Synthesis of rRNA, tRNA and Other RNA-species Concomitant with Polyribosome Formation in Aging Potato Tuber Slices

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One of the earliest consequences of slicing plant storage organs such as potato tubers into thin disks is the formation of polysomes, which in potato slices is complete after 9 hours and is dependent on transcription. Fresh disks do not incorporate ^{32}P, ^{3}H-uridine or ^{14}C-leucine into their ribosomes, whereas ribosomes and polysomes of aged disks use these precursors effectively. This development can be completely blocked by actinomycin D. Among the different RNAs synthesized during aging is 28S- and 16S—rRNA, 5S—RNA, tRNA, and a component sedimenting around 15—18S with a base-composition different from 16S—rRNA, 5S- and 4S—RNA and which supports peptide formation in an in vitro incorporation system.

It is suggested that this compound represents mRNA, which is not available immediately after slicing the tissue. These findings are consistent with the view of a derepression phenomenon in sliced storage tissue.

Thin slices of plant storage organs such as potatoes, carrots, sugar beets, red beets, Jerusalem artichokes, swedes, turnips and chicory represent suitable material for various biochemical investigations. Slicing brings about higher template availability and enhanced activity of DNA dependent RNA polymerase in sugar beet disks [1] and a vigorous formation of ribosomal and transfer-RNA species [2–7]. Concomitant protein synthesis is necessary for the formation of new cellular membranes and various enzymes working in quite different metabolic pathways (glycolysis and pentose shunt [8, 9], phenol metabolism [10, 11], fatty acid synthesis [12–14], respiration [15–17]). The induction of certain enzymes occurs simultaneously with the inactivation of other enzymes, the latter also depending on protein synthesis [8, 9, 11]. Moreover several examples of rapid genome-independent metabolic changes after slicing are well known [18–20].

The evidence points to the importance of a derepression phenomenon after slicing the storage organs: unblocking of previously blocked genes, but reasons and mechanisms are unknown. Before considering various possible derepression mechanisms in detail, it is important to know more about regulations of gene expression at the level of translation. The work reported here and in a subsequent paper can be interpreted in terms of regulation of protein synthesis through availability of RNA-fractions, which are absent or in low concentration in the intact tubers and newly synthesized after slicing.

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[1] C. DUDA, Ph. D. Thesis, Purdue University, Lafayette, Indiana, USA 1969.
[2] Y. MASUDA, Plant Cell Physiol. **7**, 573 [1966].
[3] R. J. ELLIS and I. R. MACDONALD, Plant Physiol. **42**, 1297 [1967].
[4] M. J. SAMPSON and G. G. LATIES, Plant Physiol. **43**, 1011 [1968].
[5] I. H. CHERRY, in: Biochemistry and Physiology of plant growth substances, p. 417, ed. WIGHTMAN, Setterfield 1968.
[6] C. J. LEAVER and J. L. KEY, Proc. nat. Acad. Sci. USA **57**, 1338 [1967].
[7] C. J. LEAVER and J. L. KEY, J. molecular Biol. **49**, 671 [1970].
[8] G. KAHL, H. LANGE, and G. ROSENSTOCK, Z. Naturforsch. **24 b**, 911 [1969].
[9] G. KAHL, H. LANGE, and G. ROSENSTOCK, Z. Naturforsch. **24 b**, 1544 [1969].
[10] H. HYODO and J. URITANI, Plant Cell Physiol. **7**, 137 [1966].
[11] M. ZUCKER, Plant Physiol. **43**, 365 [1968].
[12] C. WILLEMOT and P. K. STUMPF, Plant Physiol. **42**, 391 [1967].
[13] C. WILLEMOT and P. K. STUMPF, Canad. J. Bot. **45**, 579 [1967].
[14] A. B. ABDELKADER, P. MAZLIAK, and A. M. CATESSON, Phytochem. **8**, 1121 [1969].
[15] R. E. CLICK and D. P. HACKETT, Proc. nat. Acad. Sci. USA **50**, 243 [1963].
[16] R. E. CLICK and D. P. HACKETT, Biochim. biophysica Acta [Amsterdam] **142**, 403 [1967].
[17] G. G. LATIES, Plant Physiol. **40**, 1237 [1965].
[18] G. G. LATIES, Austral. J. Sci. **30**, 193 [1967].
[19] B. JACOBSEN, Ph. D. Thesis, University of California, Los Angeles 1970.
[20] B. JACOBSEN, B. N. SMITH, S. EPSTEIN, and G. G. LATIES, J. gen. Physiol. **55**, 1 [1970].

Material and methods

Potato tubers (Solanum tuberosum L., cv. "Pontiac"), stored at 2 °C, were transferred to 20 °C two to three weeks before use. Disks (1 cm in diameter, 1 mm thick) were cut from pith and medulla of the tuber and carefully freed from cell debris by several washings with sterilized water. The slices were aged in sterilized desiccators, aerated with filtered air which was passed through a water-cushion at the bottom of the desiccator containing Streptomycin sulfate and Penicillin G at a concentration of 10^{-4} M each. Thus moisture was kept high and bacterial contamination low. Temperature was maintained at 25 °C. For experiments, which required application of tracers or inhibitors, a batch of tissue was transferred from the desiccators to small Erlenmayer vessels and shaken in the dark at 25 °C. Pulse-labeling with ^{3}H-uridine, ^{32}P or ^{14}C-leucine usually was done for one hour, unless indicated otherwise.

Isolation of ribosomes [6]

Disks were brought to 2 °C, coarsely chopped, and ground with mortar and pestle in homogenizing medium (0.25 M RNAse-free sucrose; 0.05 M Tris-succinate buffer, pH = 7.8; 0.01 M $MgCl_2$ and 0.015 M KCl and 0.005 M 2-mercaptoethanol). The homogenate was filtered through miracloth and centrifuged at 20,000 *g* for 20 minutes. Deoxycholate was added to the supernatant to a final concentration of 0.5% and the solution centrifuged for 20 min at 20,000 *g*. Ribosomes were pelleted from the supernatant by layering on a step gradient (2 ml 0.5 M sucrose — 3 ml 1.6 M sucrose in 0.01 M Tris-succinate, pH = 7.8; 0.01 M $MgCl_2$; 0.015 M KCl; 0.005 M 2-mercaptoethanol) and centrifuging at 115,000 g for 3 hours at −4 °C in a Spinco model F centrifuge. After several gentle washings with distilled water the pellet was dissolved in a small volume of 0.01 M Tris-succinate buffer, pH = 7.8; 0.01 M $MgCl_2$; 0.015 M KCl and 0.005 M 2-mercaptoethanol, and centrifuged at 1500 *g* for 15 minutes. The clear supernatant was used as ribosome preparation. All manipulations were done at 2 − 4 °C.

Polysome profiles

A ribosome sample containing from 140 − 180 μg RNA/ml was applied to an exponential 10 − 48% sucrose gradient (in 0.01 M Tris-succinate, pH = 7.8; 0.01 M $MgCl_2$; 0.001 M Spermidine) and centrifuged at 105,000 *g* and −5 °C for 1.5 hours (Beckman L-2D; SW 41 rotor). After puncturing the tubes the profiles of the gradients were determined by passing the effluent through a flow cell with a 4 mm light path using a Gilford recording spectrophotometer, Model 2400. The flow rate was kept constant by a flow pump and the absorption of the effluent was recorded at 260 nm. Fractionation of the gradients into 0.2 − 0.5 ml portions was followed by precipitation of the RNA with an equal volume of 10% TCA and carrier-DNA (mg/ml). After one hour in ice the precipitate was collected on Millipore filters and washed several times with 5% TCA containing 0.4% sodium pyrophosphate. Radioactivity on the dried filters was estimated in a toluene scintillator (4 g PPO and 100 mg POPOP/*l* toluene).

Dissociation of ribosomes into subunits

The ribosomal pellet was dissolved in 1 ml TKE-buffer, pH = 7.4 (0.01 M Tris-HCl; 0.015 M KCl and 0.015 M EDTA) and layered on top of a 12 − 30% linear sucrose gradient. After centrifuging for 16 − 18 hours at 63,500 *g* and 2 − 4 °C in an SW 25.1 rotor the tubes were punctured as described above, the OD of the effluent determined, and fractions of 0.5 − 1 ml were collected with an LKB-Ultro-Rac.

It was important to improve separation of the 4S-, 5S − RNA and 40S-subunit. This was achieved in a 15 − 25% linear gradient and centrifugation time of 18 hrs at 3 °C and 140,000 *g* in an SW 41 rotor.

Extraction of ribosomal RNA from isolated ribosomes

The ribosomal pellet was suspended in 2 ml TMK-buffer (0.001 M Tris-HCl, pH = 7.5; 0.05 M KCl; 0.0015 M $MgCl_2$) and diluted with an equal volume of water. After addition of 5 ml 4 M LiCl and mixing, the suspension was kept at 2 °C overnight. The ribosomal RNA was obtained by centrifuging for 15 min at 3000 *g*, the pellet was resuspended in 2 M LiCl and again centrifuged. After two more washings the pellet was solubilized with a small volume of 1 E buffer (0.04 M Tris; 0.02 M sodium acetate; 0.001 M EDTA, adjusted to pH = 7.2 with glacial acetic acid), layered on a linear 17 − 34% sucrose gradient in 1 E-buffer and centrifuged for 24 − 30 hours at 4 °C and 25,000 rpm in an SW 25.1 rotor of the Spinco.

Base composition

The different ^{32}P-labeled RNAs were collected from the gradient, lyophilized for several hours, the residue diluted approximately 10 fold with water and precipitated overnight at −2 °C with the threefold volume of ethanol. The precipitate was collected by centrifugation and washed several times. After total evaporation of ethanol the RNA was suspended in 0.3 M KOH and incubated for 18 hours at 37 °C. The neutralized and concentrated solution was suspended in electrophoresis buffer (0.6 M acetic acid; 0.01 M EDTA; 3.4 ml pyridine/*l*; pH = 3.5); the bases were separated according to SEBRING and SALZMAN [21].

Results

Polysomes

Polysomes are barely detectable in cells of the intact tuber (Fig. 1). Their formation begins 3 − 4 hours after the tissue is sliced and reaches a maximum in another 5 − 6 hours (45—50% ribosomes as polysomes). After 48 hours of aging of the tissue

[21] E. D. SEBRING and N. P. SALZMAN, Anal. Biochem. **8**, 126 [1963].

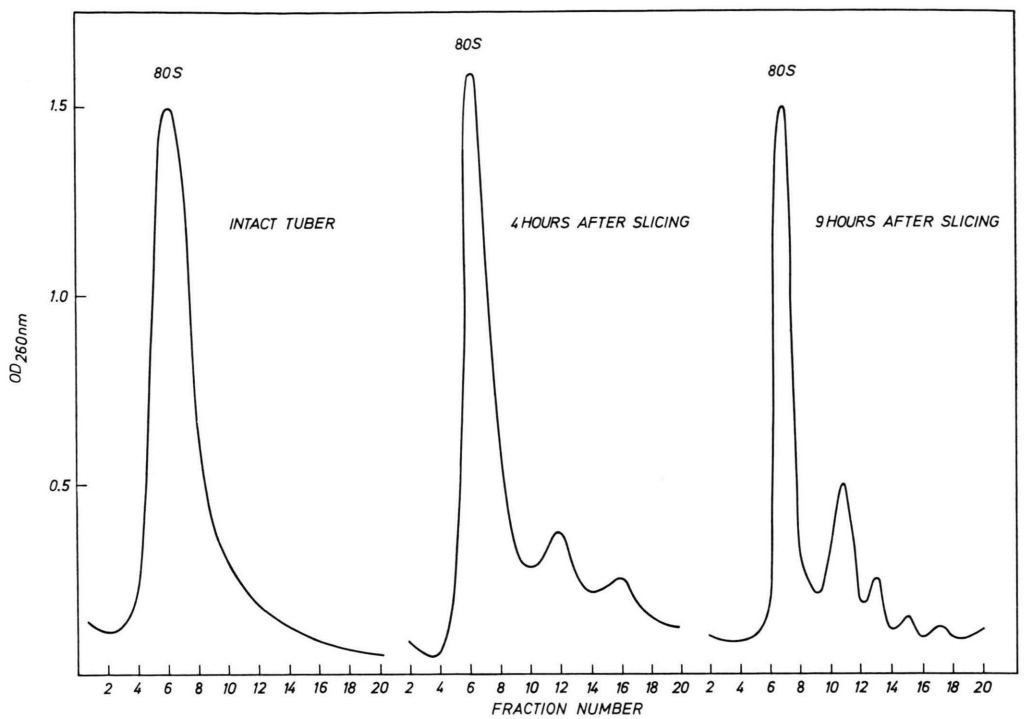

Fig. 1. Sedimentation profiles of ribosomes isolated from the intact potato tuber and tissue slices after different time of aging.

slices the polysome content drops again, until it reaches 25% at 96 hours (Fig. 2). Polysome formation subsequent to slicing is prevented by actinomycin D, a nitrogen atmosphere and low temperature (Table 1). The polysomal aggregates are easily dissociated into monosomes by DNAse-free RNAse (1.2 μg/3.6 OD ribosomal RNA).

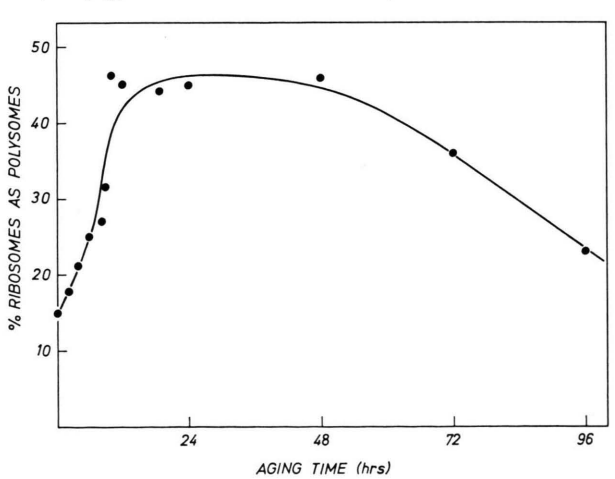

Fig. 2. Kinetics of polysome formation in aging potato tuber slices. The area under the sucrose density gradient profiles of the polysome region were used for calculation of the percentage of polysomes present.

RNA and protein synthesis

Freshly sliced potato tissue does not incorporate ^{3}H-uridine or ^{32}P into ribosomal components. Aging overnight leads to greatly increased incorporation of ^{3}H-uridine, ^{32}P and ^{14}C-leucine into the ribosomes with high specific activity in the polysome region (Fig. 3). The development of these capacities for incorporation is blocked by low dosages of actinomycin D given shortly after slicing (Fig. 4). If all RNA species are extracted with LiCl from ribosomes of pulse-labeled fresh and aged disks, the same pattern can be obtained: there is heavy incorporation of ^{32}P into 28S-, 16S-, 5S- and 4S–RNA of ribosomes from aged slices only (Fig. 5).

Treatment	Aging time [hrs]	Polysomes [%]
Control	0	15
Control	15	45–50
Actinomycin D (20 μg/ml)	15	18
nitrogen atmosphere	15	20
low temperature (+4 °C)	15	30

Table 1. Inhibition of polysome formation during aging of potato tuber slices by Actinomycin D, by nitrogen atmosphere and by low temperature.

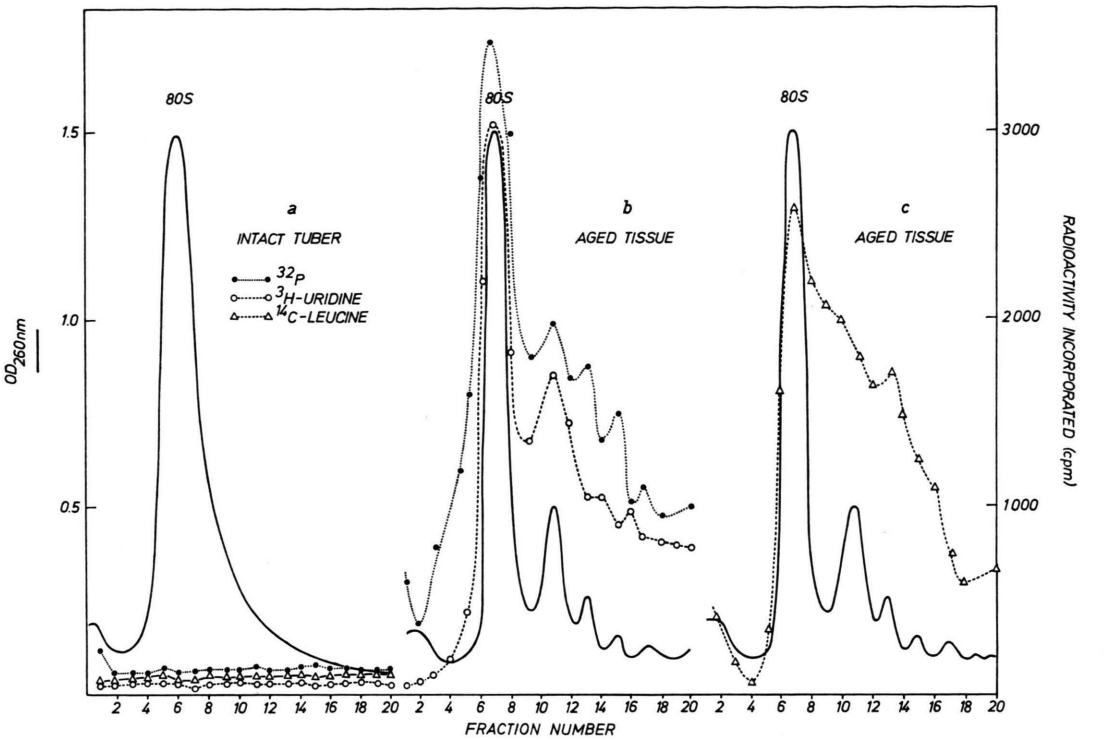

Fig. 3. Incorporation of labeled precursors into monosomal and polysomal regions of sucrose density gradients from a) ribosomes of the intact potato tuber, b) and c) ribosomes of tissue slices aged for 15 hours.

The high specific activity of the region between 5S – RNA and 16S—RNA (Fig. 5) suggested that this was the location of one or more messenger RNAs. This region was labeled in short-pulse experiments (15 min) and chased quickly with cold $PO_4^{3\ominus}$ (50 mM).

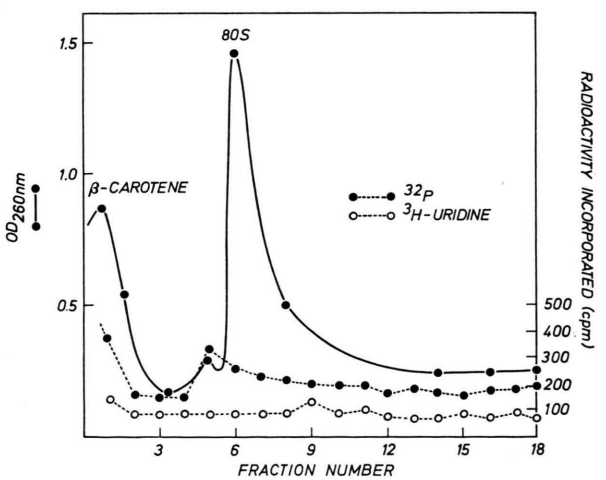

Fig. 4. Inhibition of polysome formation and incorporation activity of ribosomes from aged potato tissue slices by Actinomycin D. The inhibitor was added immediately after slicing the tissue (concentration: 20 μg/ml).

Since LiCl in the concentrations used for RNA extraction from isolated ribosomes is able to destroy the coding properties of mRNAs (P. MANDEL, pers. comm.), separation of mRNAs from polysomes of aged potato tuber slices was achieved by EDTA dissociation of ribosomes and sucrose density gradient centrifugation of the resulting subunits and RNAs. Fig. 6 a shows the extreme low incorporation of ^{32}P into the greater and smaller subunit of ribosomes from the intact tuber and the 5S-/4S – RNA region of the gradient. One-hour-pulse of aged disks, however, is sufficient to label all components dissociated from isolated ribosomes heavily (Fig. 6 b). It is apparent that the 60S-subunit is labeled to a lesser degree than the 40S-subunit, a fact which is understandable in view of the mechanism of rRNA formation (45S-precursor, preferential transport of 16S – rRNA into the cytoplasm [7]; see also Fig. 5).

Aging of the tissue slices results in the appearance of a component sedimenting between 5S – RNA and the smaller ribosomal subunit,

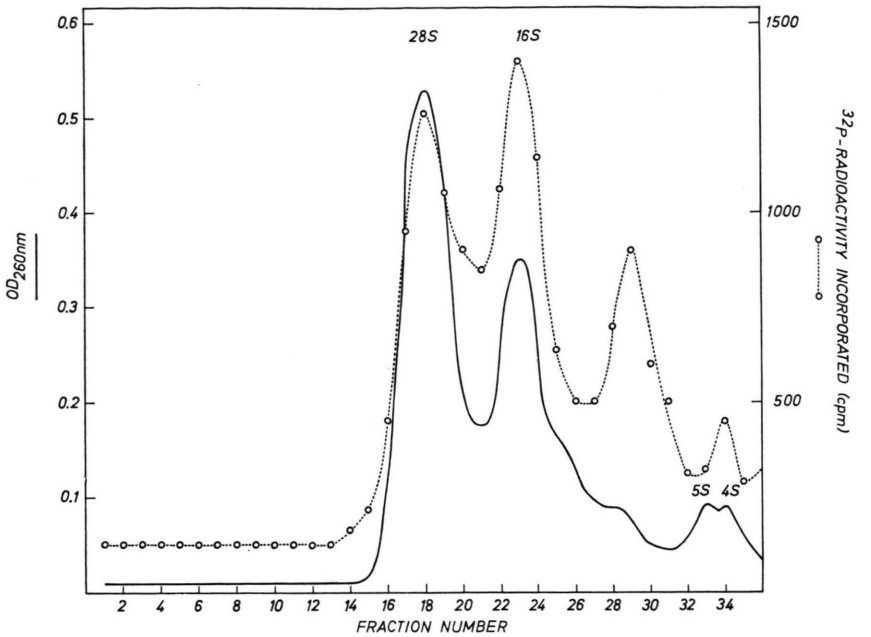

Fig. 5. Incorporation of ^{32}P into 28S- and 16—rRNA, 5S- and 4S—RNA of potato tuber slices aged for 15 hours. Pulse labeling was done for 1 hour with 0.5 mc ^{32}P/25 ml incubation medium.

designed as peak I in Fig. 6 b. This peak is barely detectable in freshly sliced potato tissue, whereas the other peaks are present (Fig. 6 b). The appearance of peak I is prevented by Actinomycin D applied shortly after preparation of the disks. The base composition of this fraction I shows it to be distinct from ribosomal RNA, 5S- and 4S—RNA (Table 2).

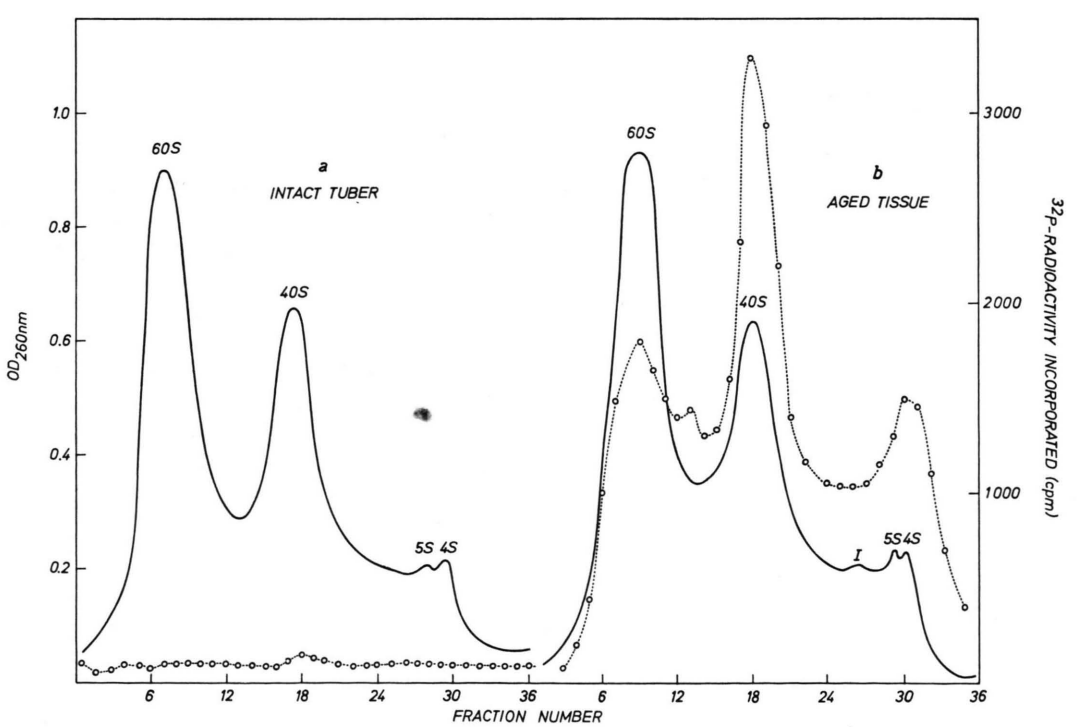

Fig. 6. Dissociation of ribosomes into subunits and 5S-/4S—RNA by EDTA. a) Sedimentation profile of dissociated ribosomal components from the intact potato tuber. b) Incorporation of ^{32}P into 60S- and 40S-subunits and 5S-/4S—RNA of potato tissue slices aged for 15 hours.

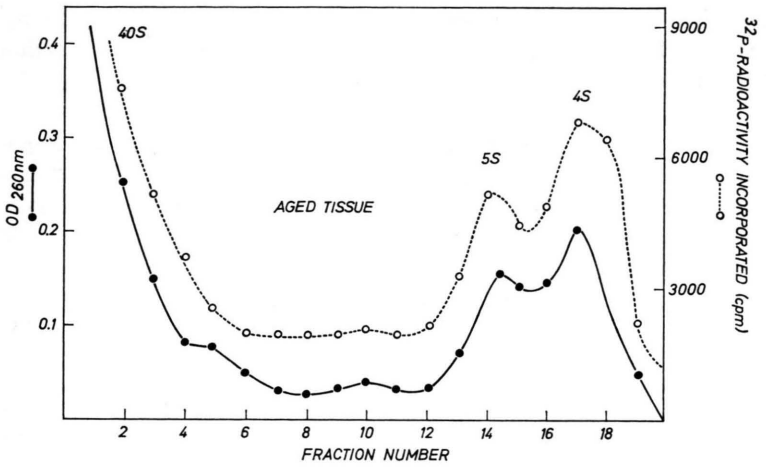

Fig. 7. Separation of 5S-/4S—RNA and fraction I from the 60S- and 40S-subunits of ribosomes from aged potato tuber slices.

	[mole-%]			
RNA	CMP	AMP	GMP	UMP
16S	21,8	24,9	26,9	26,3
I	24,7	26,8	24,2	24,5
5S	23,1	31,3	21,0	24,7
4S	23,0	29,5	21,0	26,6

Table 2. Base composition of 16S—rRNA, fraction I, 5S—RNA and tRNA isolated from aged potato tuber slices. Distribution of ^{32}P incorporated into each nucleotide as percentage of total radioactivity in the four nucleotides. Standard deviation 0,8—1,0 mole-%.

Although there was no preferential incorporation into this compound, it stimulated ^{14}C-leucine incorporation in an in vitro aminoacid incorporation system [22]. Concentration of this fraction therefore was improved by using a linear 15—25% sucrose gradient. EDTA-dissociated polysomes were centrifuged on this gradient long enough to sediment part of the 40S subunits and all of the 60S subunits. Three components remained in the gradient — one small peak around 15—18S and two peaks in the 4S-/5S-region (Fig. 7).

Discussion

The monosome-polysome profiles of sliced tissue are one measure of the profound metabolic changes known to occur in sliced aerated plant storage tissues. As demonstrated by LEAVER and KEY for carrot disks [6] and CHERRY for sugar beet slices [5], rapid polysome formation ensues slicing and this can be visualized in the electron microscope [23]. In sliced potato tissue maximal aggregation of ribosomes into polysomal structures is reached only after 9 hours (Figs. 1 and 2). This development is prevented by Actinomycin D, indicating the involvement of transcription (Table 1).

Ribosomes of fresh potato tissue are not capable of incorporating labeled precursors (^3H-uridine; ^{32}P). Likewise ^{14}C-leucine does not label the polysomal fraction of sucrose gradients. After 15 hours of aging, however, these tracers are very actively incorporated with especially high specific activity in the polysomes (Fig. 3). The simultaneously enhanced incorporation of leucine is an indication for polypeptide synthesis on these polysomes. The incorporation of ^{32}P and ^3H-uridine reflects in part synthesis of rRNA and tRNA (Fig. 5), but short pulses allow clear incorporation into rapidly labeled RNA compounds only.

One of these rapidly labeled RNA species appears in sucrose gradients of LiCl-extracted, pulse labeled ribosomes from aged disks between the 16S—rRNA and the 5S—RNA. It can be located by its high specific activity which precedes extensive labeling of the 16S—rRNA and which can be chased with cold $PO_4^{3\ominus}$ (Fig. 5). Usually a small peak of optical density coincides with this heavily labeled region. This complex is absent in freshly prepared disks and its appearance during aging can be suppressed by Actinomycin D. All these properties suggest that this component is a messenger-RNA-complex.

[22] G. KAHL, Z. Naturforsch., in press [1971].
[23] L. C. FOWKE and G. SETTERFIELD, in: Biochemistry and physiology of plant growth substances, p. 581, ed. WIGHTMAN, Setterfield 1968.

LiCl-extraction of RNAs from isolated ribosomes, however, destroys the coding properties of mRNAs (as has been shown with cerebral mRNA; P. MANDEL, pers. comm.). Therefore separation of mRNAs from ribosomes was achieved by EDTA. If isolated ribosomes from intact potato tubers are dissociated into subunits and RNAs by EDTA, sucrose density gradient profiles show the presence of the 60S- and 40S-subunits and 5S- and 4S – RNAs. EDTA-dissociation of ribosomes from aged tissue slices, however, releases an RNA-fraction, which is not present shortly after slicing, which sediments at 15—18S and which has a different base composition from that of both 16S – rRNA and 5S/4S – RNA (Table 2). The appearance of this compound during aging of the slices is prevented by Actinomycin D (20 μg/ml). Pooled from sucrose gradients, this fraction ("fraction I") is able to enhance ribosomal activity in vitro [22].

Taken altogether these findings support the hypothesis, that the RNA-compound designed as "fraction I" represents mRNA(s), which is (are) synthesized after slicing the tissue as a consequence of partial derepression of the previously repressed genome [1] of the cells.

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Activation of Protein Synthesis in Aging Potato Tuber Slices

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Whereas ribosome preparations of freshly sliced potato disks do not show appreciable activity in an in-vitro amino acid incorporation system, aging of the tissue leads to a greatly enhanced incorporation activity which reaches its maximum 24 hours after slicing. If ribosomes from freshly excised disks are provided with polyuridylic acid, their activity in the incorporation of phenylalanine is increased about 8 fold.

Moreover, an RNA-fraction can be dissociated by EDTA from ribosomes of aged potato tuber slices, which sediments at 15—18S, has a base composition different from that of 16S—rRNA, 5S- and 4S—RNA, and is not present on ribosomes of fresh slices. Its appearance is inhibited by actinomycin D and therefore most probably dependent on transcription. This compound, purified from sucrose gradients, enhances in vitro leucine incorporation into peptide material by ribosomes of fresh potato slices.

The possibility is discussed that this fraction-among other factors-is responsible for the enhanced protein synthesis after slicing plant storage organs, and is indicative of a general derepression phenomenon in these tissues.

If bulky plant storage tissues, i. e. potato tubers, are sliced into thin disks, a vigorous metabolic activation ensues. Most processes, which are enhanced after slicing, have been shown to be dependent on protein synthesis. Thus formation of various proteins is one of the earliest consequences after excision of the tissue disks. This is indicated by a rapid aggregation of ribosomes into polysomal structures [1, 2] and an enhancement of ribosomal activity in in-vitro incorporation systems.

Increased polysome formation after slicing seems to be due to greater availability of m-RNA, since poly-uridylic acid added as an artificial messenger strikingly activates ribosomes from fresh slices [1, 15]. The present paper reports, that the enhancement of incorporation activity can also be achieved with native RNA-fractions, dissociated from polysomes of aged potato disks. This result supports the concept that ribosomal action in the intact storage organs is limited by availability of newly synthesized messenger.

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