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DE NOVO SYNTHESIS OF POLY(A) POLYMERASE IN MUNG BEAN HYPOCOTYLS, INVOLVING STORED mRNA

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Key Word Index—*Vigna*, Leguminosae, mung bean, hypocotyls, poly(A) polymerase, enzyme regulation, *de novo* synthesis, stored mRNA, poly(A)$^+$RNA, product characterization

Abstract—A linear increase in poly(A) polymerase activity (3–4-fold) in hypocotyls of germinating mung beans was accompanied by a parallel increase in the levels of poly(A)$^+$ RNA (4–16 hr). The enzyme activity declined at subsequent stages of seed germination (28–52 hr) without a similar downward trend in the levels of poly(A)$^+$ RNA The inhibition of enzyme activity could, however, be alleviated by ion exchange chromatography on DE-52. This indicated the presence of some inhibitory factor which interfered with the poly(A) polymerase activity in the ammonium sulphate fraction precipitate. Thus a direct relationship is indicated between the rise in the activity of poly(A) polymerase and the increased levels of poly(A)$^+$ RNA in the hypocotyls of germinating embryos. Administration of cycloheximide (20 μg/ml) strongly inhibited poly(A) polymerase activity in the hypocotyls, thereby indicating the requirement of *de novo* protein synthesis. Conclusive proof for the *de novo* synthesis of poly(A) polymerase was achieved by labelling the enzyme *in vivo* with $^{35}SO_4^{2-}$. The ^{35}S-label was recovered in the cysteine and methionine residues of the purified poly(A) polymerase. Thus it became evident that the stimulation of poly(A) polymerase activity in hypocotyls is primarily achieved by the *de novo* synthesis of the enzyme. Transcriptional activity was, however, not mandatory for the synthesis of poly(A) polymerase, since cordycepin (250 μM) failed to block the rise in the enzyme activity Apparently, poly(A) polymerase is synthesized from its stored mRNA in mung bean hypocotyls Curiously, the cordycepin-treated hypocotyls exhibited a significant stimulation of poly(A) polymerase activity (3–4 fold) over that of the controls. It is envisaged that the drug blocks the transcription of the inhibitory factor of poly(A) polymerase and is therefore indirectly responsible for the stimulation of enzyme activity The ^{3}H-labelled reaction product of poly(A) polymerase was resistant to the hydrolytic action of RNAase A and RNAase T_1 The labelled product was also retained on the affinity matrix of oligo (dT)-cellulose This proved the polyadenylate nature of the *in vitro* synthesized reaction product The M_r of the mung bean poly(A) polymerase is 120000.

INTRODUCTION

The widespread occurrence of poly(A) polymerase (E.C. 2.7.7.19) has been reported both in plant and animal cells [1, 2]. This enzyme is responsible for the addition of poly(A) sequences at the 3′ terminus of hnRNA and mRNA [3]. Thus poly(A) polymerase is of crucial importance during RNA processing and could play a key role in the control of gene expression in eukaryotes [4, 5]. Poly(A) polymerase has been purified from a variety of animal tissues, such as calf thymus [6], rat liver nuclei [7] and hepatoma cells [8], and its M_r, subunit structure and kinetic properties determined. Surprisingly, poly(A) polymerase has not been so far purified from any plant tissue and consequently, nothing is known about its subunit structure and kinetic properties.

In wheat aleurones and excised wheat embryos, poly(A) polymerase is regulated by gibberellic acid at the post-transcriptional level [4, 5, 9]. Concomitantly, the phytohormone also enhanced the relative abundance of poly(A)$^+$RNA both in wheat and barley aleurones [10–12] In addition, high levels of poly(A)$^+$RNA have also been reported in GA_3-treated seedlings of castor beans, maize and hazel [13–15]. Inhibition of poly(A) polymerase activity by cycloheximide and amino acid analogues in wheat aleurones and embryos has indicated that *de novo* protein synthesis is necessary for the enhancement of poly(A) polymerase activity [5, 9] Nevertheless, conclusive proof for the *de novo* synthesis of poly(A) polymerase has yet to be established

In rat hepatoma cells, the activation of poly(A) polymerase is achieved through the process of phosphorylation of the enzyme [16]. Thus the regulation of enzyme activity could occur in the absence of transcriptional and translational activities of the cells.

In the present investigation, we have observed a direct relationship between the stimulation of poly(A) polymerase activity and the relative abundance of poly(A)$^+$RNA in mung bean hypocotyls. Conclusive evidence has been presented for the *de novo* synthesis of poly(A) polymerase Inhibitor studies indicated that this RNA processing enzyme is translated from its stored mRNA.

RESULTS

Stimulation of poly(A) polymerase and poly(A)$^+$RNA

Dry excised mung bean hypocotyls contained substantial poly(A) polymerase activity (1.4×10^3 picomol/

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AMP incorporated/mg protein) in an ammonium sulphate precipitable fraction (30–50% satn). A linear increase in poly(A) polymerase activity (3–4-fold) was observed in hypocotyls during embryo germination (16 hr, Fig 1). This rise in poly(A) polymerase activity was associated with a parallel increase in the levels of poly(A)$^+$ RNA (9-fold) in hypocotyls (Fig 1) At subsequent stages of germination (28–52 hr), there was a considerable decline in the activity of poly(A) polymerase in the hypocotyl tissue However, relatively high levels of poly(A)$^+$ RNA were maintained at these stages of embryo germination (Fig 1) With a view to ascertaining the molecular basis of the decline in poly(A) polymerase activity, we fractionated the ammonium sulphate fraction precipitate (30–50% satn) by DE-52 ion exchange chromatography at different stages of embryo germination (12–48 hr) A time course study revealed that there was no decline in poly(A) polymerase activity in the DE-52 fractions (Fig 2) Thus it became evident that some inhibitory factor was responsible for the reversible inactivation of poly(A) polymerase The inhibitor of poly(A) polymerase activity was conveniently separated by DE-52 chromatography

Regulation of poly(A) polymerase through de novo *enzyme synthesis*

Treatment of germinating mung bean embryos with CHI (20 μg/ml) severely inhibited (79%) poly(A) polymerase activity in the hypocotyls in comparison to the control (Fig 3) Thus enhancement of poly(A) polymerase activity in mung bean hypocotyls is dependent on *de novo* protein synthesis Conclusive proof for *de novo* synthesis of poly(A) polymerase was obtained by labelling the enzyme *in vivo* with $^{35}SO_4^{2-}$ Poly(A) polymerase was purified to *ca* 2980-fold. This was accompanied by the increase in the specific radioactivity (*ca* 68-fold) of the purified enzyme fraction (see Table 1) The purified poly(A) polymerase gave a single protein band on native acrylamide gels thereby proving the electrophoretic homogeneity of the enzyme The catalytic activity of this protein band was determined by slicing the gel and eluting the enzyme protein from each slice The single activity peak of poly(A) polymerase thus obtained (Fig 4) corresponded with the position of the protein band on native gels. The ^{35}S-labelled purified poly(A) polymerase was also fractionated on acrylamide gels Scanning of this gel (2 mm strips) revealed a single radioactivity peak coincident with the poly(A) polymerase activity peak (Fig 4) Further characterization of the labelled poly(A) polymerase revealed that the ^{35}S-label was incorporated into the methionine and cysteine residues of the enzyme (data not presented) These observations clearly proved that *de novo* synthesis of poly(A) polymerase was necessary for the enhancement of enzyme activity in hypocotyls of germinating mung bean embryos

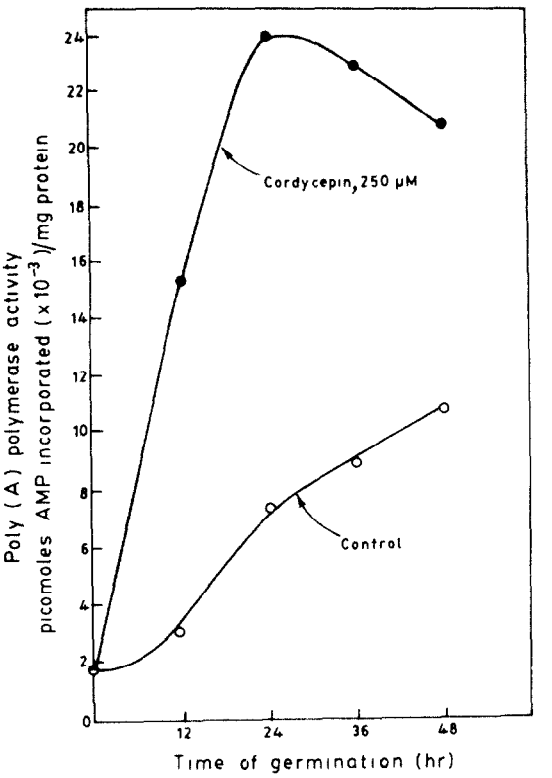

Fig 2 Time course study showing the stimulation of poly(A) polymerase activity in cordycepin (250 μM)-treated hypocotyls Mung bean seeds were germinated for different time intervals in the presence and absence of cordycepin (250 μM) The enzyme activity was measured in the DE-52 fraction

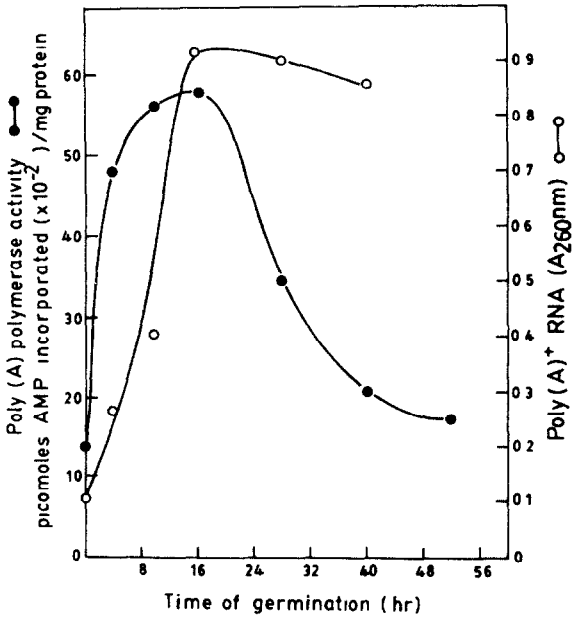

Fig 1 Time course study showing the activity of poly(A) polymerase and the levels of poly(A)$^+$RNA in hypocotyls, excised from germinating mung bean seeds Ammonium sulphate fraction precipitate (30–50% satn) was prepared from the mung bean hypocotyls for the assay of poly(A) polymerase activity In a parallel experiment, total RNA was isolated and fractionated on oligo (dT)-cellulose for the separation of poly(A)$^+$RNA

Biosynthesis of poly(A) polymerase from its stored mRNA

Cordycepin (250 μM), a potent inhibitor of transcription, failed to stop poly(A) polymerase activity in

mung bean hypocotyls; instead there was significant stimulation (3–4-fold) of enzyme activity in the drug-treated tissue (24 hr; see Fig. 2). This clearly indicated that the biosynthesis of poly(A) polymerase does not require fresh transcriptional activity during early seed germination. Apparently, poly(A) polymerase is synthesized from its stored mRNA. With regard to the stimulation of poly(A) polymerase activity in cordycepin-treated hypocotyls, we consider that the drug possibly blocked the transcription of the inhibitor of poly(A) polymerase enzyme. The low levels of inhibitor could result in the apparent enhancement of poly(A) polymerase activity.

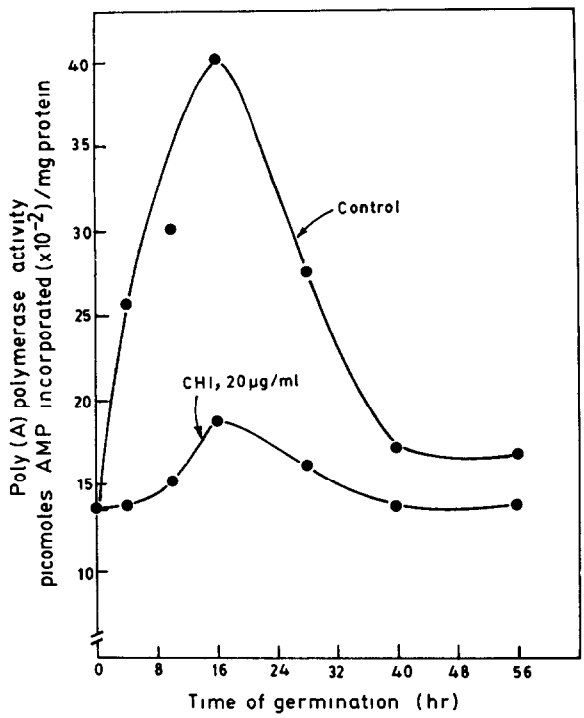

Fig 3 Inhibition of poly(A) polymerase activity in mung bean hypocotyls by cycloheximide Mung bean seeds were germinated in the presence and absence of CHI (20 µg/ml) for different time intervals Poly(A) polymerase activity was measured in the ammonium sulphate fraction precipitate (30–50% satn)

Determination of M_r of poly(A) polymerase

Polymin-P fraction was subjected to gel permeation on Sephacryl S-200 column A single molecular form of

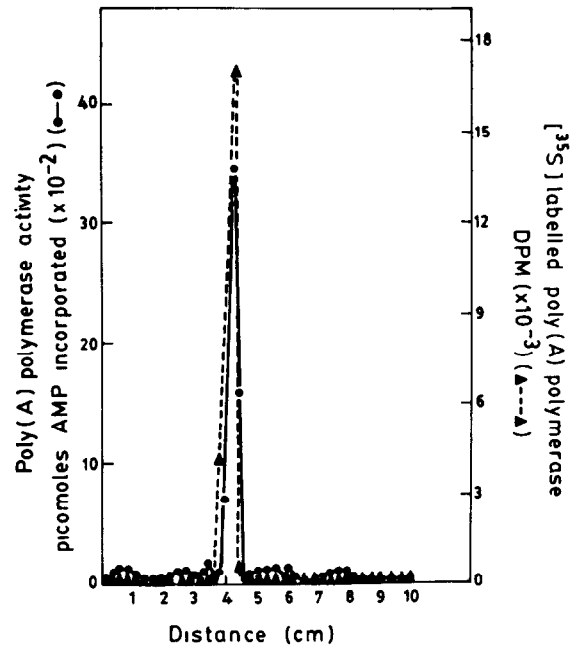

Fig 4. *In vivo* labelling of poly(A) polymerase with $^{35}SO_4^{2-}$ in germinating mung bean hypocotyls The dry excised hypocotyls were imbibed (24 hr) in a medium containing $^{35}SO_4^{2-}$ for labelling the proteins *in vivo* Poly(A) polymerase was purified to electrophoretic homogeneity from the ^{35}S-labelled and unlabelled hypocotyls and then fractionated on the native acrylamide gels (10%). The gels were sliced and eluted for determining the poly(A) polymerase activity and the ^{35}S labelling of the enzyme Staining of gel with silver nitrate revealed a single protein band The enzyme activity peak and the ^{35}S-labelled radioactivity peak were co-incident with each other

Table 1. Purification protocol for the ^{35}S-labelled poly(A) polymerase from mung bean hypocotyls

	Poly(A) polymerase activity		^{35}S-label incorporation into the protein fraction	
Purification step	(units/mg protein)	Purification (fold)	(dpm/mg protein)	Increase in specific radioactivity (fold)
Crude fraction	1 4	1 0	$1\,3 \times 10^4$	1 0
Polymin-P fraction	45 7	32.6	100×10^4	7.7
ATP-Sepharose affinity chromatography followed by DE-52 fractionation	4177 8	2984.0	$88\,9 \times 10^4$	68 4

Mung bean hypocotyls were germinated in $^{35}SO_4^{2-}$ for 24 hr. Poly(A) polymerase was purified to electrophoretic homogeneity Incorporation of ^{35}S-label into the protein fraction was determined at various steps of the enzyme purification In a parallel experiment, poly(A) polymerase was purified from the unlabelled tissue. Poly(A) polymerase activity was assayed at different steps of enzyme purification. One enzyme unit is defined as one nanomole of AMP incorporated into the poly(A) product in 60 min at 37°

poly(A) polymerase eluting between 90 and 120 ml was obtained with a M_r of 120000.

Characterization of reaction product of poly(A) polymerase

The ^{3}H-labelled reaction product was characterized by its specific binding to an oligo (dT)-cellulose affinity matrix. The reaction product was retained on the oligo (dT)-cellulose and was isolated by washing the affinity matrix with the elution buffer (Fig 5) Thus the bound radioactivity on the affinity column represented the polyadenylated reaction product The ^{3}H-labelled reaction product was also resistant to the nucleolytic action of RNAase A and RNAase T_1, thereby proving the synthesis of poly(A) stretches under our assay conditions (Table 2)

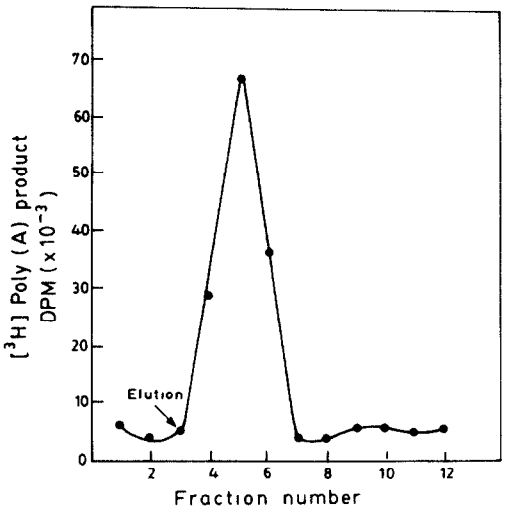

Fig 5. Characterization of ^{3}H-labelled poly(A) reaction product by affinity chromatography on oligo(dT)-cellulose The ^{3}H-labelled reaction product of poly(A) polymerase was annealed on oligo (dT)-cellulose matrix as described in Experimental The bound poly(A) product was released by washing the column with elution buffer (Tris–HCl 10 mM, pH 7 6) Radioactivity was measured in each fraction (500 μl)

Table 2 Characterization of ^{3}H-labelled reaction product of poly(A) polymerase

Additions	Acid-insoluble product (dpm)
^{3}H-labelled reaction product	5499
^{3}H-labelled reaction product + RNAase A (10 μg/ml) + RNAase T_1 (10 knutz/ml)	5340

The ^{3}H-labelled reaction product of poly(A) polymerase was treated with RNAase A and RNAase T_1 for one hr. The labelled product, insensitive to treatment with nucleases, was precipitated with TCA (5%) and collected on Whatman 3MM discs for measurement of its radioactivity content

DISCUSSION

A rapid upsurge in poly(A) polymerase activity in mung bean hypocotyls could be responsible for the parallel rise in the relative abundance of poly(A)$^+$RNA A similar positive correlation between poly(A) polymerase and poly(A)$^+$RNA has been reported in the GA_3-treated excised aleurones and embryos of wheat [5, 9, 10] Blocking of poly(A) polymerase activity by CHI in the hormone-treated tissue simultaneously lowered the levels of poly(A)$^+$RNA [9] Similarly, in pea epicotyls, the auxin-mediated inhibition of poly(A) polymerase activity was associated with a dramatic decline in the levels of poly(A)$^+$RNA [17] In germinating wheat embryos, the infection of tissue by a fungal pathogen resulted in a strong inhibition of poly(A) polymerase activity with a concurrent parallel decrease in the levels of poly(A)$^+$RNA [18, 19] Thus it seems logical to conclude that the regulation of poly(A) polymerase in plant cells does indeed modulate the total levels of poly(A)$^+$RNA

The rise in the activity of poly(A) polymerase was strongly inhibited by treatment of the mung bean hypocotyl tissue with CHI (present work) This indicated that *de novo* protein synthesis is necessary for the stimulation of poly(A) polymerase activity In cereal aleurones and embryos of wheat, both CHI and amino acid analogues strongly blocked the GA_3-stimulated poly(A) polymerase activity [5, 9] However, conclusive proof for the *de novo* synthesis of poly(A) polymerase is now presented in mung bean hypocotyls. This was essentially achieved by labelling the enzyme with $^{35}SO_4^{2-}$ *in vivo*. Poly(A) polymerase has been purified by ATP-Sepharose affinity chromatography Acrylamide gel electrophoresis of the purified enzyme on native gels revealed a single protein band, thereby proving the electrophoretic homogeneity of the enzyme preparation Fractionation of ^{35}S-labelled purified poly(A) polymerase revealed a single radioactivity peak co-incident with purified unlabelled poly(A) polymerase The ^{35}S label was ultimately recovered in the methionine and cysteine residues of purified poly(A) polymerase We conclude that the enhancement of poly(A) polymerase activity in the mung bean hypocotyls, during early germination of embryo, is indeed achieved through the *de novo* synthesis of the enzyme molecules

The unique feature about the regulation of poly(A) polymerase activity in wheat aleurones [5], wheat embryos [9, 20] and mung bean hypocotyls (present study) is that it is translated from its stored mRNA during germination of seeds This view is based on the fact that blocking of transcription by cordycepin did not curtail the induction of poly(A) polymerase By contrast there was additional enhancement of poly(A) polymerase activity in drug-treated wheat embryos [9] and mung bean hypocotyls We consider that cordycepin possibly blocks the synthesis of an inhibitory factor of poly(A) polymerase The enhanced activity of poly(A) polymerase could be observed with the masking of this inhibitor Thus the drug does not seem to have a direct stimulatory effect on the poly(A) polymerase activity In germinating excised wheat embryos, the stimulation of *o*-diphenolase is not dependent on fresh transcription, but does require *de novo* protein synthesis This suggested that *o*-diphenolase is translated from its stored messenger already present in the ungerminated wheat embryos [21] However, in this system, cordycepin failed to bring about the enhancement of *o*-diphenolase activity as has been observed

for poly(A) polymerase in wheat embryos [9] and mung bean hypocotyls

EXPERIMENTAL

The seeds of mung bean (*Vigna radiata*, Var PS-16) were procured from the Indian Agricultural Research Institute, Karnal, India. Carrier free $^{35}SO_4^{2-}$ and [^{3}H]-ATP (5Ci/mmol) were purchased from Bhabha Atomic Research Centre, India. Sephacryl S-200 was a product of Pharmacia Fine Chemicals, Sweden. ATP, PVP, Polymin-P and polyethylene glycol (M_r 20000) were obtained from Sigma, U S A The DE-52 ion-exchanger and Whatman 3MM chromatographic paper were procured from Whatman Ltd, England The M_r calibration kit was purchased from Boehringer, F R G All other reagents were of analytical grade ATP-Sepharose was prepared in our laboratory according to the procedure given in [22] Primer RNA was isolated from germinating wheat embryos as described in [23]

Isolation of hypocotyls Mung bean seeds were surface sterilized with $HgCl_2$ soln (0.02%) for 10 min and then thoroughly rinsed with sterile dist H_2O. The seeds were germinated in sterile distilled water containing chloramphenicol (50 μg/ml) In another set of experiments, the seeds were initially imbibed in the absence and presence of cycloheximide (20 μg/ml) or cordycepin (250 μM) for a period of 4 hr in the dark at 25° Thereafter, the seeds were transferred aseptically to flasks containing water, CHI (20 μg/ml) or cordycepin (250 μM) and allowed to germinate at 25° in the dark on a shaker (100 rpm) Chloramphenicol (50 μg/ml) was added in the medium as a bactericidal agent The hypocotyls were manually clipped from the seedlings at different time intervals, frozen in liquid N_2 and stored at $-60°$.

In vivo labelling of proteins in excised mung bean hypocotyls with $^{35}SO_4^{2-}$ Dry mung bean seeds were crushed in a Waring blendor and sieved through meshes of various sizes for separating large pieces of cotyledons The excised hypocotyls were manually picked and stored in an air-tight bottle at 4° The dry excised mung bean hypocotyls (30 g) were surface sterilized with $HgCl_2$ soln (0.02%) and rinsed several times (× 6) with sterile dist H_2O and then germinated in sucrose soln (2% w/v) containing $^{35}SO_4^{2-}$ (40 mCi) at a concentration of 300 μCi/ml at 25° in the dark on a shaker (100 rpm) Chloramphenicol (50 μg/ml) was added to the medium as a bactericidal agent

Enzyme preparation. Mung bean hypocotyls were homogenized in Tris–HCl (50 mM, pH 8 0) containing 2-mercaptoethanol (5 mM) Polyvinyl polypyrrolidone (2%, w/v) and acid-washed sand were added during homogenization The homogenate was centrifuged (10000 *g* for 15 min) and the pellet fraction was discarded The clear supernatant was subjected to $(NH_4)_2SO_4$ fractionation (30–50% satn) The pptd proteins were collected by centrifugation (10000 *g* for 10 min) The protein fraction was dissolved in an aliquot of Tris–HCl (20 mM, pH 8.0) containing 2-mercaptoethanol (5 mM) and dialysed against the same buffer. All operations were carried out at 4° The desalted enzyme fraction was employed for the assay of poly(A) polymerase activity

The dialysed $(NH_4)_2SO_4$ fraction precipitate was subjected to ion exchange chromatography on DE-52 The column was equilibrated with Tris–HCl buffer (20 mM, pH 8.0) containing $(NH_4)_2SO_4$ (50 mM), 2-mercaptoethanol (5 mM) and glycerol (20%) The $(NH_4)_2SO_4$ fraction ppt. was diluted to a protein concentration of 5 mg/ml. Solid $(NH_4)_2SO_4$ was added to the enzyme fraction to achieve 50 mM concentration of this salt The unbound fraction from DE-52 containing poly(A) polymerase activity was concentrated against polyethylene glycol, followed by dialysis This enzyme fraction was designated as DE-52 fraction

Assay of poly(A) polymerase. The assay mixture (300 μl) contained Tris–HCl buffer (100 mM, pH 8.0), $MnCl_2$ (2 mM), dithiothreitol (5 mM), wheat embryo primer RNA (500 μg), [^{3}H]-ATP 1 5 mM, (4 μCi) and enzyme fraction (200–500 μg protein). The enzyme assay was carried out at 37° for 30 min However, the assay of enzyme in DE-52 fraction was carried out after incubating for 60 min at 37° The reaction was terminated by plating an aliquot (40 μl) of the assay mixture onto a Whatman disc (3 MM) and immersed in chilled TCA (5%, 10 ml per disc) The unincorporated [^{3}H]-ATP was removed by rinsing with TCA (5%, × 4) followed by a wash (× 1) with Et_2O–EtOH (1 1) and Et_2O The filter discs were dried at 60° and the radioactivity was measured as described in [4]. The specific activity of poly(A) polymerase is expressed as pmol AMP incorporated/mg protein

Isolation of total poly(A)$^+$ RNA The total RNA was extracted from the frozen mung bean hypocotyls by the procedure given in [23] The RNA fraction was suspended in Tris–HCl (10 mM, pH 7 6), heated at 65° for 5 min and chilled rapidly. The RNA soln was diluted to obtain a concn of 0 5 mg/ml and NaCl (2 M) was added to obtain 0 5 M concn of the salt This sample was loaded onto an oligo (dT)-cellulose affinity column (2 0 × 1 0 cm) at 4°, washed extensively with binding buffer. The bound poly(A)$^+$RNA was eluted with elution buffer (Tris–HCl buffer 10 mM, pH 7 6) at 45°. Poly(A)$^+$RNA was determined in each fraction (1 ml) by measuring A_{260} This procedure is essentially a modified version of the method for the isolation of poly(A)$^+$RNA given in [24]

Characterization of reaction product The ^{3}H-labelled reaction product of poly(A) polymerase was treated with RNAase A (10 μg/ml) and RNAase T_1 (10 knutz/ml). The assay was carried out in the presence of Tris–HCl buffer (100 mM, pH 7 5) and NaCl (0.5 M). Omission of nucleases from the reaction mixture served as control The reaction mixture was incubated at 37° for 1 hr and was terminated by the addition of equal vol of chilled TCA (10%) containing sodium pyrophosphate (2 mM) The acid precipitable product was collected on Whatman 3MM discs for measuring radioactivity

Purification of poly(A) polymerase from mung bean hypocotyls Frozen mung bean hypocotyls (500 g) were homogenized in the presence of acid washed sand and polyvinyl polypyrrolidone (2% w/v) in buffer (800 ml) containing Tris–HCl (250 mM, pH 7 3), Na-EDTA (0 2 mM), $MgCl_2$ (5 mM), $(NH_4)_2SO_4$ (75 mM) and 2-mercaptoethanol (50 mM) The homogenate was passed through four layers of muslin cloth and centrifuged at 10000 *g* for 15 min Polymin-P soln (10% v/v) was slowly added to the supernatant fraction to obtain a final concn of 0 7% The nucleoproteins precipitated by this procedure were collected by centrifugation The Polymin-P ppt was washed with Buffer B (250 ml) containing Tris–HCl (50 mM, pH 7.3), Na-EDTA (0.1 mM) and 2-mercaptoethanol (5 mM). The nucleic acid bound proteins from the pellet fraction were eluted with Buffer B containing $(NH_4)_2SO_4$ (0 5 M). The resulting fraction was centrifuged (10000 *g* for 15 min) and the yellowish supernatant was subjected to $(NH_4)_2SO_4$ fractionation (0–65% satn). The pptd proteins were collected by centrifugation and desalted by exhaustive dialysis against Tris–HCl buffer (50 mM, pH 7 9), $MgCl_2$ (0 5 mM), 2-mercaptoethanol (5 mM), $ZnCl_2$ (1 μM) and glycerol (25%) This fraction was designated as Polymin-P fraction The dialysed Polymin-P fraction was further subjected to ATP-Sepharose affinity chromatography [25]. The bound proteins were eluted with equilibrating buffer containing ATP (3 mM). The eluted fraction from ATP-Sepharose was concd by dialysis against polyethylene glycol. The concd proteins were fractionated on DE-52, pre-equilibrated with Tris–HCl buffer (50 mM, pH 7 9) containing $MgCl_2$ (0 5 mM), $ZnCl_2$ (1 μM), 2-mercaptoethanol (5 mM) and glycerol (25%) Poly(A) poly-

merase was collected as the unbound protein fraction The column was further washed with one bed vol of equilibrating buffer containing $(NH_4)_2SO_4$ (10 mM) All the fractions were pooled by dialysis against the above mentioned Tris–HCl buffer This procedure was also followed for the purification of ^{35}S-labelled poly(A) polymerase from mung bean hypocotyls The ^{35}S-labelled purified poly(A) polymerase was fractionated on polyacrylamide gel (10%) under nondenaturing conditions The gel was dried and cut into the strips (2 mm wide) and scanned for radioactivity Simultaneously one of the gel lanes was stained for the visualization of protein bands The purified enzyme was also hydrolysed in HCl (6 M) for 2 hr at 110° The hydrolysate was chromatographed for 18 hr using BuOH-HOAc-H_2O (12 3 5) as a solvent system The dried chromatogram was radioautographed for the identification of ^{35}S-labelled methionine and cysteine residues, while the authentic marker amino acids (methionine and cysteine) were visualized by staining with ninhydrin (0 3% in BuOH)

Determination of M_r *of poly(A) polymerase* The Polymin-P fraction was subjected to molecular sieving on Sephacryl S-200 (2 09 × 70 cm) Fractions (2 ml each) were collected after the void vol (V_o = 78 ml) An aliquot of 180 μl from each fraction was employed for the assay of poly(A) polymerase activity The void vol of the column was determined experimentally by the elution profile of Blue Dextran (4 mg/ml) under identical conditions The M_r of the poly(A) polymerase was determined by calibrating the Sephacryl S-200 column with marker proteins in two batches Batch I Aldolase (M_r 158 000), Huhnerei albumin (45 000) Batch II Bovine serum albumin (Dimer, 136 000), Bovine serum albumin (Monomer, 68 000) The column was pre-equilibrated with Tris–HCl buffer containing 2-mercaptoethanol (5 mM), $ZnCl_2$ (1 μM) and $MgCl_2$ (0 5 mM) The same buffer was used for the elution of proteins

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