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Particle-bound Phytochrome: Association with a Ribonucleoprotein Fraction from *Cucurbita pepo* L.*

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Summary. In the absence of ethylenediaminetetraacetic acid (EDTA) and added Mg^{2+}, the phytochrome, RNA, protein, cytochrome c oxidase and NADPH-cytochrome c reductase in $20000 \times g$ pellets from hypocotyl hooks of red-irradiated *Cucurbita* seedlings are more or less coincident in a single, broad band on linear sucrose gradients. The inclusion of 3 mM EDTA in the extraction, resuspension and gradient media has three major effects: (a) The phytochrome profile splits into two main bands; (b) the main RNA population shifts to a sharp peak which co-sediments with the "lighter" phytochrome band at 31S; (c) the main NADPH-cytochrome c reductase peak shifts to a lower density. This indicates that the EDTA dissociates a rough-endoplasmic-reticulum fraction into separate membrane and ribonucleoprotein (RNP) components, and that part of the phytochrome is associated with the latter. The 31S RNP fraction is 35–40% RNA, has a 260/235 nm absorption ratio of 1.36 and the RNA dissociates into small fragments in sodium dodecyl sulfate. More than 90% of the phytochrome and RNA in the isolated 31S fraction becomes pelletable upon the addition of 10 mM Mg^{2+}. Higher Mg^{2+} levels release the phytochrome and some of the other protein present from the RNA which remains pelletable. The data indicate that the 31S RNP fraction may be degraded ribosomal material with extraneously bound protein, including phytochrome. Several aspects of phytochrome-binding to particulate fractions which have been reported in the literature are consistent with an interaction of P_{fr} with ribosomal material—degraded or otherwise.

Introduction

Several authors have reported that phytochrome is bound to particulate material in homogenates of plant tissue [11, 12, 18–23, 25]. The form of the pigment (P_r or P_{fr}), the concentration of divalent cations, and the pH of the extract are important variables in the interaction of phytochrome with the particulate fraction(s). The identity of the fraction(s) has remained elusive, however. Marmé *et al.* [11, 12] reported the isolation of a phytochrome-containing component which could be separated from cytochrome c oxidase activity by manipulation of the Mg^{2+} concentration. This component banded at 15% (w/w) sucrose on a gradient after 6 h centrifugation at $100000 \times g$. Addition of Mg^{2+} to the isolated fraction caused the phytochrome to become readily pelletable and resulted in the formation and aggregation of 50-nm structures which appeared as vesicles in negative stain. The data were interpreted as indicating that the P_{fr} form of phytochrome binds stereospecifically to a cellular membrane; that removal

* *Abbreviations*: EDTA = ethylenediaminetetraacetic acid; ER = endoplasmic reticulum; P_{fr} = far-red-absorbing form of phytochrome; P_r = red-absorbing form of phytochrome; RNase = ribonuclease; RNP = ribonucleoprotein; SDS = sodium dodecyl sulfate.

of Mg^{2+} causes "partial solubilisation" of the phytochrome-containing membrane; and that this fraction reforms membranous vesicles upon the re-addition of Mg^{2+} [11, 12].

An alternative interpretation is suggested in the present report. Evidence is presented that the phytochrome in this fraction is associated with a slowly sedimenting ribonucleoprotein particle rather than with a "partially solubilized" membrane [11]. The data further indicate that this RNP fraction represents degraded ribosomal material with adsorbed protein, including phytochrome.

Material and Methods

Plant Material. Zucchini seedlings (*Cucurbita pepo* L. cv. Greyzini; Rumseys Seeds, Parramatta, N.S.W., Australia) were grown in the dark on moist paper towels, at 30°. Harvesting and all subsequent manipulations were performed under green safelight [14] until phytochrome measurements were complete. Hypocotyl hook segments, 5 mm long, cut from immediately below the cotyledons of 4-day-old seedlings, were used as starting material.

Irradiation Procedures. The isolated hooks were irradiated at 25° immediately after excision using a Zeutschel M3 monochromater (Heinz Zeutschel, Tübingen, Germany) and Schott & Gen. (Mainz, Germany), D.I.L. (Doppel-Linienfilter) interference filters. Red (3800 erg cm^{-2} s^{-1}) and far-red (2500 erg cm^{-2} s^{-1}) wavelengths were provided using 660 and 730 nm filters, respectively. Irradiations of 3 min were saturating as determined spectrophotometrically, and were used routinely.

Extraction and Sucrose Gradient Centrifugation. Prechilled tissue was finely minced with a razor blade and homogenised in a mortar and pestle in ice-cold extraction medium (pH 7.6) containing 35 mM N-morpholino-3-propansulfonic acid (MOPS), 250 mM sucrose, 3 mM EDTA and 14 mM 2-mercapto-ethanol. In some experiments, specified in the text, the EDTA was omitted. Likewise 10 mM $MgCl_2$ was included in the medium where specified. A solution: tissue ratio of 3:1 (v/w) was used in all cases, giving a final homogenate pH of 7.0–7.1. The brei was squeezed through nylon cloth and pre-centrifuged at $500 \times g$ for 10 min. The resultant $500 \times g$ supernatant was the starting solution for various further centrifugation programs as specified in text and figure legends. In most experiments a $20000 \times g \times 30$ min centrifugation followed. Pellets were normally resuspended in 25 mM MOPS, 3 mM EDTA, 250 mM sucrose and 14 mM 2-mercaptoethanol, pH 7.0 (except Fig. 2e and f, where the pH was 7.2). Again the EDTA was omitted in some experiments. Percent pelletability is the amount of a given component in the resuspended pellet expressed as a percentage of the total amount of that component in the pellet plus the supernatant.

For routine density gradient analysis, samples were layered onto 10–50% (w/w) linear sucrose gradients containing 25 mM MOPS, 3 mM EDTA and 14 mM 2-mercaptoethanol, pH 7.0 (except Fig. 2e, f where the pH was 7.2). EDTA was once more omitted where specified. Where $20000 \times g$ pellets were examined, the equivalent of 6 g fresh weight of tissue (25–30 mg protein) was applied in 3 ml to each 34-ml gradient. Centrifugation was at 27000 rpm in a Beckman SW27 rotor for the periods specified. Fractions of 1 ml each were collected and assayed for phytochrome, RNA, protein, cytochrome c oxidase, and NADPH-cytochrome c reductase. Percent sucrose (w/w) was measured refractometrically.

For sedimentation-velocity analysis of the phytochrome-RNA peak, 1.5 ml of a resuspended $20000 \times g$ pellet were applied to a 10 to 27% (w/w) linear sucrose gradient and centrifuged for 3 h at 40000 rpm in a Beckman SW41 rotor. Lymphocyte ribosomes dissociated by preincubation in high KCl buffer with 0.2 mM puromycin [27] were run as markers on a parallel gradient. The ribosomes, prepared according to Kay *et al.* [6], were a kind gift from Dr. R. Wettenhall of this institution. For sedimentation-velocity analysis of the RNA species after dissociation in SDS [27], pooled fractions from the 31S peak of a 4-h SW27 gradient were used. Following ethanol precipitation the pellet was resuspended in 50 mM Tris (Tris(hydroxymethyl)-aminomethane), 0.1% (w/v) SDS, 1 mM EDTA, pH 7.8, and incubated at 37° for 5 min. Samples of 0.5 ml were applied to a 5–19% (w/w) linear sucrose gradient

(Tris 50 mM, pH 7.8) and centrifuged at 60000 rpm for 3 h in a Beckman SW65 rotor. Rat-liver ribosomes identically treated were run as markers either on the same or a separate parallel gradient. These ribosomes, prepared according to Martin *et al.* [13], were also a gift from Dr. R. Wettenhall. The ultraviolet absorbance profiles were measured using an ISCO density gradient fractionator (Instrumentation Specialities Co., Lincoln, Neb., USA).

Assays. Phytochrome was measured at 0° with a Ratiospect (Agricultural Speciality Co., Hyattsville, Md., USA) using $CaCO_3$ as a scattering agent [2]. The measuring beams were 728 and 802 nm and the actinic beams 656 and 737 nm (Schott D.I.L. filters).

RNA was measured by the procedure of Fleck and Munro [4] using rat-liver RNA as a standard. The rat-liver RNA, prepared according to Perry *et al.* [16], was another kind gift from Dr. R. Wettenhall of this institution. Protein was determined by the method of Lowry *et al.* [10] using bovine serum albumin as a standard.

The mitochondrial marker enzyme cytochrome c oxidase was assayed according to Smith [26]. NADPH-cytochrome c reductase, an ER marker, was measured according to Lord *et al.* [9]. The latter enzyme activity was not affected by 1 μM antimycin A, indicating the absence of interference by contaminating mitochondrial enzymes.

Replication. All experiments were repeated at least once. Estimates of "pelletability (%)" are the means of two experiments having duplicate or triplicate measurements (Table 1, Fig. 3). Standard errors were less than 5%. Other figures are repressentative gradients.

Results

Phytochrome and RNA Pelletability at 20000 × g

Table 1 illustrates the effect of irradiation *in vivo* and of Mg^{2+} and EDTA concentrations in the extraction medium on the pelletability of phytochrome and RNA at 20000 × *g*. These results agree in principle with previous observations made under similar conditions [11, 12, 21]. P_r in far-red-treated plants is mostly non-pelletable; photoconversion to P_{fr} causes up to a 10fold increase in pelletability; and reconversion to P_r before extraction does not totally reverse the red-light-enhanced pelletability. The pelletability observed in the absence of Mg^{2+} in red-irradiated tissue is further increased some 15–20% in the presence of 10 mM Mg^{2+}. Likewise the pelletability of extractable RNA is enhanced by a further 10–12% by Mg^{2+}. As would be expected there is no effect of pre-irradiation on RNA pelletability. Other activities measured—protein, cytochrome c oxidase and NADPH-cytochrome c reductase—showed no trends in response to the variables tested.

Table 1. Percent pelletable phytochrome and RNA from hypocotyl hooks of *Cucurbita* seedlings (20000 × *g* × 30 min, pH 7.0) as a function of previous, *in-vivo* irradiation and the presence or absence of 10 mM $MgCl_2$ and 3 mM EDTA in the extraction medium

Irradiation[a]	Pelletability (%)							
	Phytochrome				RNA			
	$-Mg^{2+}$		$+Mg^{2+}$		$-Mg^{2+}$		$+Mg^{2+}$	
	−EDTA	+EDTA	−EDTA	+EDTA	−EDTA	+EDTA	−EDTA	+EDTA
Far-red	—	5	—	6	—	30	—	39
Red	43	39	57	54	33	29	42	42
Red + far-red	—	21	—	24	—	28	—	41

[a] Red = 660 nm; far-red = 730 nm; duration = 3 min each wavelength.

Although earlier studies with *Cucurbita* have often included 3 mM EDTA in the media [11, 12], it can be seen here that the chelator has only minor effects on the initial pelletability of phytochrome and RNA from red-irradiated tissue (Table 1). This indicates, firstly, that in the absence of added Mg^{2+} chelatin of any endogenous divalent cations causes only slight reductions in pelletability, and secondly, that the effects of added Mg^{2+} are almost saturated at about 7 mM, *i.e.* that concentration in excess of added EDTA. Effects of the inclusion or otherwise of 3 mM EDTA in the media do become apparent, however, when $20000 \times g$ pellets are examined on sucrose gradients.

EDTA and in-vivo Irradiation: Effects on Sucrose Gradient Profiles

Fig. 1a, b shows the distribution profiles of a pellet from red-irradiated tissue where extraction, resuspension and centrifugation were all performed in the absence of either added Mg^{2+} or EDTA. This pellet contains 35–40% of the total extractable phytochrome (Table 1). All the activities tested, although not entirely coincident, are localised more or less in a single braod band in the region of 35–40% (w/w) sucrose. The inclusion of 3 mM EDTA in the extraction, resuspension and gradient media, but still without added Mg^{2+}, has three major effects (Fig. 1c, d): (1) The phytochrome profile splits into two main bands, the relative magnitudes of which are pH dependent [20]; (2) the main RNA population shifts dramatically and becomes localised in a sharp peak which is entirely coincident with the light phytochrome band; and (3) the main peak of NADPH-dependent cytochrome c reductase activity shifts to a lower density at 28% (w/w) sucrose.

The predominantly non-pelletable phytochrome extracted from non-irradiated, dark-grown tissue has different sedimentation properties (Fig. 1e, f) to the pigment in the $20000 \times g$ pellets from the red-irradiated material (Fig. 1a–d). The major phytochrome peak in the former case remains at the interface between the gradient and the loading buffer and is clearly separated from the main RNA band. The implied dependency of the phytochrome-RNA association on the form of the pigment is discussed more fully in the following paper [20].

The apparent association of NADPH-cytochrome c reductase and RNA in the absence of EDTA (Fig. 1a, b) suggests the presence of an intact rough ER fraction. Conversely, the separation of those two activities in the presence of 3 mM EDTA (Fig. 1c, d) into a sedimentable ribonucleoprotein (RNP) fraction and a major reductase peak of lower isopycnic buoyant density, is indicative of an EDTA-induced dissociation of ribosomes from the ER membrane. Such an effect is well documented [9, 24, 28]. The behaviour of at least part of the phytochrome under these conditions is qualitatively parallel to that of the RNP fraction suggesting the possibility of an association between the two.

Time of Centrifugation: Effect on Sucrose Gradient Profiles

The sedimentation behaviour of the various fractions in $20000 \times g$ pellets from red-irradiated tissue in the presence of 3 mM EDTA is shown in Fig. 2. The heavier phytochrome band reaches isopycnic equilibrium within 90 min and remains at this buoyant density for up to 12 h of centrifugation. The cytochrome c oxidase, NADPH-cytochrome c reductase, major protein and heavy RNA peaks

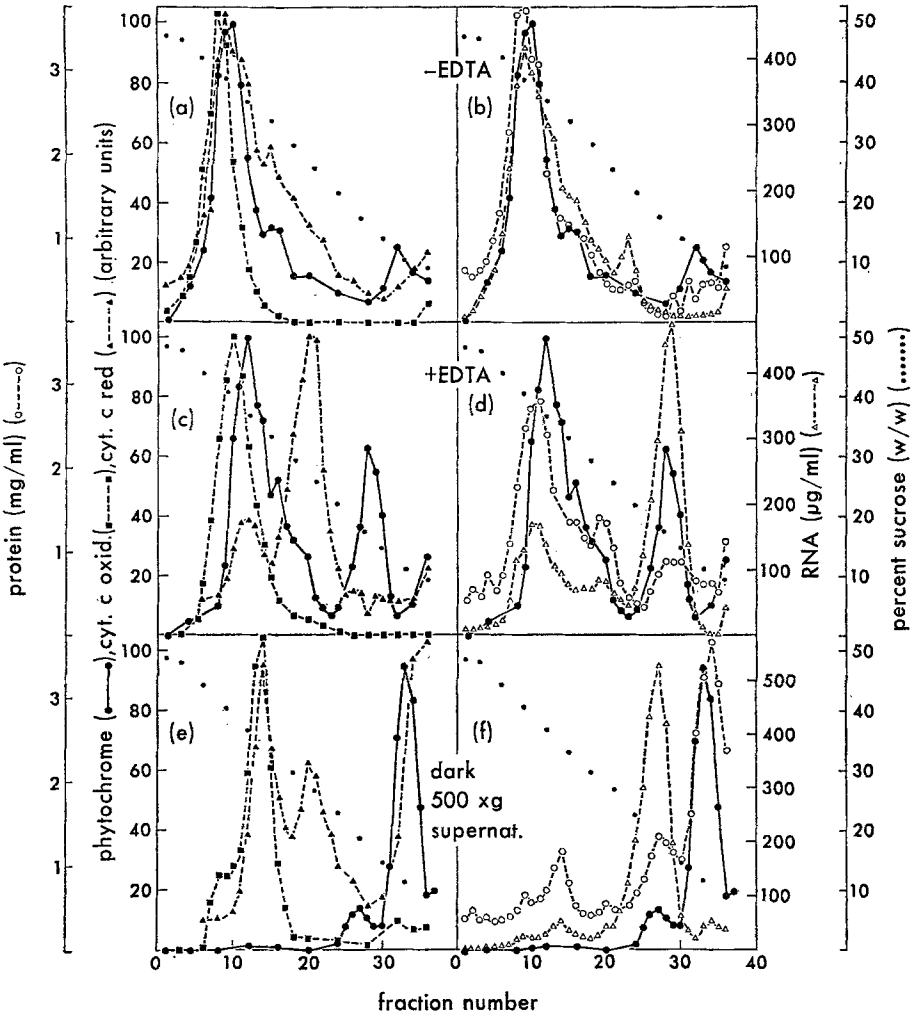

Fig. 1a—f. The effects of EDTA and *in vivo* irradiation on sucrose gradient profiles of phytochrome and other cellular components from hypocotyl hooks of *Cucurbita* seedlings. The distributions of phytochrome, cytochrome c oxidase, cytochrome c reductase, RNA and protein are shown following 4 h centrifugation on 10–50% (w/w) linear sucrose gradients, pH 7.0, at 27000 rpm in a Beckman SW27 rotor. Each pair of adjacent panels represents a single gradient. The phytochrome, cytochrome c oxidase and cytochrome c reductase profiles are directly compared in the left-hand panels (a, c, e) and the phytochrome, RNA and protein profiles in the right-hand panels (b, d, f). Gradient a/b: Distribution profiles of a $20000 \times g$ pellet from red-irradiated hooks using extraction, resuspension and gradient media containing neither EDTA nor Mg^{2+}. Gradient c/d: Distribution profiles of a $20000 \times g$ pellet from red-irradiated hooks using extraction, resuspension and gradient media free of Mg^{2+} but containing 3 mM EDTA. Gradient e/f: Distribution profiles of a $500 \times g$ supernatant from non-irradiated hooks extracted and centrifuged in Mg^{2+}-free media containing 3 mM EDTA

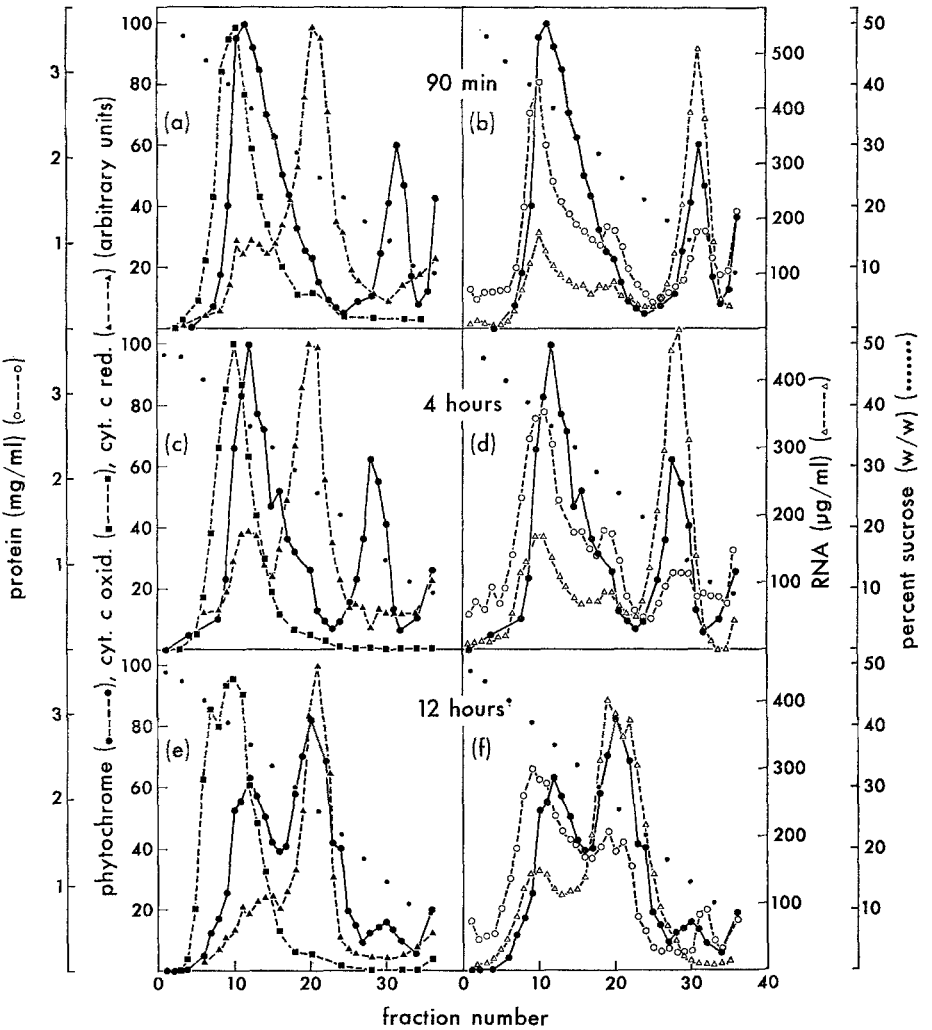

Fig. 2a—f. The effect of increasing time of centrifugation on the sucrose gradient profiles of phytochrome and other cellular components in $20000 \times g$ pellets from red-irradiated hypocotyl hooks of *Cucurbita* seedlings. Extraction, resuspension and gradient media were Mg^{2+}-free and contained 3 mM EDTA. The distribution of phytochrome, cytochrome c oxidase, cytochrome c reductase, RNA and protein are shown following 90 min (a, b), 4 h (c, d) and 12 h (e, f) centrifugation on 10–50% (w/w) linear sucrose gradients at 27000 rpm in a Beckman SW27 rotor. Each pair of adjacent panels represents a single gradient. The phytochrome, cytochrome c oxidase and cytochrome c reductase profiles are directly compared in the left-hand panels (a, c, e) and the phytochrome, RNA and protein profiles in the right-hand panels (b, d, f). The 90-min and 4-h gradients were run at pH 7.0. The 12-h gradient was run at pH 7.2 to enhance the lighter phytochrome peak. This effect is discussed elsewhere [20]

behave similarly. This indicates some form of association of the pigment in the heavy band with a membrane fraction (or membrane fractions) as yet unidentified. The lack of agreement between the phytochrome and the major cytochrome c oxidase and reductase profiles is evidence against a unique association between the pigment and the mitochondrial or ER membranes *per se*. However, the apparent coincidence of the phytochrome with the heavy reductase shoulder is of interest since the latter is likely to represent a residual rough-ER fraction from which all the ribosomes have not been dissociated [9, 24, 28]. The presence of RNA in that part of the gradient is consistent with this suggestion.

The lighter phytochrome band continues to sediment for at least 12 h of centrifugation. Co-migrating with the pigment is the major RNA peak and associated protein (Fig. 2b, d, f). This suggests a direct association between the phytochrome and the RNP fraction. This fraction is 35–40% RNA, has a 260/235 nm absorption ratio of 1.36 and a 260/280 nm ratio of 1.87. These values are consistent with those observed for ribosomal material with substantial levels of extraneously bound proteins [17]. Ribosomes are known to have a high capacity for nonspecific adsorption of proteins.

Addition of Mg^{2+} to the RNP Fraction

To test the effects of adding Mg^{2+} to the RNP band [11, 12], the peak fractions from a 4-h SW27 gradient similar to that in Fig. 2c, d were pooled and divided into aliquots. Mg^{2+} was added to give a range of final concentrations, and the samples were centrifuged at $20000 \times g$ for 30 min. The Mg^{2+}-containing fractions become turbid and at 10 mM Mg^{2+} over 90% of the phytochrome and RNA and more than 80% of the protein is pelleted (Fig. 3). Without added Mg^{2+} less than 5% of any of these components is pelletable. As the Mg^{2+} concentration increases above 10 mM the amount of pelletable phytochrome decreases dramatically such that at 100 mM or higher virtually all the pigment is released to the supernatant. The RNA in contrast remains more than 85% pelletable up to 500 mM Mg^{2+}. Protein is released from the pellet with increasing Mg^{2+} but to a lesser degree than phytochrome. Such effects of Mg^{2+} on ribosomes are well documented [17]. At relatively low concentrations, the cation induces the aggregation and precipitation of ribosomal material including associated protein. High Mg^{2+} levels also precipitate ribosomes, but in addition, simultaneously displace much of the extraneously bound protein to the supernatant. Indeed, this procedure is used for the preparation of ribosomes free of bound proteins [17].

Characteristics of the RNP Fraction

A sedimentation coefficient of 31S for the phytochrome-RNP band was estimated using lymphocyte ribosomes and their subunits as markers (Fig. 4). This indicates that the fraction does not consist of intact ribosomes or even subunits. Aberrant sedimentation patterns of partially degraded ribosomal material are well documented, however, particularly where EDTA has been used [17]. The chelator dissociates the subunits and increases susceptibility to RNase attack [3, 17]. Since the present pattern was only observed in the presence of EDTA (Fig. 1) and no precautions were taken to minimise RNase activity, the

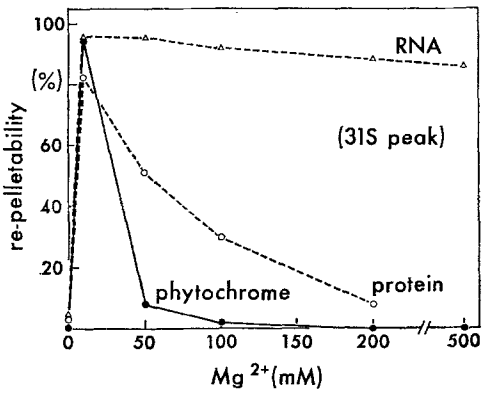

Fig. 3. Pelletability of the phytochrome, RNA and protein in the 31S ribonucleoprotein fraction in response to added Mg^{2+}. Peak fractions from the 31S band of a 4-h SW27 gradient similar to that in Fig. 2, c/d were pooled and divided into aliquots. Mg^{2+} was added to give a range of final concentrations (pH 7.0) and the samples were centrifuged at $20000 \times g$ for 30 min

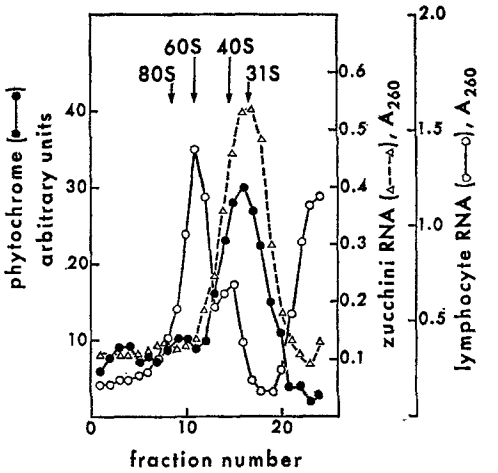

Fig. 4. Sedimentation velocity analysis of the "light" phytochrome/RNA fraction observed in Fig. 2. The distribution of *Cucurbita* (zucchini) phytochrome and RNA and of lymphocyte ribosomal subunit RNA are shown following 3 h centrifugation on separate 10–27% (w/v) linear sucrose gradients at 40000 rpm in a Beckman SW41 rotor. The position of the 80S marker was determined in a separate parallel experiment (curve not shown)

31S fraction might well represent degraded ribosomal particles. The fragmentation of the RNA component in SDS supports this view (Fig. 5). Whereas the 18S and 28S markers show the expected sedimentation pattern, the *Cucurbita* RNA has hardly entered the gradient. This indicates that the 31S fraction is an aggregate of very small RNA fragments with associated protein. Degradation of ribosomal RNA during ribosome isolation is a common problem [2, 5, 7, 8, 15, 17].

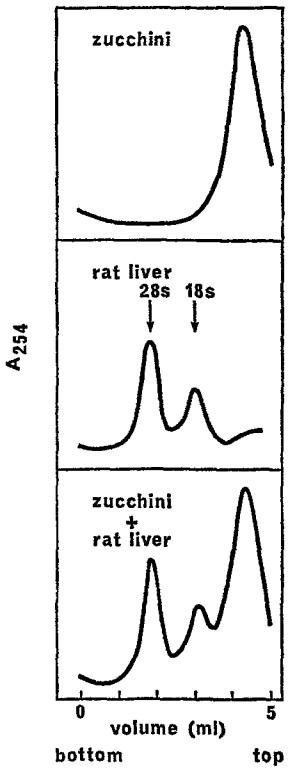

Fig. 5. Analysis of the RNA of the 31S ribonucleoprotein fraction following incubation in SDS. Pooled fractions from the 31S peak of a 4-h SW27 gradient (Zucchini = *Cucurbita*) and an aliquot of rat liver ribosomes were incubated either separately or together in SDS prior to centrifugation on 5–19% (w/w) linear sucrose gradients for 3 h at 60000 rpm in a Beckman SW65 rotor. The resultant ultraviolet absorbance profiles are shown

Discussion

The concomitant, EDTA-induced appearance of phytochrome and RNA in the light peak (Fig. 1); the close agreement between the two profiles across this peak (Figs. 1, 2, 4); the complete co-migration of the pigment and RNA in this band under all centrifugation conditions (Figs. 2, 4); and the Mg^{2+}-induced co-precipitation of the two species (Fig. 3) all argue for a direct association between phytochrome and the 31S RNP fraction. The continued sedimentation of this band over a 12-h period is strong evidence against its representing an association between phytochrome and a so-called "solubilised membrane" fraction which is at isopycnic equilibrium as was previously claimed [12]. Likewise, the well-known Mg^{2+}-induced aggregation and precipitation of ribosomal material [17] provides a plausible alternative explanation to the previously proposed Mg^{2+}-dependent "vesicularisation" of the putative "solubilised membrane" [11, 12].

The ribosomal origin of the 31S RNP fraction is further indicated by the following considerations: (a) Since more than 85% of the total cellular RNA

is ribosomal [8], this would appear to be the only component capable of providing the amount of RNA observed in the 31S fraction [20]. (b) The simultaneous, EDTA-induced shifts in location of the RNA and cytochrome c reductase on sucrose gradients (Fig. 1) indicate dissociation of ribosomal material from rough ER with the resultant decrease in microsomal membrane density [9, 24, 28]. (c) The effect of Mg^{2+} in enhancing the pelletability of the RNA whether in the initial $20000 \times g$ pellet (Table 1), a $20000–50000 \times g$ pellet [20] or in the isolated 31S fraction (Fig. 3), is reminiscent of the effect of divalent cations in inducing precipitation of ribosomes and r-RNA [17]. (d) Likewise, the release by high Mg^{2+} concentrations of phytochrome and other proteins from the RNA while retaining the pelletability of the latter (Fig. 3), resembles the displacement by the cation of extraneously adsorbed proteins from ribosomes [17]; as does the release of phytochrome by high levels of KCl [17, 20]. Ribosomes are notorious for their capacity to bind high levels of extraneous proteins by non-specific electrostatic adsorption [1, 3, 5, 8, 17]. For example 1 mg of ribosomes can bind up to 1.1 mg of hemoglobin [17]. Both the RNA content and the ultraviolet absorption ratios measured for the 31S fraction are consistent with this possibility. (e) Substantial additional quantities of RNA and phytochrome are pelleted at $160000 \times g$ from a $20000 \times g$ supernatant and these are exclusively located in the 31S fraction [20]. This procedure permits the pelleting of ribosomes and even ribosomal subunits. (f) A recently reported correlation between microscopically detectable ER and phytochrome in tissue extracts tends to support the present findings although only 6% of the total pigment was involved in that case [29]. The "heavy" phytochrome band observed in the present study might well be accounted for by an association of the pigment with the ribosomal component of the rough ER.

The sedimentation coefficient of 31S (Fig. 4) and the dissociation of the RNA into small fragments in SDS (Fig. 5) clearly indicate that, if the RNP fraction is indeed of ribosomal origin, it is in a highly degraded form [3, 5, 7, 15, 17]. This is to be expected since the extraction conditions used are extremely unfavourable to the preservation of ribosomal integrity. EDTA is known to disrupt ribosomal structure by withdrawing Mg^{2+} and causing the dissociation of the subunits [3, 7, 8, 17]. This is accompanied by an increased susceptibility to RNase attack and, in some cases, the activation of a latent, ribosome-associated RNase which initiates degradation of the particles. Furthermore, no precautions were taken to minimise RNase activity which is known to be high in many plant extracts even at 0° [3, 5, 8]. The retention of a 31S "core" of highly fragmented RNA would not be unexpected since the RNA even of intact ribosomes can be considerably degraded without any appreciable effect on the physical properties of the whole ribosome [5, 7, 8, 17]. This is attributed to nuclease cleavage of sterically accessible regions of the RNA chains without disruption of the secondary and tertiary structures.

The present data suggest therefore that the 31S RNP fraction is degraded ribosomal material with extraneously bound protein, including phytochrome. Likewise the pigment in the "heavy" band might well represent the association of phytochrome with residual ER-bound ribosomal material. Several aspects of phytochrome binding thus far reported, both *in vivo* and *in vitro* [11, 12, 18–22,

25] might be wholly or partly explained on the basis of the interaction of P_{fr} with ribosomal material—degraded or otherwise. Whether such an association is artifactual or biologically meaningful is yet to be decided.

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