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The metabolism of aged seeds. The free and membrane-bound polyribosomes of germinating rye grains of different ages

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Abstract

Grains of winter rye harvested in 1976, 1978, 1982 and 1984 were studied. Free and membrane-bound polyribosomes were isolated from embryos of imbibing and germinating grains. There was no correlation between grain viability and the amout of ribosomes. The highest incorporation of radioactive precursors (both total and specific radioactivity) was found in the RNA and ribosomal proteins from the grains with the highest viability – harvested in 1984. Lower radioactivity levels were observed in the 2 to 6 year old grains. There was no incorporation of radioactive precursors into ribosomal proteins in dead seeds.

Key words: RNA, ribosomes, rye, ageing

INTRODUCTION

Seeds gradually lose their vigor and viability as the result of long-term storage. It has been reported in numerous studies on this topic that all of the organelles and structures of the embryo undergo deterioration to a greater or lesser extent (Villiers 1974, Bray and Dasgupta 1976, Anderson 1977). This also applies to cytomembranes and ribosomes (Berjak and Villiers 1972a and b, Roberts and Osborne 1973, Zalewski 1985 and 1986). For example, during imbibition of aged grain, Golgi apparatus and endoplasmic reticulum do not appear and ribosomes have an altered rRNA and protein structure (Roberts and Osborne 1973, Zalewski and Weidner 1982). It is not known, however, to what degree does the gradual loss of viability correlate with the biogenesis of ribosomes and their association with the endoplasmic reticulum (ER) during germination of the grain. This is the period

when storage substances undergo intense metabolism and new compounds needed for the development of the embryo are synthesized. Therefore, every unfavorable change within the ribosome or ER can influence the degree of association of these two structures, and at a later stage, the quantity and quality of the proteins synthesized by them. The above statements, not all of them proven, were the reasons for undertaking studies on the synthesis of rRNA, ribosome proteins and the determination of the proportions of smooth and rough ER during the first stages of germination of rye grains from different harvest years.

MATERIAL AND METHODS

The winter rye grains cv. Dańkowskie Złote were studied. The grain was harvested at full ripeness, dried in a well ventilated room and stored in a hygrostat at a temperature of 18-20°C and an average air humidity of 50-55%. Grain harvested in the following years was studied: 1976 – denoted further as D, 1978 – C, 1982 – B, 1984 – A.

Conditions of imbibition and germination. Before imbibition and germination the grain was sterilized in a 2% solution of sodium hypochlorite for 3 minutes, after which it was washed carefully in sterile water, wiped dry with sterile gauze and placed in sterile Petri dishes. Imbibition was conducted for 8 hours at 2°C in redistilled water, while germination was for 24 or 48 hours at a temperature of 22°C. Germination was conducted in the presence of (5-^{3}H) uridine (0.37 MBq cm^{-3}) and a ^{14}C-mixture of amino acids (0.37 MBq cm^{-3}), in petri dishes in the dark and with a constant supply of labeled precursors. After the first 10 hours, the unincorporated precursors were washed away. The grain was washed with sterile water and germination continued in water. In order to make sure that the experiment was being conducted under sterile conditions, samples of the incubation mixture were taken and transferred to full mediums used in bacteria cultures. After 24 hours of incubation at 37°C no bacteria or fungi cultures were obtained.

After the set time of imbibition or germination, embryos (sprouts) were isolated by hand and stored in closed containers at −25°C.

Isolation and fractionation of polyribosomes from the rye embryos and sprouts were carried out according to Larkins and Davies (1975) with the following modifications during centrifugation. Free and membrane-bound polyribosomes were sedimented by centrifugation for 90 minutes in a 65Ti angle-head rotor at 95 000 × g. The ribosome pellet was suspended in a small volume of buffer "B" and applied to a linear sucrose concentration gradient, 125-500 mg cm^{-3}. Ribosomes were fractionated by centrifugation for 75 min at 122 000 × g in a SW-41 rotor. The contents of each tube were separated into 46-47 fractions, to each of which 2 cm^{3} distilled water were added and the

absorption was measured at 260 nm. The concentration of the ribosomes was calculated according to Gualerzi and Cammarano (1969).

The whole procedure of isolation and fractionation of ribosomes was conducted at a temperature of 2-4°C.

The radioactivity was measured using 10 cm^3 tritosol per 1 cm^3 as a scintillator with an efficiency of 47% for ^3H and 87% for ^{14}C (Fricke 1973). A Beckman LS-1801 counter was used.

RESULTS

Grain storage for different lengths of time under the same conditions of temperature and humidity caused unequal losses of its viability (Table 1). After 8 years of storage (the analyses were carried out from May to July 1985), the rye grain harvested in 1976 had completely lost its germination capacity.

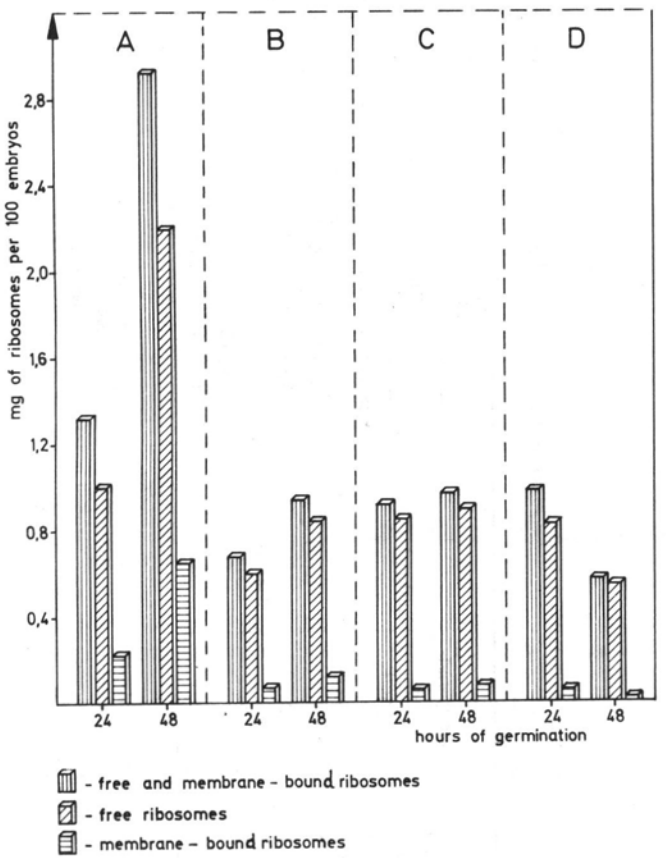

Fig. 1. Content of free and membrane-bound ribosomes after 24 and 48 hours of germination of rye grain of different ages. A, B, C and D as in Table 1

Table 1
Quantitative changes in the ribosomes of germinating rye grains of different ages

Grain sample	Germination capacity	R mg × 100 embryos $^{-1}$ (8h, 2°C)	Fraction composition Rf (%)								Fraction composition Rm-b (%)							
			P		Ph		Pl		M		P		Ph		Pl		M	
			24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
A	94	1.030	75.20	81.41	29.12	23.80	46.08	57.61	24.80	18.59	66.53	83.92	27.52	30.03	29.01	53.89	33.47	16.08
B	52	0.592	53.06	56.95	17.55	18.13	35.51	38.82	46.94	43.05	55.85	68.02	21.70	33.68	34.15	34.36	44.15	31.98
C	22	0.855	29.66	56.14	8.46	17.34	21.20	38.80	70.34	43.86	62.05	66.94	18.73	30.79	43.32	36.16	37.95	33.06
D	0	0.902	66.83	66.89	18.58	18.53	48.25	48.36	33.17	33.11	54.97	0.00	16.69	0.00	38.28	0.00	45.03	100.00
LSD P = 1%	3.93	0.105	3.88	4.13	1.82	1.58	2.89	3.60	3.79	2.43	4.70	3.91	2.11	3.00	3.10	3.99	2.55	3.94

R — total ribosomes in embryos (free and bound polyribosomes + monoribosomes + ribosome subunits); Rm-b — membrane-bound ribosomes; Rf — free ribosomes; P — polyribosomes (material sedimenting before monosomes); M — monoribosomes; Ph — heavy polyribosomes (sedimenting faster than septamers); Pl — light polyribosomes (sedimenting before monosomes); A — grain harvested in 1984 (control); B — grain harvested in 1982; C — grain harvested in 1978; D — grain harvested in 1976.

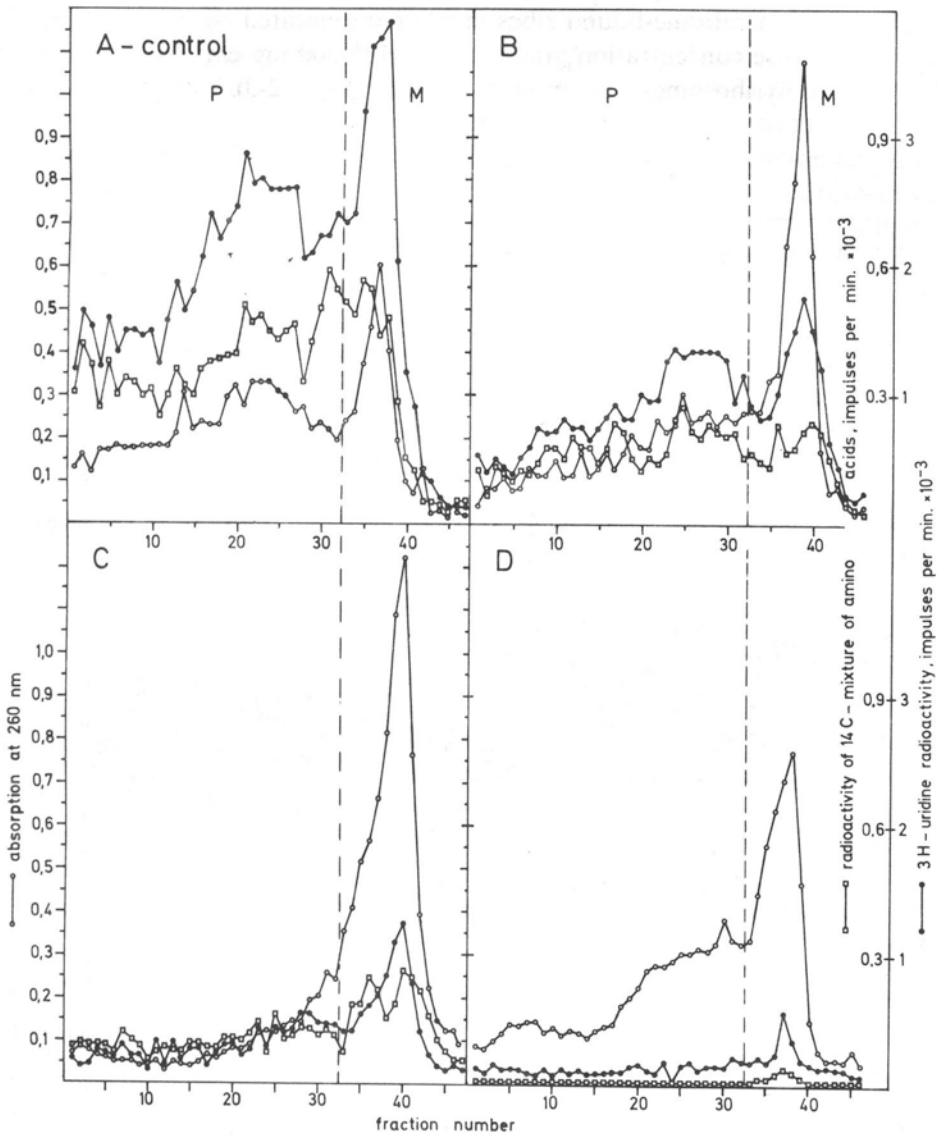

Fig. 2. Sedimentation profiles of free ribosomes isolated from rye embryos of grains of different ages after 24 hours of germination at 22°C. A, B, C, D, P, M – as in Table 1

As results from the data presented in Table 1, the ribosomes content in imbibing embryos was not related to the viability of the grain. A slightly different picture was obtained when the grain was allowed to germinate for 1-2 days. In the grain with the highest viability, the increases in the free and membrane-bound ribosomes content were the highest (Fig. 1). In the remaining samples (B and C), 24 to 48 hour germination led to slight increases in the ribosomes content in embryos, while in dead grains, even to a drop in their amount.

Free and membrane-bound ribosomes were separated by centrifugation in a linear sucrose concentration gradient from 125-500 mg cm^{-3} into two basic fractions: polyribosomes and monoribosomes (Figs. 2-3).

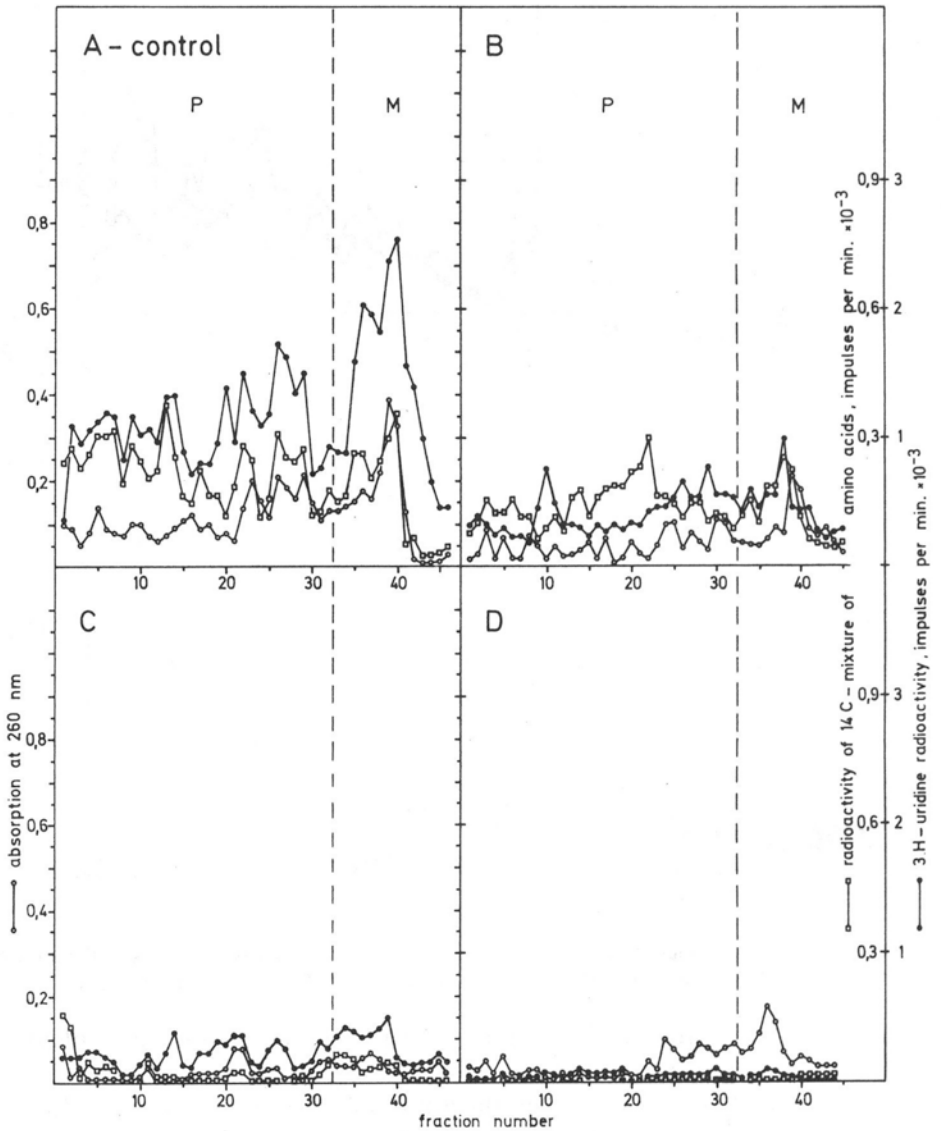

Fig. 3. Sedimentation profiles of membrane-bound ribosomes isolated from embryos of rye grains of different ages after 24 hours of germination at 22°C. A, B, C, D, P, M – as in Table 1

From the sedimentation profiles of the free ribosomes (Fig. 2) isolated from grain after 24 hrs of germination, it can be seen that approximately 75% of the ribosomes from grain with high viability were associated with mRNA while only one quarter of them were in the form of 80S monosomes. In the grain with lowered viability, 52% (B) and 22% (C), the monoribosome fraction amounted to 46.94 and 70.34% of the total amount of fractionated ribosomes, respectively (Table 1). It was interesting to see that in the dead grain after 24 hrs of germination the polyribosome content was relatively high and did not practically change after a further 24 hrs of germination. In the remaining samples an increase in the number of ribosomes associated with mRNA was noted at this time, along with a decrease in the amount of monosomes. Similar results were obtained on analysing the qualitative and quantitative composition of polyribosomes bound to ER membranes during the first two days of seed germination (Table 1, Fig. 3). In this case, however, the number of ribosomes bound to the ER was more clearly correlated with grain viability. In the dead grain, the amount of membrane-bound ribosomes dropped (from 8.3% to 4.5%) on germination from 24 to 48 hours. In addition, in the sedimentation profile of the membrane-bound ribosomes isolated from this sample (D) after 24 hours of imbibition at 22°C, monosomes made up 45.03% of the ribosomes, while after a further 24 hours, 100% (Table 1).

In order to obtain more information on the state of rRNA and ribosomal protein biosynthesis, germination of the grain was conducted in the presence of labeled precursors, that is, ^{3}H-uridine and ^{14}C-amino acid mixture. As is seen from the data in Table 2, the total radioactivity per 100 sprouts was the highest in the grain with full viability (A) and increased in the course of germination. In the grain stored for 2 (B) and 6 (C) years, these values were significantly lower and the increases in total and specific radioactivity during germination smaller

Table 2

Total radioactivity of the particular ribosome fractions isolated from germinating rye grains (24h, 48h, 22°C) of different ages

Grain sample	Total radioactivity (impulses x min^{-1} x 100 $shoots^{-1}$							
	Rf				Rm-b			
	24 h		48 h		24 h		48 h	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
A	128631	23956	146626	44893	28011	4920	37164	12219
B	33478	5999	45238	11376	6295	2159	6817	3623
C	26164	6286	26115	4558	2869	1252	3390	1786
D	4804	—	3562	—	—	—	—	—
LSD P=1%	251	1816	9620	3115	1977	361	2886	995

Symbols — as in Table 1.

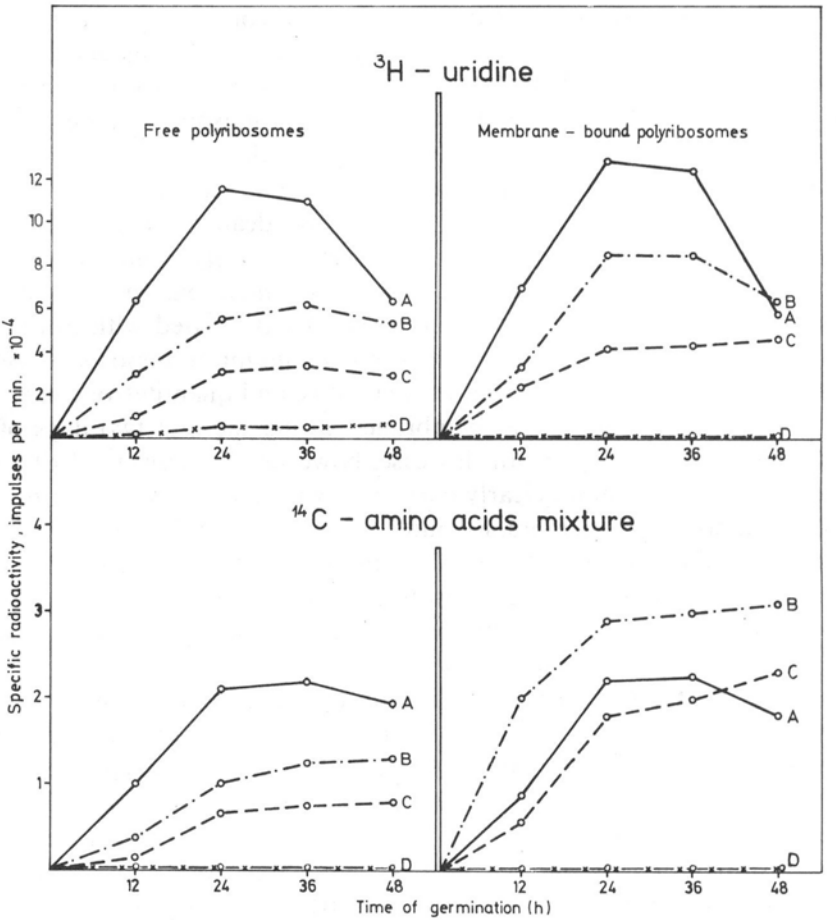

Fig. 4. Incorporatiom of ^{3}H-uridine (0.37 MBq cm^{-3}) and ^{14}C amino acid mixture (0.37 MBq cm^{-3}) into ribosomes isolated from embryos of germinating rye grain of different ages. A, B, C, D – as in Table 1

(Table 2, Fig. 4). In the dead grain, low incorporation of ^{3}H-uridine into polysomal RNA (mRNA + rRNA) was noted while there was complete absence of incorporation of labeled amino acids into ribosomal proteins.

DISCUSSION

It can be concluded from the presented data that about 78% of the cytoplasmic ribosomes in the cells of embryos of fully viable rye grains are in the form of free ribosomes, the rest are bound with the membranes of the ER (Table 1). Similar results were obtained by Dobberstein et al. (1974) in studies on the association of ribosomes with membranes in the tissues of bean seeds.

The relatively high proportion of polyribosomes in the total amount of fractionated free ribosomes obtained from dead grains after 24 and 48 hours of imbibition at 22°C is noteworthy. The low specific and total radioactivities of these fractions indicate that both the ribosomes and mRNA were synthesized during the formation and maturation of the grain. In the remaining samples, a distinct increase in the number of free ribosomes was noted during germination for 24 and 48 hours. The simultaneous increase in the absolute radioactivity is proof that the biogenesis of these ribosomes occurred during germination. The drop in the number of membrane-bound ribosomes noted in the germinating grain which had been stored for several years (Fig. 1) can have two basic causes:

– The degradation of the cytomembranes system in ageing embryos (Mietlickij 1987) or even by small changes in the ER, especially in the proteins responsible for the association of the larger subunit of the ribosome with the receptors of the ER membrane.

– Unfavorable modifications in the structure of the protein component of the ribosome. It has been found that in aged cereal caryopses, the ribosomes of embryos have altered rRNA (Roberts and Osborne 1973, Bray and Dasgupta 1976) and protein (Zalewski and Weidner 1982) structures.

The modifications of the protein part of the ribosome include decreases in its total amount of protein and changes in their physico-chemical properties. It is still unknown to what extent these changes influence the formation of the complexes initiating protein biosynthesis and association of the 60S subunit with the ER. On the basis of the data from literature, it can be supposed that the decrease in the number of polyribosomes bound with the ER in the samples of the aged grain undoubtedly influences the reduced amount of synthesized secretion proteins, and so disturbs the whole protein metabolism of the germinating rye grain.

It is interesting that in the grain which had lost its germination capacity, low levels of transcription of RNA were noted. Similar results have been obtained earlier in studies on the ageing of soybeans (Anderson 1977), wheat (Zalewski and Weidner 1982) and rye (Zalewski 1985).

Acknowledgment

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Metabolizm starych nasion. Wolne i związane z błoną polirybosomy kiełkującego ziarna żyta w różnym wieku

Streszczenie

Analizowano ziarniaki żyta ozimego, pochodzące ze zbiorów w latach 1976, 1978, 1982 i 1984. Z pęczniejącego i kiełkującego, w obecności znakowanych prekursorów białek i RNA, ziarna izolowano rybosomy wolne i związane z retikulum endoplazmatycznym. Nie stwierdzono wyraźnej korelacji między ilością rybosomów i żywotnością ziarna. RNA i białka rybosomowe, kiełkującego ziarna żyta o pełnej żywotności, charakteryzowały się największą radioaktywnością absolutną i właściwą. W ziarnie przechowywanym przez 2 lata i przez 6 lat wartości te były znacznie mniejsze, a w ziarnie martwym nie zauważono wcielania znakowanych prekursorów do białek rybosomalnych.