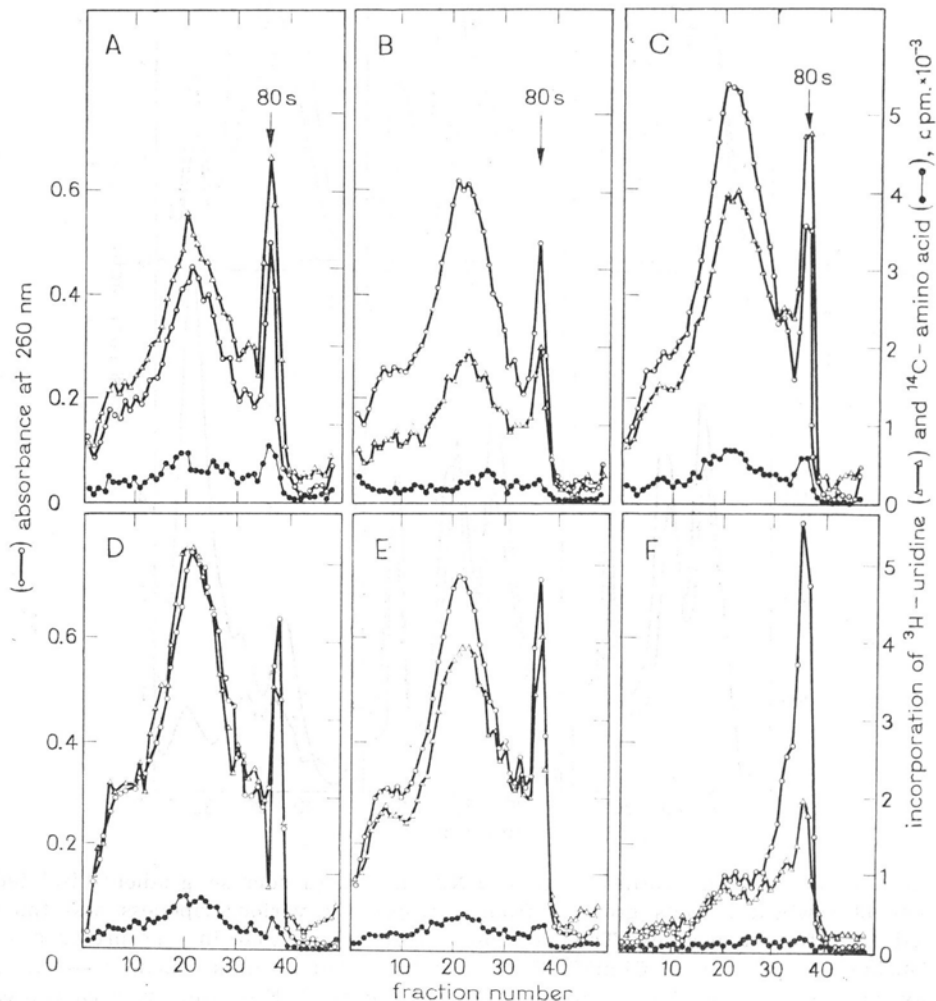


Fig. 2. Sedimentation profiles of total ribosomal fraction (in 12.5-50% sucrose gradient) isolated from 48-h sprouts grown from wheat caryopses collected at various stages of development and ripening and from fully ripe caryopses subjected to various degrees of ageing. The seeds were germinated in the presence of  $^3\text{H}$ -uridine (10  $\mu\text{Ci/ml}$ ; 29 Ci/mM) and of a mixture of  $^{14}\text{C}$ -labelled amino acids (10  $\mu\text{Ci/ml}$ ; 35 mCi/mA  $^{14}\text{C}$ ). Triangles denote  $^3\text{H}$ -uridine incorporation into both the basic fractions, monosomes and polyribosomes, darkened circles denote  $^{14}\text{C}$ -amino acids incorporation. The arrow indicates the site of monosome sedimentation (80S).

Sample symbols A, B, C, D, E, F the same as in Fig. 1

constituted as little as a dozen per cent or so, and their subunits occurred in minimal amount (Fig. 2).

Beside  $^3\text{H}$ -uridine,  $^{14}\text{C}$ -labelled amino acids were used. Incorporation of this mixture into the polyribosomes was, however, low. The observed tendencies in the synthesis of polyribosomal RNA and ribosomal proteins were similar as in the investigations on total RNA synthesis. In the embryos of seeds which lost their germination capacity (sample F), during soaking (48 h,  $22^\circ\text{C}$ ) a significant polyribosome synthesis was also observed. The profile of polyribosomes and distribution of radioactivity were, however, quite different than in the remaining samples (Fig. 2). The proportion of monoribosomes in the total ribosomal fraction in the discussed sample was very large amounting to about 60 per cent. This is evidence of continuous low aggregation of mono- into polyribosomes, and of a low rate of protein synthesis in the embryos from sample F caryopses.

#### DISCUSSION

The intensity of synthesis of early transcription products in germinating (2 to 6 h) embryos of wheat caryopses of varying ripeness and ripe ones stored under various air humidity conditions varies widely (Weidner et al. 1980). Ribonucleic acids were synthesised fastest in embryos of fully ripe grain (several months after the harvest — control). Resumption of the process of transcription in embryos of unripe caryopses or those stored for a prolonged period was in the early period of germination greatly retarded. These results manifest the germination capacity of the caryopses and the increments of the sprout mass. The more aged or unripe was the grain taken for analysis the less intensive was the synthesis of early transcription products and the lower were the germination capacity and sprout mass increment.

Quite different tendencies in the synthesis of total and polyribosomal RNA were noted when the germination period was longer (48 h) as was the case in the present study. Intensive  $^3\text{H}$ -uridine incorporation into polyribosomal and total RNA was, namely, observed both in embryos from caryopses of milk (sample A) ripeness and those from grain stored under air humidity of 30 and 60 per cent (samples D and E).

Enhanced RNA synthesis in unripe caryopses and not completely senescent ones did not occur probably earlier than between 24 and 48 h of germination. Up to 24 h of germination the transcription process in both these samples was greatly delayed as compared with the control, as has been earlier ascertained (Wiśniewski and Kulka 1979, Weidner and Kulka 1980).

The present results are partly contradictory to the so far accepted view concerning nucleic acids metabolism in ageing seeds. Up to date investigations indicated that with depression of viability the ability of embryos and of the embryo axis to synthesise RNA also decreases (Ching 1972, Roberts and Osborne 1973, Henri et al. 1974, Van Onckelen et al. 1974, Bray and Dasgupta 1976, Wiśniewski and Kulka 1979).

It results from the investigations of Roberts et al. (1967) and Roberts and Osborne (1973) that DNA also undergoes unfavourable structural changes and fragmentation to oligonucleotides in old seeds. It was, however, established in the present study that even nongerminating caryopses synthesise all the basic kinds of cellular RNA. This means that part of the DNA chains or at least some of their segments are accessible to transcription.

In our opinion, the cause of intensive  $^3\text{H}$ -uridine incorporation into total and polyribosomal RNA preparations from embryos of caryopses collected at milk ripeness and of those stored for a prolonged time (A, D and E) lies in the reduced amount of latent RNA forms (preformed mRNA, tRNA and ribosomes). Latent RNA forms localised mainly in the embryo play a highly important role, particularly in the initial period of seed germination. If unripe or aged caryopses are capable of germination, and for various reasons the amount of latent RNA forms in the embryos is decreased, protein biosynthesis must be based in a much higher extent on newly synthesised ribonucleic acid molecules.

All latent kinds of RNA indispensable for protein synthesis in the early period of germination are produced in the stages of development and ripening of the grain (Ajtkhozhin et al. 1976, Spiegel et al. 1975, Wasilewska 1978). These latent RNA forms, however, do not yet accumulate in the embryos in the period of milk ripeness but at later stages of ripening (Ajtkhozhin et al. 1976, Peumans and Carlier 1977, Peumans et al. 1978).

The destruction of cellular membranes stressed by numerous authors (Berjak and Villiers 1972, Floris 1970, Roberts 1972, Villiers 1973), the deep structural changes in the cytoplasm and other unfavourable events in ageing seeds must lead to a breakdown of the latent RNA forms, and particularly of the highly polymerised molecules of preformed mRNA and ribosomes. This seems to find confirmation in the observed degradation of high molecular weight RNA fractions in embryos and in the aleurone layer of rapidly ageing cereal caryopses (Roberts and Osborne 1973, Van Onckelen et al. 1974, Wiśniewski and Kulka 1979, Weidner et al. 1980).

To sum up, both caryopses collected at milk ripeness and those subjected to ageing must be deprived to a large extent of the latent



RNA forms, and this in our opinion is the direct cause of enhanced RNA synthesis in the second 24 h of germination in samples A, D and E.

As regards the observed but little intensive synthesis of RNA in embryos from caryopses collected at wax ripeness (sample B), King (1976) also noted a reduced germination capacity at this phase of grain ripeness. According to this author, this was due to the maximal accumulation of abscisic acid in the grain at wax ripeness, which prevents sprouting in the ear of the maternal plant.

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*Synteza kwasów rybonukleinowych i białek rybosomalnych podczas kiełkowania niedojrzałych oraz zestarzałych ziarniaków pszenicy*

Streszczenie

Kiełkowaniu poddawano ziarniaki zebrane w dojrzałości młecnej, woskowej i pełnej oraz zestarzałe, przechowywane przy wilgotności powietrza 30%, 60% oraz 80%. Im bardziej ziarniaki były niedojrzałe oraz ziarniaki o dojrzałości pełnej zestarzałe, tym niższą charakteryzowały się zdolnością kiełkowania i przyrostami suchej masy kiełków. Po 48 godz. kiełkowania w obecności radioaktywnych prekursorów, z ziarniaków usuwano zarodki i przeznaczano je do izolowania ogólnej frakcji rybosomalnej oraz RNA. Preparaty ogólnego RNA oraz frakcji rybosomalnej rozdzielano przez ultrawierwienie w gradiencie sacharozowym. We wszystkich próbach, niezależnie od zdolności do kiełkowania, profil radioaktywności był wyraźnie skorelowany z profilem gęstości optycznej. Świadczy to, że ziarniaki syntetyzują w tym okresie wszystkie podstawowe gatunki komórkowego RNA. Na podkreślenie zasługuje stwierdzenie wyraźnej syntezy trzech zasadniczych frakcji ogólnego RNA (25S, 18S i 4-5S RNA) jak również frakcji polirybosomów w zarodkach ziarna, które całkowicie utraciło zdolność do kiełkowania. Wysoką radioaktywność stwierdzono w preparatach ogólnego RNA i w polirybosomach pochodzących z 48 godz. kiełków wyrosłych z ziarniaków zebranych w dojrzałości młecnej oraz w kiełkach ziarniaków zestarzałych przechowywanych w wilgotności powietrza 30% i 60%. Zdaniem autorów, przyczyną intensywnego wcielenia  $^3\text{H}$ -urydyny do RNA jest obniżona ilość form zapasowych RNA (preformowany mRNA, tRNA i rybosomy) w zarodkach ziarniaków niedojrzałych oraz zestarzałych. Aby ziarniaki te mogły wykiełkować przy zmniejszonej ilości form zapasowych, konieczne jest oparcie mechanizmów biosyntezy białka w znacznie większym stopniu o nowo zsyntetyzowane molekuly kwasów rybonukleinowych.