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Temperature Characteristics and Adaptive Potential of Wheat Ribosomes

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ABSTRACT

The translational efficiency of wheat ribosomes was studied as a function of an *in vivo* temperature pretreatment of wheat seedlings (*Triticum aestivum* L.). Ribosomes were isolated from heat-pretreated (36°C) and reference (4°C, 20°C) wheat seedlings. The efficiency of the ribosomes in translating polyuridylic acid was assayed. Ribosomes from heat-pretreated seedlings exhibit a threefold enhanced incorporation rate of phenylalanine as compared to ribosomes from wheat seedlings adapted to 20 or 4°C. This difference develops within 24 hours after onset of the heat treatment of seedlings following a 3 hour lag phase. The temperature induced changes can be traced back to the cytoplasmic ribosomes, since cycloheximide inhibits translation almost completely. Thermal inactivation of ribosomes occurs at 45°C, irrespective of the temperature pretreatment of the wheat seedlings. Specific differences in the yield of ribosomes, in the polyribosomal profiles, and in the apparent Arrhenius' activation energy of protein synthesis were observed depending on the age and the temperature pretreatments. The results presented here are considered an important molecular correlation to phenotypical temperature adaptation of *in vivo* protein synthesis in wheat (M Weidner, C Mathée, FK Schmitz 1982 Plant Physiol 69: 1281-1288).

Wheat seedlings exhibit phenotypical, *i.e.* nongenetic temperature adaptation of protein synthesis. The optimum temperature of [^{14}C]leucine incorporation into the total protein fraction depends on the growing temperature (preadaptation treatment) of the seedlings. In 10 d old plants kept for 48 h at either chilling (4°C), or medium (20°C) or high (36°C) temperature, the protein synthesis optima are at 27, 31, and 35°C, respectively. Further, the temperature coefficient μ, calculated from Arrhenius plots of *in vivo* protein synthesis rates is shifted to higher values with rising preadaptation temperature. The respective values are 59.6 kJ·mol^{-1}, 76.4 kJ·mol^{-1}, and 99.1 kJ·mol^{-1} (24–26). To evaluate the molecular basis of this temperature adaptation phenomenon, ribosomes from wheat seedlings pretreated at different temperatures *in vivo* were investigated with regard to their translational efficiency, thermal stability, inhibitor susceptibility, and the apparent activation energy of polyphenylalanine synthesis. In addition, polyribosomal profiles were recorded.

MATERIALS AND METHODS

Plant Materials and Chemicals. Seed (*Triticum aestivum* L. var Kolibri) was obtained from the F. von Lochow Breeding Co. (Northeim, FRG). Wheat germ (type I), heparin (sodium salt), lincomycin (hydrochloride), and cycloheximide were supplied by Sigma (Taufkirchen, FRG). ATP (disodium salt), GTP (dilithium salt), creatine phosphokinase (EC 2.7.3.2, from rabbit muscle), and poly(U) (potassium salt) were bought from Boehringer-Mannheim (Mannheim, FRG). Sucrose (for density gradient ultracentrifugation) was obtained from Merck (Darmstadt, FRG). L-Phenyl-2,3-[^{3}H]alanine (1.11 TBq·mmol^{-1}) was provided by Amersham-Buchler (Braunschweig, FRG). Membrane filters (cellulose acetate, pore size 0.45 μm) were supplied by Sartorius (Göttingen, FRG). All other reagents were 'analytical grade'.

Growth Conditions. Wheat seedlings were grown on Vermiculite at 20 ± 0.5°C under continuous irradiation (Sylvania Cool White fluorescent tubes, light intensity approximately 100 μE·m^{-2}·s^{-1}). Eight d old seedlings were subjected to 4, 20, or 36°C temperature pretreatments for 48 h, unless stated otherwise.

Isolation of Ribosomes and Measurement of *in Vitro* Protein Synthesis. Wheat shoots were frozen in liquid N_2 pulverized and ground in 4 volumes (ml g^{-1}) of extraction buffer consisting of 200 mM Tris-HCl (pH 8.5), 200 mM sucrose, 50 mM $MgCl_2$, 60 mM KCl and 5 mM DTT. The extract was strained through four layers of gauze and centrifuged for 10 min at 29,000 *g*. This process and the subsequent operations were carried out at 2 to 4°C. The supernatant was filtered through Miracloth and centrifuged again as above. The ribosomes from 7.5 ml of the resulting supernatant were pelleted through a 4 ml layer of 1.75 M sucrose (in 40 mM Tris-HCl [pH 8.5], 10 mM $MgCl_2$, and 20 mM KCl) in 75 × 15 mm tubes by centrifuging for 2 h at 240,000 g_{av} (6). The pellets, rinsed once, were resuspended in 20 mM Hepes-KOH (pH 7.6), containing 5 mM Mg-acetate, 125 mM K-acetate, and 6 mM 2-mercaptoethanol. This ribosome preparation was used for measuring the translation rates. The wheat germ postribosomal supernatant was prepared from the 30,000 *g* supernatant of a wheat germ extract (16) by centrifugation for 2 h at 240,000 g_{av}. The supernatant was passed through a Sephadex G-25 column, preequilibrated with grinding buffer (16) and the protein-rich, faintly yellow fractions were pooled, frozen in liquid N_2, and stored at −20°C. The standard wheat germ system (16) was slightly modified to optimize translation of poly(U) by ribosomes from wheat shoots. In a final volume of 25 μl, the reaction mixture contained 20 mM Hepes-KOH (pH 7.6), 3 mM Mg-acetate, 100 mM K-acetate, 2 mM DTT, 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, 1 μg creatine phosphate kinase, 500 μM spermidine, 5 μl wheat germ postribosomal supernatant, 1 μCi L-phenyl-2,3-[^{3}H]alanine 30 Ci/mmol, 2.5 μg tRNA prepared from wheat shoots (23), 10 μg poly(U), and 5 μl ribosomes. The reaction was stopped by addition of 0.5 ml ice-cold 10% TCA. The acid-insoluble material was collected on a cellulose acetate filter (exclusion limit 0.45 μm) and rinsed with 10% TCA. The filters were washed successively with 5% TCA, ethanol/diethylether (3:1, v/v) and diethylether. The dried filters were counted for radioactivity, using *p*-terphenyl/POPOP/toluene (4g/100 mg/1L) as scintillation mixture.

For fractionation of polyribosomes 7 d old wheat seedlings were temperature-treated at 4, 20, or 36°C for 24 h. Extraction was performed as described previously, except that the time for ultracentrifugation was set at 75 min. The ribosomal pellet was rinsed once and suspended in 0.5 ml of 40 mM Tris-HCl (pH 8.5), containing 20 mM KCl and 10 mM $MgCl_2$. Seven A_{260}-units of ribosomal material were layered on a 100 to 500 mg·ml^{-1} sucrose gradient (in Tris-buffer) and centrifuged at 188,000 g_{av} for 65 min in a Beckman rotor SW 41 Ti. Gradients were withdrawn with a density gradient removing apparatus (Auto Densi Flow, Haake-Buchler, Saddle Brook NJ) and scanned at 254 nm with a Serva-Chromatocord absorbance monitor (Serva, Heidelberg, FRG). The peak areas of the polysome profiles were calculated planimetrically using a Kontron MOP-AMO2 image analyzer (Kontron, Eching, FRG).

RESULTS

***In Vitro* Translation Rates in Dependence on Incubation Time and Ribosomal Concentration.** The translational efficiency of ribosomes, obtained from wheat plants adapted to medium (20°C) and high (36°C) temperature, was tested, employing a wheat germ postribosomal supernatant. The translation of endogenous mRNA was excluded, because no other amino acids besides phenylalanine were present in the assay cocktail. All data, presented here, result from subtraction of the 'minus poly(U)'-values (background) from the poly(U)-stimulated phenylalanine incorporation rates. The use of poly(U) offers certain advantages as compared to natural mRNA when, as in our case, interest is focused on translation rates rather than on translation products. Saturating conditions with respect to messenger concentration can be more easily (and economically) established in the test system, thus improving reproducibility. Since poly(U)-translation does not depend on the initiation step, the temperature characteristics thereof do not interfere with the investigation of temperature effects and thermal stability of the ribosomes themselves.

When the concentrations of all components of the translation system are carefully adjusted to saturation levels, the rate of polyphenylalanine synthesis is strictly proportional to incubation time up to 30 min at a given ribosome concentration (normally 5 A_{260} units). Only an incubation time from ≧60 min leads to a pronounced decrease of the phenylalanine incorporation rate (Fig. 1). These results indicate that conditions can be established where the rate of polyphenylalanine synthesis is limited by and proportional to the amount of ribosomes present (Fig. 1, inset). The curves deviate from linearity at ribosome concentrations higher than approximately 3 A_{260} units. Maximum phenylalanine incorporation is reached at 6 to 7 A_{260} units of ribosomes. The optimum ribosome concentration for the *in vitro* test system is not different for ribosomes isolated from 20 and 36°C-pretreated wheat shoots. There is a marked difference in the translation potential of ribosomes from heat-pretreated and nonheat-pretreated wheat seedlings, independent of incubation time and actual ribosome concentration. The ribosomes from plants, pretreated at 36°C exhibit approximately 3-fold higher rates of poly(U)-translation than ribosomes from wheat seedlings adapted to 20°C. To exclude a messenger shortage, poly(U) is added again after 60 min of incubation. This does not result in an enhancement of protein synthesis; the slight decrease in incorporation can be traced back to the mere dilution of the sample. On the other hand, supplementary addition of wheat germ ribosomes leads to a marked enhancement of phenylalanine incorporation in the sample originally containing ribosomes from nonheatadapted seedlings. Wheat germ ribosomes were prepared in the same manner as ribosomes from wheat plants, except that wheat germ was ground with 5 volumes (ml g^{-1}) of extraction buffer and quartz sand.

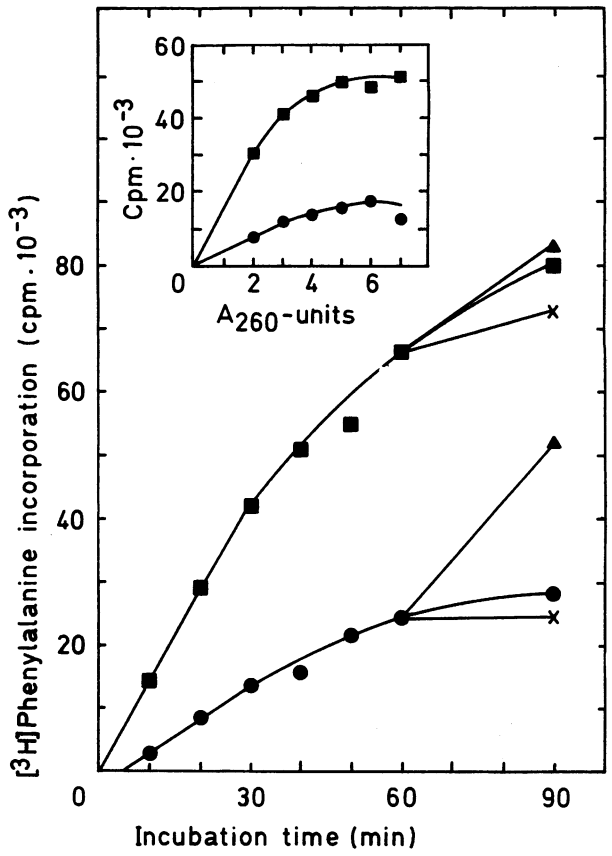

FIG. 1. Time course of [^{3}H]phenylalanine incorporation by a cell-free protein synthesizing system which is composed of a wheat germ postribosomal supernatant and wheat ribosomes isolated from 20°C (●) and 36°C (■) pretreated wheat seedlings, respectively (inset, same symbols). The translational efficiency of the ribosomes was measured at 25°C incubation temperature. Background ('minus poly(U)'-incorporation rates) was subtracted. After 60 min of incubation, 5 μl (10 μg) of poly(U) (×) or 5 μl (25 A_{260}-units·ml^{-1}) of wheat germ ribosomes (▲) were added. Inset, [^{3}H]phenylalanine incorporation in relation to ribosome concentration, measured at 35°C after 30 min incubation.

These results provide strong evidence that the translation of poly(U) is not limited by any assay component but only by the translational efficiency of the wheat ribosomes. Only a slight increase in phenylalanine incorporation can be observed in ribosomes from heat pretreated shoots following the addition of wheat germ ribosomes. The enhancement is more pronounced when the dilution of the assay mixture is taken into account. It can be demonstrated that the translational activity of the test system with wheat germ ribosomes guarantees a maximal incorporation of [^{3}H]phenylalanine of approximately 160,000 cpm. Summarizing, under the test conditions chosen, the poly(U)-stimulated incorporation rates are a reliable measure for the translational capability of the ribosomes and the approximately 3-fold increase observed in case of 36°C-ribosomes results from an enhanced translational efficiency as compared to 20°C-ribosomes. This finding is not restricted to heat pretreated wheat plants, because ribosomes isolated from heat pretreated barley plants show enhanced translational efficiency as well (approximately 1.5-fold; data not shown).

***In Vitro* Translation Rates as a Function of Duration of the Heat-treatment *in Vivo*.** Wheat seedlings were exposed to 36°C for different periods of time in order to evaluate the time-course of the enhancement in translational efficiency of the ribosomes. The time schedule of the experiments was arranged in such

manner that the age of the plants was identical (10 d) at the end of the different heat treatment periods. After a lag-phase of about 3 h, the stimulation of translational efficiency develops within 24 h and remains constant at least until 48 h after onset of the heat treatment (Fig. 2).

Inhibition of *in Vitro* Protein Synthesis. The application of inhibitors of protein synthesis should provide information about the share of cytoplasmic (80S) and chloroplastic and mitochondrial ribosomes in the enhancement of translational efficiency. Cycloheximide in a concentration of 0.1 mM reduces the translation rate to approximately 30%, a 1 mM solution leads to a rate of maximal 10% of the control, while lincomycin is practically noninhibitory at these concentrations (Table I). Ribosomes from heat-adapted wheat plants were inhibited somewhat less than those from seedlings grown continuously at 20°C. In conclusion, the results presented here reflect the behavior of cytoplasmic ribosomes, while the contribution of 70S ribosomes is negligible.

Thermostability of Ribosomes *in Vitro*. The temperature stability of ribosomes was investigated by applying a 5 min preincubation period of the ribosomes at temperatures up to 55°C prior to testing polyphenylalanine synthesis for 60 min at 25°C. The resuspension buffer for the ribosomal pellet was supplemented with RNase inhibitor heparin in a concentration of 1 mg·ml^{-1} because of ribonuclease activity, especially at high temperature, during preincubation. Ribosomal integrity is maintained up to 45°C, independent of the preceding temperature pretreatment of the plants (Fig. 3). At temperatures beyond 45°C, a rapid decline of protein synthesis capability occurs, indicating thermal degradation of the ribosomes. Ribosomal activity is reduced to 60% subsequent to a 50°C preincubation; preincubation at 55°C results in cessation of translation.

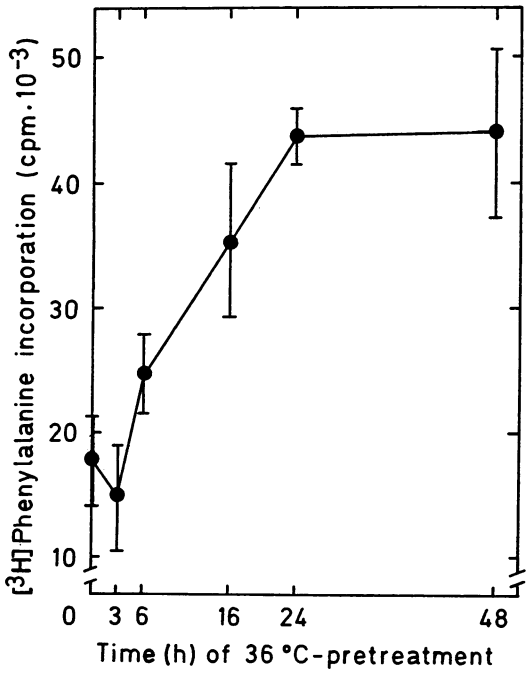

FIG. 2. Time curve for the adaptive enhancement of translational efficiency of wheat ribosomes induced by a 36°C-pretreatment of the seedlings. Incorporation of [^{3}H]phenylalanine was measured after 60 min at 25°C. Bars, SE (n = 3).

Table I. *Effect of Inhibitors of Protein Synthesis on [^{3}H]Phenylalanine Incorporation, Employing Ribosomes Isolated from 20°C and 36°C Pretreated Wheat Seedlings*

Incorporation was measured after 60 min at 25°C. All values are means ± SE from three experiments.

	Relative [^{3}H]Phenylalanine Incorporaton Rate	
	20°C-ribosomes	36°C-ribosomes
	%	
Control	100	100
+ 0.1 mM cycloheximide	20 ± 2.5	37 ± 4.5
+ 1.0 mM cycloheximide	1 ± 5.7	10 ± 1.0
+ 0.1 mM lincomycin	101 ± 6.1	97 ± 3.6
+ 1.0 mM lincomycin	108 ± 9.7	101 ± 4.9

Temperature-curves and Apparent Arrhenius Activation Energy of *in Vitro* Translation. In order to evaluate whether wheat ribosomes reflect the *in vivo* adaptive shift (24) in the temperature coefficient (μ) and in the optimum temperature of protein synthesis in wheat, the temperature-curves of polyphenylalanine synthesis have been determined, using ribosomes from 4, 20, and 36°C-pretreated plants. The age-dependent changes in ribosomal efficiency were also investigated employing ribosomes from younger wheat shoots (8 d old instead of 10 d) grown at 20°C. No difference in ribosomal efficiency can be observed between plants pretreated at chilling and medium temperatures (Fig. 4). Ribosomes isolated from 8 d old plants exhibit only a slightly higher translational efficiency. This indicates that plant age does not pronouncedly affect translational efficiency during the temperature pretreatment periods.

Q_{10} values and the apparent Arrhenius activation energies of *in vitro* protein synthesis are given in Table II. The Q_{10} values are about 3 for ribosomes from plants grown at 20°C for 8 d as well as for plants subjected subsequently to a 48 h temperature treatment at 4 and 20°C. A significantly lower Q_{10} value of 2.2 can be estimated for translation with ribosomes from heat adapted seedlings. Accordingly, apparent Arrhenius activation energy is approximately 82 k J·mol^{-1} for 4 and 20°C-pretreated plants as well as for 8 d-plants, but only 55 kJ·mol^{-1} for 36°C-preadapted plants.

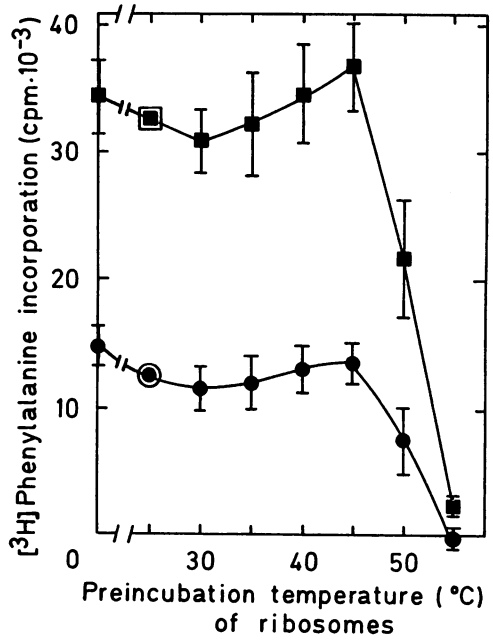

FIG. 3. Thermostability of wheat ribosomes determined following a 5 min preincubation period at temperatures as indicated. [^{3}H]phenylalanine incorporation was measured at 25°C (60 min). Symbols are as for Figure 1. Data were standardized on the mean of 25°C-preincubation values (◉, ■). Bars, SE (n = 5).

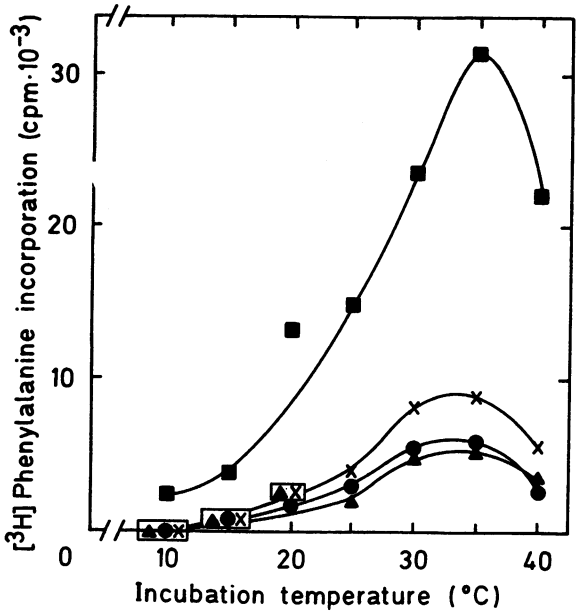

FIG. 4. Temperature dependence of polyphenylalanine synthesis. Ribosomes were isolated from 10 d old plants, pretreated at 4°C (▲), 20°C (●), and 36°C (■) for 48 h and also from wheat seedlings grown for 8 d at 20°C (×). [^{3}H]phenylalanine incorporation was measured after 30 min.

The temperature optima of polyphenylalanine synthesis do not differ between these various origins of ribosomes. Maximum incorporation rate of phenylalanine is reached at approximately 35°C (Fig. 4). Hence, an unidentified component of the translation system besides the ribosomes must be rate limiting beyond 35°C, considering the fact that *in vitro* temperature stability of ribosomes is maintained up to 45°C (Fig. 3).

Yield of Ribosomes. The quantity of ribosomes isolated from a given amount of wheat shoot material differs considerably depending on the age of plants and also depending on the *in vivo* temperature pretreatments (Table III). During aging of the seedlings (8 d→10 d) the yield of ribosomes decreases by about 30%. High temperature treatment of the wheat seedlings leads to a smaller loss in ribosomal quantity of only 15%. On the other hand, chilling of the seedlings at 4°C leads to an increase of ribosome yield of more than 10%.

Polyribosome Profiles. Polyribosomes were isolated from 8 d old wheat seedlings which were pretreated for 24 h at chilling (4°C), medium (20°C), and high (36°C) temperature prior to fractionation by density gradient centrifugation. In this experiment, the preadaptation period was limited to 24 h, which is the minimum time for completion of the adaptive changes in ribosomal efficiency (Fig. 2). In case of 20°C plants the proportion of monosomes (including subunit material) amounts to about 25% of the total ribosomal yield. After a 24 h high temperature treatment, the share of monosomes is reduced to 15%. This shift in the ratio of monosomes to polysomes is even more pronounced after a 4°C treatment; monosomes amount to only 10% of the total (Fig. 5). This result shows that low as well as high temperature treatments of the wheat seedlings possibly stimulate polyribosome formation as an expression of enhanced translation of mRNA.

Difficulties may arise in the quantitative interpretation of polyribosomal patterns because of a loss of small ribosomal material (monosomes, subunits) during sucrose pad centrifugation. The recovery of ribosomal material is proportionately reduced with increasing percentage of monosomes and subunits present in the material, which is being investigated (6, 7, 10). Furthermore, the main portion of membrane-bound polyribosomes assumably is lost with the mitochondrial pellet. However, the qualitative differences in the ribosomal profiles are obvious, even when the proportion of monosomes and subunits is underrepresented and the membrane-bound polyribosomes are not included. In young plants, like *e.g.* wheat seedlings of this age, free ribosomes are predominant (1, 14).

DISCUSSION

A heterologous cell-free translation system has been employed to determine the translational efficiency of wheat ribosomes isolated from seedlings preadapted to different temperatures.

The ability of wheat ribosomes to synthesize polyphenylalanine is enhanced about 3-fold after a 48 h high-temperature (36°C) treatment of the wheat shoots. Enhancement of ribosomal efficiency by exogenous factors has been reported also for ribosomes isolated from maize seedlings after a 3 h exposure to light, which were approximately twice as efficient in poly(U)-directed phenylalanine incorporation as ribosomes from plants kept in the dark (22). The activities of maize ribosomes were increased about five times by an *in vivo* GA_3 treatment (20).

Small but reproducible efficiency losses of ribosomes could be observed within 48 h when the wheat seedlings age. Some corresponding data are available about functional deterioration of ribosomes during aging. The translational activities of ribosomes from young and old mouse livers were examined in an assay system dependent on rabbit globin mRNA. Old ribosomes showed a 30 to 40% reduced activity as compared to young ribosomes (11).

The question arises as to a possible relationship of the temperature effect on the ribosomal level, described here, to the HSR.[1] Several points may be mentioned, which make it unlikely that both phenomena are closely related or that the heat induced enhancement of translational activity is just a manifestation of

[1] Abbreviations: HSR, heat shock response; E_a, apparent activation energy.

Table II. *Q_{10}-Values and E_a for the Linear Region of the Temperature Curves (Arrhenius Plot), Calculated from 15°C and 25°C Values of the Rates of Polyphenylalanine Synthesis*

Ribosomes were isolated from 10 d old wheat seedlings pretreated at 4, 20, or 36°C for 48 h and also from seedlings grown for 8 d at 20°C. [^{3}H]Phenylalanine incorporation was measured after 60 min. All values are means ± SE from five experiments (4°C pretreated seedlings only four experiments).

Temperature Pretreatment of Seedlings	Translation Rates of Ribosomes at Incubation Temperature		Q_{10}	E_a
	15°C	25°C		
	cpm			*kJ·mol^{-1}*
8d 20°C	5873 ± 1379	16915 ± 4235	2.9 ± 0.3	76 ± 8
8d 20°C + 48h 4°C	2900 ± 860	10183 ± 3019	3.5 ± 0.4	90 ± 9
8d 20°C + 48h 20°C	3502 ± 1045	10902 ± 3593	3.1 ± 0.4	81 ± 9
8d 20°C + 48h 36°C	24462 ± 6573	52903 ± 17290	2.2 ± 0.3	54 ± 11

Table III. *Yield of Isolated Ribosomes*

A_{260}-units of resuspended ribosomal pellets were determined. The value of the 8 d old wheat seedlings was set to 100%. This corresponds to approximately 7 A_{260}-units per g fresh wt of plant material. The ratio of A_{260}/A_{280} is 1.8 to 1.9 in all cases. The values are means ± SE of 18 experiments.

Temperature Pretreatment of Seedlings	Ribosomes Yield
	%
8d 20°C	100
8d 20°C + 48h 4°C	112 ± 15.5
8d 20°C + 48h 20°C	71 ± 9.3
8d 20°C + 48h 36°C	85 ± 9.3

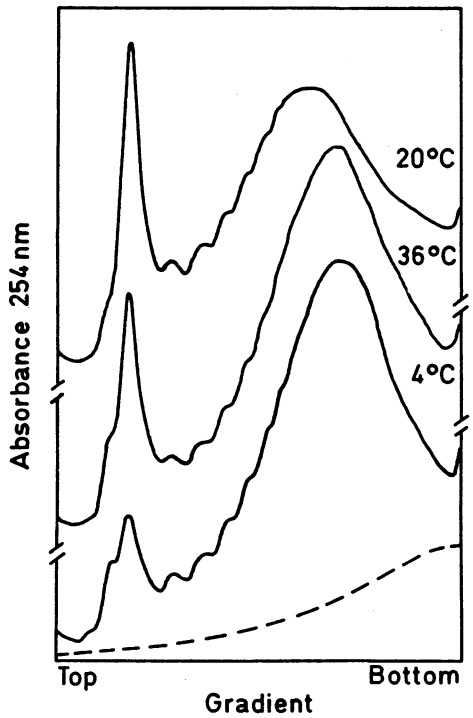

FIG. 5. Polyribosome profiles of 8 d old wheat shoots, pretreated 24 h at 20, 36, and 4°C. (---) Gradient blank.

the HSR (12). First, the *in vivo* increase in ribosomal efficiency of wheat seedlings develops gradually within approximately 24 h after onset of the heat treatment, subsequent to a 3 h lag-phase. This time course is quite different from the rapid HSR, where this would already be the time frame for recovery. Second, an enhancement of overall translational efficiency is not typically observed in HSR. Instead, a switch-over from the translation of 'control'-mRNAs to HS-mRNAs, *i.e.* a qualitative rather than a quantitative change takes place. It cannot be excluded though, that both phenomena exhibit similar features with regard to alterations in the phosphorylation pattern of ribosomal proteins (*e.g.* Protein S6). Phosphorylation levels are presently investigated (see below).

The functional stability of wheat ribosomes is maintained up to 45°C under test conditions and does not exhibit any differences at all between ribosomes from heat-pretreated and nonheat-pretreated plants. Hence, the temperature where functional breakdown of the ribosomes takes place evidently is much lower than the thermal transition point for degradation when melting occurs (28). This result is in good agreement with data from rat liver ribosomes. Ribosomal subunits, maintained at various temperatures for 5 min, were temperature-stable up to 45°C in case of the small subunit and approximately 53°C in case of the large subunit (15). Evidently, in ectothermic plant as in the case of endothermic animals (3) the thermal stability of ribosomes is not critical for the heat induced breakdown of the organisms.

The temperature optimum of *in vitro* protein synthesis carried out by 80S wheat ribosomes is reached at 35°C incubation temperature, irrespective of the preceding temperature treatment of the seedlings. Because the ribosomes are thermostable up to 45°C, a component of the wheat germ postribosomal supernatant must have been inactivated at this temperature. This result confirms findings, where warming of a reticulocyte lysate supernatant to 42°C causes a marked decrease in protein synthesis which was due either to formation of an inhibitor or thermal inactivation of an essential factor in the supernatant (9). Aminoacyl-tRNA synthetases were found to exhibit high thermolability (5, 25) and must be considered prime candidates for heat-inactivation of the protein synthesis apparatus at a relatively low temperature.

Apparent Arrhenius activation energies of about 82 $kJ \cdot mol^{-1}$ (20 $kcal \cdot mol^{-1}$) were calculated for translation rates measured with ribosomes from 10 d old wheat seedlings preadapted to chilling (4°C) and medium (20°C) temperature as well as for 8 d old 20°C-plants. High temperature reduces the E_a value to 55 $kJ \cdot mol^{-1}$ (13 $kcal \cdot mol^{-1}$). This result demonstrates a reduced immanent increase in reaction rate with rising temperature of heat adapted plants and ribosomes which, on the other hand, are characterized by a strong adaptive increase in translational efficiency. A value of 17 $kcal \cdot mol^{-1}$, reported for ribosomes from potato mitochondria (21) is in the same order of magnitude.

Polyribosome profiles have been widely used as an indicator of protein synthesis activity (13, 18, 27). Since initiation frequently appears to be the site for the physiological regulation of protein synthesis (8), polyribosome profiles have been considered a reliable measure for protein synthesis activity under varying experimental conditions (13). The polysomal profiles presented here show differences depending on the temperature pretreatment of the seedlings. The polysomal peaks are increased after heat- and, more pronounced, after chilling-treatment of the seedlings, indicating an amplification of protein synthesis initiation. However, decreased termination would also cause a buildup of larger polysomes. Temperature-dependent changes in the polysomal population also have been observed during germination of maize seeds at high temperatures (41°C). Polysome amounts were decreased compared to seeds imbibing at 28°C (17).

Some inconsistencies are recognized when the various effects of high and low temperature on wheat ribosomes are compared. Heat leads to a strongly enhanced translational efficiency but yields only minor changes in the polysomal level and the ribosomal quantity. Cold, on the other hand, causes a significant increase in the polysomal level and the ribosomal quantity, while translational efficiency is not stimulated.

In the course of phenotypical temperature adaptation, the rate of *in vivo* protein synthesis in wheat is enhanced 2- to 4-fold as a consequence of a heat pretreatment of the wheat seedlings (24, 26). Accordingly, the adaptive enhancement of translational efficiency of wheat ribosomes, induced by the same heat pretreatment of the seedlings as above, is possibly a causal relationship, essential for the functioning of the adaptation process. Nevertheless, certain discrepancies must be conceded when this correlation between temperature adaptation on the ribosomal level and on the level of *in vivo* [^{14}C]leucine incorporation into protein is evaluated in detail. First, the optimum temperature of *in vivo* protein synthesis which shifts between 27.5°C and 35°C, depending on the state of adaptation, cannot be connected to the thermal stability of the wheat ribosomes which maintain a functional condition at temperatures as high as 45°C. For this aspect of phenotypical temperature adaptation the aminoacylation step

may be crucial (25). Second, the decrease of the temperature coefficient of *in vivo* protein synthesis after adaptation of the wheat seedlings to chilling temperatures is not paralleled by a reduction of E_a of poly(U)-translation by ribosomes isolated from plants pretreated at 4°C. Furthermore, the E_a-value of polyphenylalanine synthesis is reduced rather than increased by a preceding heat treatment of the wheat seedlings. This means, the marked stimulation of translational efficiency observed occurs on the basis of an adaptive increase of the maximum rate (V_{max}) of poly(U)-translation, overriding the contrary effect of the decreased E_a. In other words, ribosomal efficiency can hardly be responsible for the alterations of temperature coefficient μ of *in vivo* protein synthesis.

Finally the question arises as to the structural correlate of the adaptive ribosomal efficiency changes. Ribosomal RNAs as well as ribosomal proteins can be affected by temperature treatment *in vivo*. Some relevant data are available about ribosomal proteins. At least 17 ribosomal proteins from cold-hardened black locust seedlings are different from those of nonhardy ones (2). Investigation of phosphorylation of ribosomal proteins has become increasingly attractive, because protein phosphorylation is affected *in vivo* by various environmental stimuli, suggesting that this process may be involved in the regulation of protein synthesis at the translational level (1). Alterations of protein S6 phosphorylation can be induced by heat shock, *e.g.* in tomato plants and in plant cell cultures (12, 19). Phosphorylation of this protein is associated with ribosomal conformational changes (4).

At present, ribosomal subunits as well as the patterns of ribosomal proteins including the modifications of phosphorylated proteins are under investigation in order to elucidate further the molecular basis of phenotypical temperature adaptation of protein synthesis in wheat.

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