

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  $\mu\text{g}/\text{ml}$ ). Pooled from sucrose gradients, this fraction ("fraction I") is able to enhance ribosomal activity *in vitro*<sup>22</sup>.

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<sup>1</sup> 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<sup>1,2</sup> 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<sup>1,15</sup>. 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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## Material and methods

The plant material and its treatment has been reported in detail and ribosomes were isolated essentially as described previously<sup>3</sup>.

### *In vitro* incorporation

The ribosomal pellet was dissolved by gentle stirring in a small volume of TKM-buffer (0,05 M Tris-HCl, pH=7,8; 0,015 M KCl; 0,01 M MgCl<sub>2</sub>) and used as such for the *in-vitro* incorporation test. The test mixture consisted of: 0,5 mM Tris-HCl, pH=7,6, 0,05 mM KCl, 8 mM MgCl<sub>2</sub>, 0,01 mM phosphoenolpyruvate, 50  $\mu$ g pyruvate kinase, 5 mM ATP, 0,5 mM GTP, 1 mg of the high speed supernatant protein, 50  $\mu$ g bovine serum albumin, 0,02 mM 2-mercaptoethanol, 1  $\mu$ C-leucine-UL (240 mc/mM).

The reaction was started by the addition of 1–2 mg ribosomal protein in a total volume of 1 ml. Reaction temperature was 30 °C. After appropriate time intervals the incubation was stopped by the addition of 5 mg bovine serum albumine as carrier and 2 ml 10% trichloroacetic acid. The protein was collected by centrifugation, washed with 5% TCA, heated for 15 min at 90 °C, again centrifuged and washed with 5% TCA, collected on glass fiber filters and washed with ethanol: ether (1:1) and diethylether successively. The oven-dried filters were counted in a Beckman liquid scintillation system.

### *Preparation of high speed supernatant*

Initially the aminoacyl-sRNA-synthetases were prepared by a two-step ammonium sulfate precipitation. But for unknown reasons this preparation was far less active than a dialyzed high speed supernatant obtained from ribosomes, even though sRNA was present in abundance. Therefore the supernatant of the final centrifugation step during isolation of ribosomes was extensively dialyzed against several changes of 0,005 M Tris-HCl, pH 7,6; 0,008 M MgCl<sub>2</sub> and 0,002 M 2-mercaptoethanol in the cold. Small portions of this solution were kept frozen at –20 °C. None of the preparations used was older than two weeks.

The RNA content of the ribosomal suspensions was determined by UV-absorption, protein estimations were made according to the micro-biuret procedure of ITZHAKI et al.<sup>4</sup>.

The preparations were checked for homogeneity by drying an aliquot of the clear ribosomal suspensions on a copper grid and adding one drop of 1% uranyl-acetate. After the surplus has been drawn off with filter paper, the preparation was visualized with a Siemens Elmiskop IA electron microscope.

### *Concentration of RNA-fraction I*

This RNA-compound was separated by sucrose density gradient centrifugation as already described<sup>3</sup>. Fractions from twelve gradients were collected, extensively dialyzed against several changes of ice-cold TKM-buffer and lyophilized for some hours. The residue was dissolved in 0,05 M Tris-HCl, pH 7,8, con-

taining 0,01 M KCl, and the RNA was precipitated with three volumes of ethanol at 0 °C overnight. The RNA was collected by centrifugation, washed, air-dried and stored at –20 °C. This preparation is active for at least two days.

## Results

Ribosomes from freshly prepared potato slices slowly incorporate amino acids into polypeptide material. Aging of tissue for 15 hours is sufficient to enhance this incorporation activity about fivefold (Fig. 1). Characteristically, most of the incorpora-

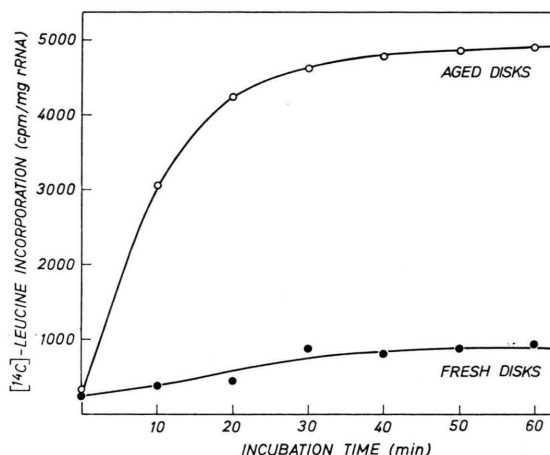


Fig. 1. Kinetics of incorporation of [<sup>14</sup>C]-L-leucine into peptide material by isolated ribosomes from freshly sliced and aged potato tuber disks. Aging time: 15 hours. Assay conditions were essentially as described in Material and Methods.

tion was complete after only 20 minutes. Best results were obtained by using a reconstituted amino acid mixture as substrate. Phenylalanine was incorporated only poorly; leucine was markedly better.

The ability of isolated ribosomes to incorporate <sup>14</sup>C-leucine varies according to the time the tissue has aged before the ribosomes are isolated (Fig. 2). Maximum incorporation is observed with ribosomes from tissue aged for 24 hours; after this it drops back to lower levels (Fig. 2).

Isolated ribosomes from freshly excised tissue are not inactive per se, but evidently lack one or several stimulating factors. If fresh ribosomes are incubated together with artificial messenger (in this case 100  $\mu$ g poly-uridylic acid per reaction) the incorporation of <sup>14</sup>C-phenylalanine can be markedly increased (Fig. 3).

As has been reported<sup>3</sup>, ribosomes from aged tissues are easily dissociated into subunits and some

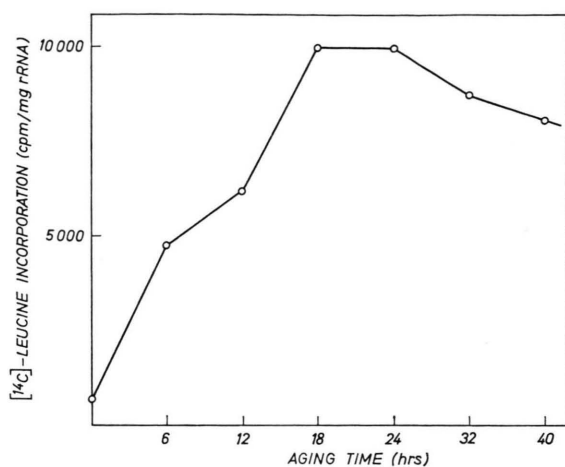


Fig. 2. In vitro protein synthesis by ribosomes from potato tuber disks aged for different periods of time.

RNA-components, two of which are most likely 5S- and 4S-RNA. One component, tentatively characterized and designed as fraction I, was pooled from a series of sucrose gradients (Fig. 5), concentrated and checked for a possible activity in the in-vitro-system (Fig. 4).

The addition of fraction I doubled the incorporation capability of ribosomes from fresh potato slices. Fraction I lost this property after one week of storage at  $-20^{\circ}\text{C}$ . In contrast to the rapidly occurring effect of poly-U, enhancement of  $^{14}\text{C}$ -leucine-incorporation into TCA-insoluble material

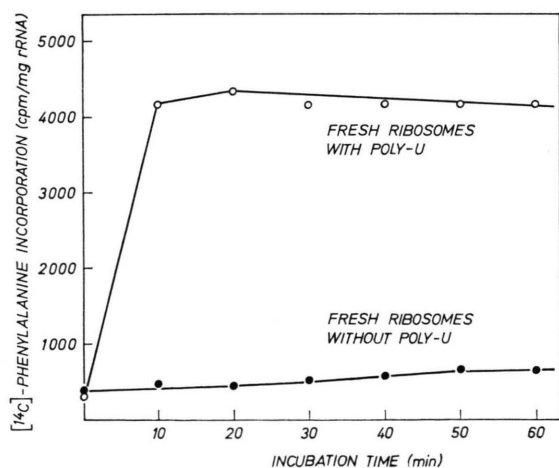


Fig. 3. Kinetics of  $^{14}\text{C}$ -L-phenylalanine incorporation by ribosomes of the intact potato tuber in the presence (O-O) and in the absence (●-●) of poly-U ( $100\ \mu\text{g/ml}$ ). The incubation medium was identical to that described in Material and Methods except  $1\ \mu\text{C}$  [ $^{14}\text{C}$ ]-phenylalanine ( $280\ \text{mCi/mmole}$ ) was used as substrate and the concentration of  $\text{MgCl}_2$  was doubled.

by fraction I only gently increased with time and reached its maximum after 60 min of incubation. Stimulation of ribosomal activity with different preparations of fraction I ranged from 65 to 130% over the controls.

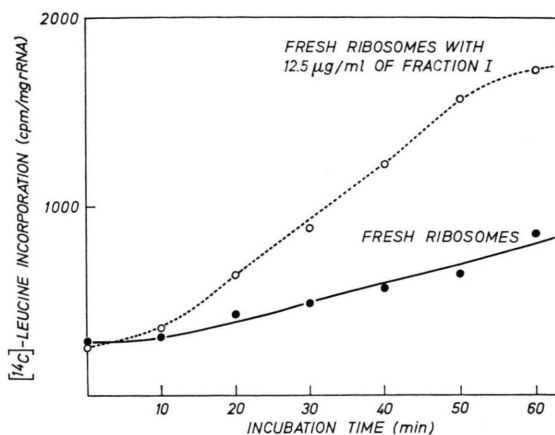


Fig. 4. Kinetics of incorporation of  $^{14}\text{C}$ -L-leucine by ribosomes of freshly sliced potato tuber tissue in the absence (●-●) and in the presence (O-O) of RNA-fraction I. This RNA fraction was pooled from sucrose gradients as described in Material and Methods.

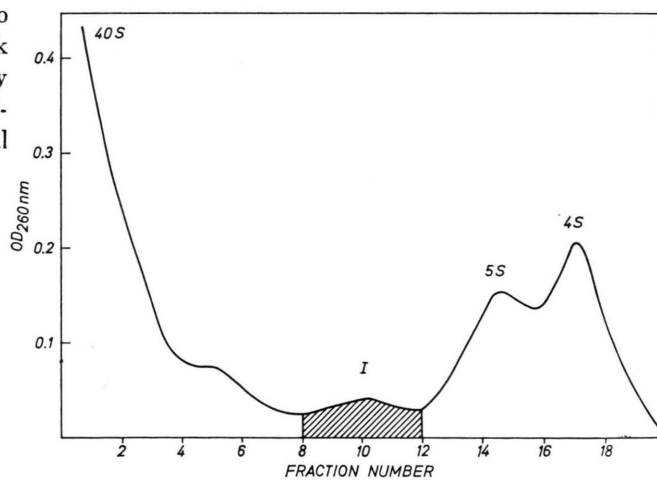


Fig. 5. Sedimentation profile of the 40S-subunit, 5S- and 4S-RNA and fraction I, liberated from ribosomes of aged potato tuber disks by EDTA. Isolated ribosomes were dissolved in  $10\ \text{mM}$  Tris-HCl, pH 7.4;  $15\ \text{mM}$  KCl and  $15\ \text{mM}$  EDTA and centrifuged in a linear 15–25% sucrose gradient for 18 hours at  $+3^{\circ}\text{C}$  and  $140000\ g$  in a SW 41 rotor (Spinco model centrifuge).

## Discussion

The rapidly occurring polysome formation in potato tuber tissue in response to slicing is roughly

paralleled by an enhanced ribosomal capacity to incorporate aminoacids into peptide material in vitro. The increase in ribosomal activity is characteristic for sliced tissue from various plant storage organs. It has been reported by LEAVER and KEY<sup>1</sup> for carrot disks and by ELLIS and MACDONALD<sup>2</sup> for red beet slices. Whereas cells of carrot slices evidently reach maximal incorporation after as little as 3–4 hours of aging<sup>1</sup>, ribosomes of red beet and potato slices attain the maximum only after 24 hours, and afterwards decrease in this activity<sup>2</sup> (Fig. 2). Also, the very high incorporation rates of ribosomes from aged carrot slices<sup>1</sup> never have been found even with the most carefully isolated ribosomes from aged potato slices (Fig. 2). Thus slicing induces the same trend at the ribosomal level of the cells from different organs, but speed and also intensity of the activation vary to some extent.

With ribosomal preparations from carrot and red beet slices, it has been demonstrated that bacterial contamination is not a major factor in these experiments and is not responsible for the enhanced incorporation rate of aged disks. Electron microscopic examination of the ribosomal pellet from fresh and aged potato disks proved it to consist exclusively of ribosomes with globular appearance and an average diameter of 150 Å. Several bacteriostatics (Penicillin G, Chloramphenicol) did not influence the in vitro amino acid incorporation by ribosomes of aged potato disks.

In freshly sliced red beet tissue, ELLIS and MACDONALD<sup>2</sup> found the content of activating enzymes and t-RNA in the high speed supernatant enough to permit good incorporation rates. Since interference of an inhibitor has also been ruled out, low ribosomal activity shortly after slicing must be due to

other factors. In view of the stimulating effect of polyuridylic acid on ribosomes from fresh tissue (Fig. 3)<sup>1,15</sup>, lack of native messenger-RNA may be one of these factors restricting protein synthesis in the intact organs. Slicing would then lead to mRNA synthesis. It is suggested that fraction I (Fig. 4, Fig. 5) contains mRNA, although there is only indirect evidence for this, since the RNA component of fraction I – possibly heterogenous – has not been investigated in detail. Thus stimulatory effects of high molecular weight RNAs others than mRNA are well known and probably due to protection of endogenous m-RNA on the ribosomes<sup>5,6</sup>. Last not least, higher radioactivity in TCA-precipitable material does not allow firm conclusions as to the nature of the product.

Despite these uncertainties it remains an attractive possibility, that slicing initiates differential gene activity, resulting in formation of ribosomal, transfer and messenger RNA which altogether enable the ribosomal population to carry out protein synthesis. An indication of genetic derepression is the higher template availability and enhanced DNA-dependent RNA polymerase activity found in sliced sugar beet tissue<sup>7</sup>, both of which are responsible for the synthesis of rRNA<sup>1-3,8-12</sup>, tRNA and messenger RNA<sup>13,14</sup>, part of which may appear as fraction I on ribosomes of aged potato slices. The increase in number of ribosomes and provision of messenger RNAs ultimately results in the striking change in metabolism of aging plant storage tissue slices.

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