Phytochemistry, 1974, Vol. 13, pp. 1303 to 1310. Pergamon Press. Printed in England.

PHYTOCHROME CONTROLLED ACID RNase: AN "ATTACHED" PROTEIN OF RIBOSOMES

GEORGE JOHN ACTON

Department of Botany, Glasgow University, Scotland

(*Received Revised* 18 *September* 1973)

Key Word Index—*Lupinus albus*; Leguminosae, hypocotyls, phytochrome; ribonuclease, ribosomes, autodegradation, binding

Abstract—The light-dependent increment in RNase activity (which is ribosome bound in cell extracts) is distributed as a gradient increasing from base to hook of lupin hypocotyls. No evidence was found of non-specific or of specific activation of pre-formed enzyme molecules following isolation, either before or after (latent activity) destruction of particles. The autodegradation capacity of ribosomes isolated from irradiated cells was almost double that of ribosomes from etiolated tissue. It is proposed that association between the bulk of the light-controlled RNase fraction and lupin ribosomes results from binding of soluble protein. It is not clear whether binding is specific or an artifact of isolation.

INTRODUCTION

MUCH attention has been focused on the possible role of particulate RNases in plants. Some authors have interpreted the association of nucleolytic activity with ribosomes (polysomes; microsomes) isolated from higher plants as having physiological significance.[1–6] On the other hand, other studies indicate that RNase activity associated with sub-cellular particles isolated from higher plants may result from contamination with soluble enzymes from the supernatant fraction.[7–9] The major aim of the work presented here was to determine if binding of the phytochrome-controlled acid RNase fraction of lupin hypocotyls[10,11] (resolved as one of two ribosome-bound isoenzymes,* ref. 11) is meaningful in terms of the *in vivo* system. Two facts support the view of a role at the ribosome site. Firstly, most of the light-mediated *increase* in the level of RNase activity is found in particulate cell fractions (taking total activity of etiolated hypocotyl homogenates as 100% then *ca* 90% is soluble and 10% particulate. On this basis total activity of homogenates of irradiated hypocotyls (24 hr, W) is *ca* 150% of which 45–60% is particulate).[11] Secondly, the enzyme is unusually firmly bound (neither high pH, nor repeated washing of pellets, nor passage of ribosomes through sucrose greatly disturbed the RNase-ribosome association.)[11]

* Resembles the E C class ribonuclease II (E C 3 1 4.23)

[1] CHERRY, J. H., HAGEMAN, R. H. and HANSON, J. B. (1962) *Radiation Res.* **17**, 740
[2] KESSLER, B. and ENGELBERG, N. (1962) *Biochim. Biophys. Acta* **55**, 70
[3] GAGNON, C. and DE-LAMIRANDE, G. (1972) *Arch. Biochem. Biophys.* **150**, 573
[4] CHANG, C. W. (1970) *Can. J. Biochem.* **48**, 450
[5] HOWE, R. C. and URSINO, D. J. (1972) *Can. J. Botany* **50**, 691
[6] BIRMINGHAM, B. C. and MACLACHLAN, G. A. (1972) *Plant Physiol.* **49**, 371.
[7] WILSON, C. M. and SHANNON, J. C. (1963) *Biochim. Biophys. Acta* **68**, 311
[8] HSIAO, T. C. (1968) *Plant Physiol.* **43**, 1355
[9] WYEN, N. V. and FARKAS, G. L. (1971) *Biochem. Physiol. Pfl. Jena* **162**, 220.
[10] ACTON, G. J. (1972) *Nature New Biol.* **236**, 255
[11] ACTON, G. J., LEWINGTON, R. J. and MYERS, A. (1970) *Biochim. Biophys. Acta* **204**, 144.

Before proceeding with this investigation it was necessary to make reasonably certain that greater amounts of assayable activity were the result of light increasing the number of active molecules in the intact cell The alternative possibility, namely that increases in assayable activity were an artifact of rupturing cells, could arise if light decreased the level of some unrelated inhibiting factor or decreased stability of ribosomes leading to earlier activation of a latent RNase (also present on particles from etiolated tissue) Experiments designed to test these possibilities will be discussed first

RESULTS

Distribution of light-sensitive ribosome-bound RNase

To determine whether the size of response was equal throughout the hypocotyl, intact etiolated seedlings were irradiated (or left in darkness; controls), cut into four equal portions and ribosomes isolated and assayed Results (Table 1, intact) show that the light-mediated increase in the level of extractable RNase activity [expressed as the relative change (D = 100%) in units/g fr wt] was maximal in the apical-quarter region [essentially similar patterns were obtained when results were expressed as units/hypocotyl (four segments), units/mg soluble protein or units/mg ribosomes] The explanation is not simple Etiolated plants have a RNase gradient increasing from apex to base (Table 1, intact) The action of light is to produce a gradient in the opposite direction, namely increasing from base to hook (Table 1, intact)

TABLE 1 DISTRIBUTION OF RIBOSOME-BOUND RNASE IN ETIOLATED AND IRRADIATED HYPOCOTYLS

Material	RNase activity (units/g fr wt) Dark Intact	Dark Excised	Light Intact	Light Excised	Change (D = 100%) Intact	Change (D = 100%) Excised
Apical quarter	5 8	7 5	15 1	16 3	260	220
Sub-apical quarter	7 7	8 9	13 9	14 2	180	160
Sub-basal quarter	9 3	9 6	13 0	12 6	140	130
Basal quarter	10 4	8 0	10 6	9 9	103	125

Results from two comparable experiments presented Experiment 1 seedlings irradiated intact 5-day-old etiolated seedlings irradiated (24 hr W) or left in dark (controls), hypocotyls cut into four equal parts of ca 2 5 cm, and ribosomes isolated and assayed Experiment 2 irradiation of excised sections hypocotyls excised from 5-day-old etiolated seedlings (safelight), cut into four equal sections grouped and floated on H_2O in Petri-dishes (30 sections 10 ml H_2O) and irradiated (24 hr W) or left in dark (controls) prior to isolating and assaying ribosomes. Whole hypocotyl controls Experiment 1 D = 8 3 W = 13 8 units g fresh wt %D = 170 Experiment 2 D = 8 3 W = 12 0 units g fresh wt %D = 145 Light-mediated change is % D control (D = 100%) to nearest 5%

Effect of wounding and ageing upon bound RNase activity

In order to obtain rapid entry of applied compounds into hypocotyl cells (necessary for pulse-chase labelling experiments) excised sections were floated on water prior to treatment To determine the size of the response in dark-excised sections, etiolated hypocotyls were cut into four equal parts using a dim green safelight and the sections obtained floated on sterilized water under light or in the dark (controls) for 24 hr The dark controls (Table 1, excised) showed the same apex to base distribution as intact seedlings Neither cutting nor ageing measurably increased the level of ribosome-bound RNase activity in the dark The combination of cutting and ageing under light did not increase activity over intact controls (Table 1) except in the basal segment The dark-excised apical quarter section was selected as the most suitable material for the following experiments

Effectiveness of continuous white and far-red irradiation

Kinetics of the rise in activity following continuous irradiation show that development of activity was preceded by a lag period of 6 hr (FR) or 6–9 hr (W) (Fig. 1). Activity rose at a linear rate between 9 and 36 hr in both cases but the rise was more rapid under white light Maximum relative increases (36 hr) over dark controls were 80% (W) and 66% (FR) on a hypocotyl basis, and 100% (W) and 75% (FR) as units/mg protein (Fig. 1).

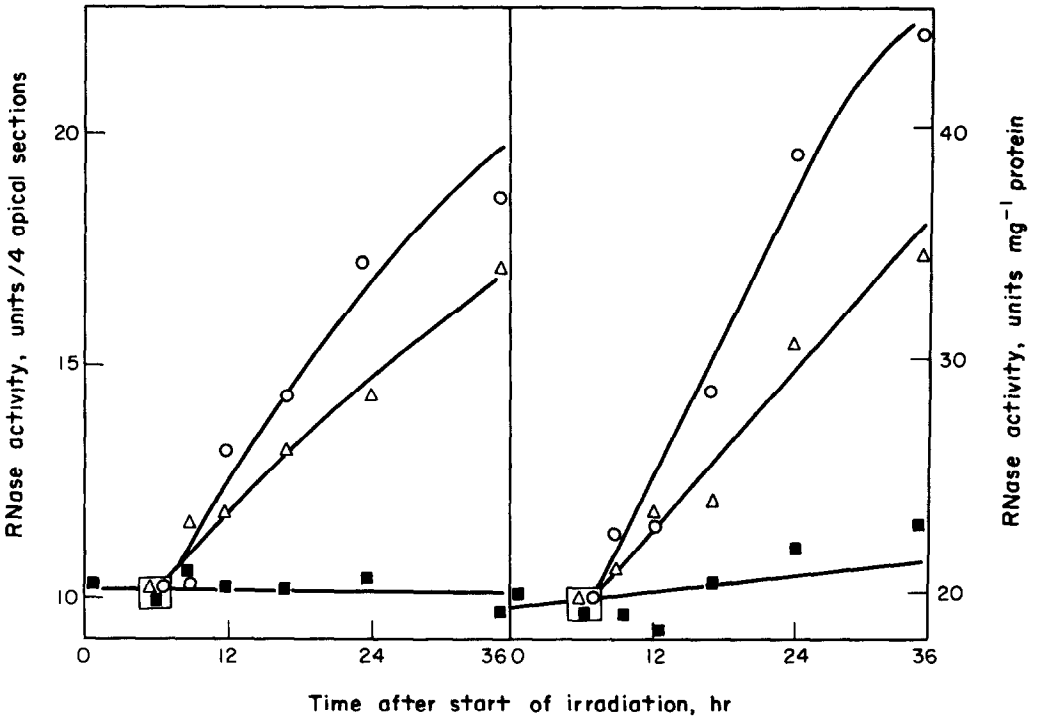

FIG 1 KINETICS OF RISE IN RNASE ACTIVITY IN CONTINUOUSLY IRRADIATED SECTIONS
Apical-quarter sections (2·5 cm) from 5-day-old etiolated seedlings were excised (green safelight), floated on H_2O, and transferred to light at zero time or kept in the dark (controls) Sections were combined and ribosomes isolated at subsequent intervals over 36 hr Circles, W treated, triangles, FR treated, squares, dark controls

Activators and inhibitors

Different proportions of ribosomes from etiolated and irradiated apical-quarter sections were mixed prior to assay for RNase activity. Mixing of equal proportions of extracts from etiolated (14·0 units/mg protein) and irradiated (77·0 units/mg protein) sections gave assayable RNase activity of 44·5 units/mg protein (expected if no interaction 45·5). Similarly, mixing 3:1 proportions gave 25·5 (3D:1L, expected 27·5) and 63·0 (1D:3L, expected 61·2) units/mg protein. Activities of extracts in which the enzyme had been previously solubilized by disintegrating ribosomes with EDTA and KCl (Ref. 11) were also additive (D = 23·5, L = 175·5, 1D:1L = 98·5 units/mg protein, expected 99·5). These results show that freely available specific inhibiting or activating factors are not present on ribosomes.

Activation of RNase occurs when ribosomes isolated from *Escherichia coli*,[12] rat liver[13] and yeast[14] are partially degraded. No activation was observed following disintegration of ribosomes (EDTA plus KCl) isolated from etiolated lupin hypocotyls [11] This indicates that greater activity of ribosomes isolated from irradiated tissue could not result solely from greater instability of these preparations leading to release of a latent enzyme The opposite approach to this problem is to isolate highly stable ribosomes and to incubate under conditions leading to very slow ribosome breakdown. Similar activity between preparations at onset of incubation followed by a rapid rise in the preparation from irradiated tissue could mean that the light-mediated increase resulted from release of a latent RNase. Ribosomes were isolated (T–M Medium C) and immediately incubated (20 mM acetate buffer at pH 5·6 with 12 mM $MgCl_2$) under conditions which should cause less rapid destruction (at 14 since high temp. leads to ribosome breakdown[15] and with additional Mg^{2+} to maintain tight folding). Aliquots of 1 ml were added to 2 ml ethanol at 5 min intervals over 1 hr and amounts of acid-soluble nucleotides released from ribosomes estimated (routine RNase assay) The shape of the velocity vs time plots obtained closely resemble those previously reported for autodegradation of isolated plant ribosomes [3,8] The main feature of the results is that reactions were almost linear with time for preparations from both etiolated and irradiated sections. The lack of a sharp rise in enzyme activity during incubation of ribosomes from irradiated tissue indicates that light stimulation of RNase activity is not an artifact of ribosome instability.

A second feature of the results is that hydrolysis of endogenous RNA at 14° of preparations from irradiated sections (7·9 units RNase/mg ribosomes at 2·2 mg/ml final concn) proceeded at nearly twice the rate of that of preparations from etiolated tissue (5·1 units/mg ribosomes at 2·2 mg/ml final concn). This difference in rate of autodegradation was also observed at 10° and 20°. Clearly, ribosomes from light-treated sections were less stable *in vitro* than those from dark-grown tissue.

Effect of light on gross synthesis of ribosomal protein

There are two possible ways in which the light-sensitive isoenzyme could be a true ribosomal component; the enzyme could be a structural ribosomal protein, or the enzyme could be a soluble protein attached at clearly defined binding sites (a conditional ribosomal protein). That light might have a gross effect on synthesis of structural proteins was tested by incubating sections with L-lysine-[4,5-^{3}H] under one growth regime (L or D) and with L-lysine-[U-^{14}C] under the alternative regime (D or L) followed by isolating ribosomes and "stripping off" their loosely attached proteins Results (Table 2) do not show a higher L.D isotope ratio for structural protein (compared with the ratio for soluble protein), implying that light has had no detectable specific effect on the synthesis of structural proteins.* Reversing the isotopes and continuous feeding of label gave similar results (Table 2) However, the W treatment stimulated synthesis of soluble proteins of isolated ribosomes.

* This means the rate of incorporation of label into both protein fractions following irradiation has either (a) increased by the same extent, (b) decreased by the same extent, or (c) stayed the same

[12] SPAHR, P F and HOLLINGWORTH, B R (1961) *J Biol Chem* **236,** 823
[13] TASHIRO, Y (1958) *J Biochem (Tokyo)* **45,** 937
[14] DANNER, J (1965) *Diss Abs* **25,** 4385
[15] WEST, S H and HANSON J B (1960) *Weeds* **8,** 341

TABLE 2. EFFECT OF CONTINUOUS LIGHT ON SYNTHESIS OF RIBOSOMAL PROTEIN IN APICAL-QUARTER SECTIONS

Experiment	Irradiation	Labelling sequence D	L	% Uptake* ^3H	^{14}C	% Incorporation† ^3H	^{14}C	Ribosomal protein (dpm × 10^{-5}) ^3H	^{14}C	L/D	Supernatant protein (dpm × 10^{-5}) ^3H	^{14}C	L/D
1	FR	^3H	^{14}C	6	13	9	12	2 11	1 24	0 59	19 80	12 60	0·64
2	FR	^{14}C	^3H	7	15	10	15	2 70	1 84	1 47	28 30	18 10	1 56
3	W	^3H	^{14}C	79	85	12	18	22 10	17 60	0 80	158 00	183 00	1·16

* Proportion of label taken up

† Proportion of label taken up and incorporated into ribosomal fraction

Experiments 1, 2 batches (25 sections) dark-excised apical-quarter sections incubated for 2 hr in D with 50 μCi-L-lysine-[4,5-^3H] or 10 μCi-L-lysine-[U-^{14}C], washed in 1 mM cold L-lysine and transferred to clean Petri-dishes containing cold lysine One labelling treatment irradiated (FR, 18 hr), other left in D prior to isolating ribosomes Pellets (16 × 10^4 *g*) re-suspended in T–M Medium B, ribosomes stripped of attached proteins by incubating with streptomycin (1 78 mM final concn), centrifuged (10^5 *g*, 2 hr) and radioactivity of ribosomal protein (pellet) and supernatant determined Experiment 3, as above except continuous incubation with tracer Batches of 50 sections incubated with 50 μCi-L-leucine-[4,5-^3H] (D) or 30 μCi-L-leucine-[U-^{14}C] (W, 24 hr). RNase activities (units/mg protein) of 16 × 10^4 *g* pellets from irradiated sections as per cent activity D controls (D = 100%), Experiment 1 = 121, Experiment 2 = 132, Experiment 3 = 242

Binding of soluble protein

The second possibility, namely that the isoenzyme is a soluble protein of the cell sap which is a necessary component, intrinsic to the ribosome, is more difficult to test. That it belongs to the "loosely bound" fraction of proteins is demonstrated by: (1) Compounds with a high affinity for ribosome binding sites (streptomycin,[16] spermine[17]) readily released the enzyme (a single wash in streptomycin at 1·78 mM final concn or in spermine at 0·02–0·5 mM final concn at 4° for 1 hr released only half the exogenous protein (protein:RNA ratios fell from *ca* 1·8 to 1·3) but 65–70% of the associated RNase activity*). However, there was a residual fraction of RNase (up to 10% of the initial amount bound) which was not removed, even by more severe treatments (2) Lupin ribosomes can both bind and exchange RNase protein in an external pool. Specific radioactivities in dpm per 1000 enzyme units of enzyme on particles obtained by homogenizing fresh tissue in maceration medium containing labelled "RNase-like" protein† show that 40–50% of RNase on the particles came from protein added to the medium [Table 3, Expt 1, Expt 2 (i)]. Also mixing increasing amounts of non-radioactive "RNase-like" protein with ribosomes isolated with soluble ^{14}C-labelled protein already attached [Table 3, Expt 2 (ii)] led to a drop in specific activity greater than could be explained by additional cold enzyme diluting out the label (Table 3).

To determine whether binding represented some type of specific protein-ribosome interaction, a binding curve was constructed from the data in Table 3. The shape of the curve obtained indicated constant binding rather than co-operativity. For 5, 10, 15 and 35 mol RNase added, 3, 3·5, 3·8 and 4·2 mol of RNase, respectively, were bound. This suggests each ribosome has only 3–4 binding sites (or binding site equivalents).

* There was no evidence of release of latent enzyme or of selective release from ribosomes from irradiated material

† ^{14}C-labelled lupin ribosomal proteins with MW *ca* 25 000 prepared by EDTA treatment and purified 60-fold by double chromatography (7300 dpm and 900 units RNase activity/mg protein, see Experimental)

[16] PETERMAN, M L (1964) *The Physical and Chemical Properties of Ribosomes*, p 43, Elsevier, Amsterdam

[17] MADISON, J T and DICKMAN, S R. (1963) *Biochemistry* **2**, 321

TABLE 3 BINDING AND EXCHANGE OF ^{14}C-LABELLED SOLUBLE PROTEIN

Experiment	Protein added Amount (mg)	Labelled	Cold	Radio-activity (dpm)	% Label attached*	Ribosomal pellet RNase activity (units)	Specific radioactivity (dpm/1000 units)	% Total RNase labelled Found	Expected†
1	0·25	+		470	26	137	3470	44	
2 (i)	0·25	+		630	35	154	3890	48	
(ii)	None (washed pellet control)			520		124	4190	52	
	0·56		+	555		171	3250	40	44
	0·84		+	450		186	2410	29	40
	1·96		+	410	—	222	1860	23	33

* Proportion of ^{14}C-label added (dpm added = 100%) in maceration medium

† If no exchange between labelled protein attached to pellet and cold protein in T M re-suspension medium

Experiment 1, labelled 25000 MW ribosomal fraction proteins (see Experimental) dissolved in maceration medium used to isolate post-mitochondrial supernatant (14000 *g*, 15 min) from fresh irradiated (W, 24 hr) apical-quarter sections (1·2 g). Crude "ribosomal" fractions (10^5 *g*, 2 hr) isolated and aliquots of resultant supernatant and re-suspended pellet (in T M Medium B) assayed for RNase and solubilized and counted. Experiment 2 (i) re-suspended unwashed pellets prepared as in Experiment 1 and immediately assayed. (ii) pellets re-sedimented (10^5 *g*) before assaying (pellets from first 10^5 *g* spin re-suspended in T M containing varying amounts (none for washed pellet control) unlabelled 25000 MW protein and immediately centrifuged). Relative enzyme activities of first 10^5 *g* pellets. Experiment 1 %D = 265. Experiment 2 %D = 290 taking RNase of D control in units mg protein as 100%.

DISCUSSION

This study establishes the existence of a gradient of light-stimulated RNase activity, increasing from base to hook of non-greened lupin hypocotyls following irradiation. Amounts of spectrophotometrically assayable phytochrome also increase towards the hook in hypocotyls (in lupins the 2·5 cm apical region at 8 days after germination in total darkness had an average phytochrome content (P_{tot}, uncorrected) of $12·4 \times 10^{-3}$ Δ (Δ_A) per 10 segments, whereas the sub-apical 2·5 cm (see Table 1) contained $6·1 \times 10^{-3}$ Δ (Δ_A) per 10 segments, a base to apex gradient was also observed in etiolated sunflower hypocotyls[18]). A RNase gradient was also found in onion roots [19] In this case extractable activity increased with distance from the root cap

The results presented differ from other reports on ribosome-bound RNases in plants in two major respects. Firstly, neither cutting (wounding) nor ageing of sections increased *bound* activity in either light or darkness Ageing raises the level of activity of a light-insensitive predominantly particle-bound RNase (WL II) extractable from detached wheat leaves Part of this increase was identified with the fraction sedimented at 10^5 *g* [20] Secondly, in hypocotyls light elevates the level of extractable ribosome-bound RNase activity [10 11] This is in contrast to studies on detached greened leaves in which white light has either little effect,[20] or markedly inhibits,[21] the development of RNase activity associated with the fractions sedimented at 10^5 *g* It appears that development of ribosome-bound RNase activity during photomorphogenesis is a unique process

Another striking difference between the present report and a number of previous stu-

[18] BRIGGS, W R and SIEGELMAN, H W (1965) *Plant Physiol* **40**, 934
[19] PILET, P-E, PRAT, R and ROLAND, J-C (1972) *Plant and Cell Physiol* **13**, 297
[20] SODEK, L and WRIGHT, S T C (1969) *Phytochemistry* **8**, 1629
[21] UDVARDY, I, FARKAS, G L, MARBÉ, E and FORTI, G (1967) *Physiol. Plant.* **20**, 781

dies[3,5,9,21–24] is the lack of release of a fully latent enzyme upon destruction of lupin ribosomes. Recovered activity in supernatant plus pellet slightly exceeds activity of untreated ribosomes following washing with spermine, but this is more likely to be a result of freeing a fully active enzyme rather than activation of a latent RNase On the other hand, increased autodegradation capacity of lupin ribosomes (presumably resulting from more bound nucleolytic activity) was observed under conditions in which metabolic activity had been suddenly reduced (in this case inhibition of cell elongation by light) This is in general agreement with recent, but speculative, proposals that autodegradation of ribosomes is low during periods of high metabolic activity in order to conserve RNA.[3,24]

On the basis of the present work it seems clear that association of the bulk of the light-sensitive RNase in lupin hypocotyls results from ribosomes binding soluble or solubilized protein. However, a constant binding curve need not indicate a preferential interaction. In addition, it is not easy to assign a plausible physiological role to 3–4 RNase mol bound to each ribosome. At the present state of knowledge it therefore seems equally justified to class the enzyme as either a conditional ribosomal protein (i.e. a true ribosome component) or as an extraneous protein, adsorbed onto ribosomes during the process of their isolation.

EXPERIMENTAL

Materials and growth conditions (25°) *Lupinus albus* L seeds (batch HU/J/71, this batch gave a low light-mediated increase in ribosome-bound acid RNase) were soaked and germinated in vermiculite Hypocotyl sections (quarter sections *ca* 2·5 cm) cut from 5-day-old etiolated seedlings (selected for uniformity) using a green safelight (after Withrow and Price[25]), surface sterilized in "Chloros" for 1 min, rinsed and placed in sterile Petri-dishes containing sterilized dist H_2O In later experiments sterilization was replaced by adding streptomycin-SO_4 at 20 μg/ml to H_2O FR source consisted of all-dielectric interference filters (centred 730 nm, bandwidth 13 nm at half peak, 55% T at peak) placed below 2 × 500 W incandescent bulbs, intensity 7·74 × 10^{-10} Einsteins per cm^2 per sec at working distance W source consisted of warm-white daylight (1·1) fluorescent tubes giving 12000 lx at working height Sources were fan cooled

Isolation of ribosomes (3°) Excised treated tissue was homogenized in 0·4 M sucrose (to minimize rupture of mitochondria and developing plastids) plus Nonidet A-10 (0·02% w/v) in T–M Medium A (50 mM Tris–acetate at pH 7·5 with 50 mM $MgCl_2$) Resulting homogenates were centrifuged at 3 × 10^4 *g*, 30 min, and supernatant spun at 10^5 *g*, 2 hr, to give a "ribosome-enriched" pellet Pellets were re-suspended in T–M Medium B (50 mM Tris–acetate at pH 7·5 with 10 mM $MgCl_2$), layered on 5 ml 35% w/w sucrose (RNase-free) in T–M Medium B and centrifuged at 16 × 10^4 *g*, 2 hr, in a fixed-angle rotor After wiping walls of tubes, pellets (clear or pale yellow) were suspended in T–M Medium B to give a final concn *ca* 0·2 or 2 mg/ml ribosomes For isolation of ribosomes with max stability T–M Medium C (50 mM Tris–acetate at pH 7·5 with 500 mM $MgCl_2$) was substituted for T–M Media A and B In binding expts (Table 3) homogenates were fractionated as described in footnotes Replacement of Nonidet by 0·3% w/v DOC in maceration medium gave similar amounts of extractable bound RNase from etiolated sections (Nonidet = 11·0 units, DOC = 8·7 units/mg ribosomes) More ribosomes were extracted from irradiated sections (W, 24 hr) by DOC, in addition, activity per unit of ribosomes and per unit of protein was lower (Nonidet = 21·9 units, DOC = 9·2 units/mg ribosomes) Extraction using either detergent gave the same result on a fr wt or hypocotyl basis (light-mediated changes: Nonidet = +2·53 units, DOC = +2·31 units/four sections) DOC did not free ribosomes of bound RNase as reported in peas[22]

Identification of ribosomes Purity of washed 10^5 *g* pellets prepared with media containing KCl has previously been checked[11] Since KCl accelerates disintegration of plant ribosomes,[8] KCl-free media were used in all expts reported here Purity of the ribosomal fraction was checked using etiolated apical-quarter sections which are low in RNase and media containing 1% v/v freshly added DEP to inhibit RNase Samples (10–20 ml re-suspended 16 × 10^4 *g* pellets, 6–8 mg/ml ribosomes) were centrifuged in a BXV zonal rotor spun 6·5 hr (4°) at 21 000 rev/min, integrated field-time 9·5 × 10^6 *g*-min, in a linear with vol 10–30% sucrose gradient After displacement of gradients (40% sucrose), one major peak consistent with 80 *S* particles (protein RNA ratio *ca* 1·3) was obtained

[22] MATSUSHITA, S. and IBUKI, F (1960) *Biochim Biophys Acta* **40,** 358
[23] URSINO, D J, STURANI, E and ALBERGHINA, F A. M (1969) *Biochim Biophys Acta* **179,** 500
[24] STARK, G F and JAENICKE, L (1971) *Z Naturforsch* **26b,** 328
[25] WITHROW, R B and PRICE, L (1957) *Plant Physiol* **32,** 244

As a further check, 16×10^4 *g* pellets were re-suspended in T–M Medium B made to 0·4% DOC, clarified (2×10^4 *g*, 10 min) and profiles on the analytical centrifuge (4°, 4×10^4 rev/min) gave one main peak (schlieren optics) with a sedimentation coefficient corrected to 20° in H_2O of 80. To minimize losses of enzyme activity interfering with routine RNase estimations, ribosomal pellets were prepared as fast as possible, immediately re-suspended, frozen at −20° for up to 3 days and thawed once immediately before use

Partial purification of enzyme Unwashed 10^5 *g* pellets were incubated with 0·25 M EDTA-1 M KCl soln and extracted as previously described[11] Fractions of 3·5 ml corresponding to proteins having MW between 21 000 and 28 000 were pooled and re-chromatographed (Sephadex G100, see Ref 11) to give the partially purified 25 000 MW protein fraction (0·2 mg/ml protein)

Incorporation of labelled amino-acids In dual-labelling experiments (Table 2) dark-excised 2·5 cm apical-quarter sections were placed in the dark in sterile Petri-dishes (25 per dish) with 30 ml sterile double-dist H_2O containing either L-lysine-[4,5-^{3}H] (7·5 Ci/mmol) or L-lysine-[U-^{14}C] (556 mCi/mmol) For continuous feeding the equivalent L-leucine isotopes were used In the preparation of labelled 25 000 MW protein (Table 3) L-amino acids (mixture)-[U-^{14}C] (54 mCi/mAt) were dissolved in the sterile H_2O Batches of 50 apical-quarter sections were incubated with 30 μCi [^{14}C]-amino acid mixture during irradiation (W, 36 hr), washed and 10^5 *g* pellets isolated prior to extraction The possibility of bacterial contamination was reduced by surface sterilization or with streptomycin SO_4 and by washing sections 6× before and after treatment In addition 2 mm was removed from each end of each section immediately before macerating It is unlikely that bacteria contributed to incorporation of label into the lupin ribosome fraction because (a) no bacteria were present at onset of irradiation, (b) there was no evidence of 70 *S* particles and (c) contaminating bacteria would raise RNase activity, comparison of preparations from equivalent portion of intact etiolated plants and identically treated sections always gave similar levels of RNase activity

Solubilization of proteins for liquid scintillation counting Both ribosomal and "attached" protein were freed from attached amino acids by solution and re-precipitation with acid and alkali as described by Trewavas[26] After the final ppt with 5% w/v trichloroacetic acid and washing with acetone, 1 ml aliquots were dissolved in 0·5 M hyamine hydroxide, neutralized (0·02 ml HOAc) and counted All dpm values given were above background

Assays for RNase, protein, ribosomes and RNA These were as described previously[11] except that ribosomes were incubated with 3 mg/ml (final concn) yeast-RNA in routine RNase assays The unit of RNase is the amount required to degrade 1 μg RNA in 1 min at 37° pH 5·6

Acknowledgements—I thank Professor R Hertel for discussions during preparation of the manuscript, Dr T H Birkbeck for analytical ultracentrifugation and Dr B Frankland for measurements of phytochrome Supported by a Royal Society scientific investigation grant-in-aid

[26] TREWAVAS, A J (1968) *Arch Biochem Biophys* **123,** 324