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REGULAR PAPER

The *Zea mays* glycine-rich RNA-binding protein MA16 is bound to a ribonucleotide(s) by a stable linkage

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Abstract Expression of the gene encoding the maize glycine-rich RNA-binding protein MA16 is developmentally regulated and it is involved in environmental stress responses. The MA16 protein shows a wide spectrum of RNA-binding activities. On the basis of in vivo labelling, where a [^{32}P]phosphate label was linked to the MA16 protein, Freire and Pages (Plant Mol Biol 29:797–807, 1995) suggested that the protein may be post-translationally modified by phosphorylation. However, further analysis showed that the [^{32}P]phosphate label was sensitive to different treatments, suggesting that modification distinct from protein phosphorylation might occur in the MA16 protein. Biochemical analysis revealed that this [^{32}P]phosphate labelling was resistant to phenol extraction and denaturing SDS-PAGE but sensitive to micrococcal nuclease, RNase A and RNase T1 treatments. The mobility of [^{35}S] labelled MA16 protein on SDS-PAGE did not significantly changed after the nuclease treatments suggesting that the [^{32}P]phosphate label associated to MA16 protein could be a ribonucleotide or a very short ribonucleotide chain. In addition, immunoprecipitation of labelled extracts showed that the ribonucleotide(s) linked to the MA16 protein was removed by phosphorolytic activity. This activity could be catalysed by a phosphate-dependent ribonuclease. The C-terminus of MA16 protein harbouring a glycine-rich domain was predicted to be an intrinsically disordered region.

Keywords Exosome · Exoribonuclease · Nucleotidylylation · Nucleus · Nucleolus · Intrinsic disorder

Abbreviations

MN	Micrococcal nuclease
PNPase	Polynucleotide phosphorylase
Pi	Inorganic phosphate
PDX	Phosphate-dependent exoribonuclease

Introduction

Expression of the gene encoding the MA16 protein is induced in immature maize embryos by incubating with the abscisic acid hormone (Gomez et al. 1988), or in vegetative tissues under stress conditions such as drought, wounding or treatment with heavy metals (Gomez et al. 1988; Didierjean et al. 1992; Lobreaux et al. 1993). The protein contains a consensus RNA recognition motif (RRM) in the amino-terminus and arginine–glycine-rich motifs in the carboxy-terminal region. Homologous proteins have been identified in different plant species and in other kingdoms from cyanobacteria (Sato 1995) to human (Derry et al. 1995).

The RRM domain comprises 80–90 amino acids within which the most highly conserved sequences are an octapeptide termed RNP1, and a hexapeptide designated RNP2 (Bandziulis et al. 1989; Kenan et al. 1991). Although the

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functional target of many putative RNA-binding proteins remains unknown, the presence of these motifs in a newly-deduced protein sequence is a very strong indicator of nucleic-acid-binding ability. The predicted RNA-binding property of the MA16 protein was confirmed by using ribohomopolymer binding assays (Ludevid et al. 1992), and co-purification (Freire and Pages 1995). The MA16 protein showed a wide spectrum of RNA-binding activities for several RNAs including rRNA and translatable mRNAs. The binding was heparin and salt sensitive, which is indicative of electrostatic interactions. The protein was also found associated to its own mRNA, suggesting a possible feedback control (Freire and Pages 1995).

The carboxy-terminal region of the MA16 protein has high glycine content with charge alternation (arginine/aspartic and arginine/glutamic) and aromatic amino acids, exclusively tyrosines. The positively charged domain can lead to non-specific interactions due to its electrostatic nature (Freire and Pages 1995).

Sequence analysis predicts that the glycine region is close to the GAR (glycine arginine rich) domain (as reported by Girard et al. 1992), and to the RGG boxes domain (arginine, glycine, glycine) (as described by Kiledjian and Dreyfuss 1992). The GAR domain is present in several nucleolar proteins: nucleolin (Lapeyre et al. 1987), fibrillarin (Ochs et al. 1985), SSB1 (Jong et al. 1987), NSR1 (Lee et al. 1991) and GAR1 (Girard et al. 1992). The GAR domain appears to facilitate nucleolar localisation and is necessary for nucleolar targeting of Arabidopsis fibrillarin (Pih et al. 2000). Among the RGG box-containing proteins, the heteronuclear ribonucleoproteins (hnRNP) are restricted to the nucleoplasm. Nevertheless, both GAR and RGG box proteins have been shown to shuttle between the nucleus and the cytoplasm (Borer et al. 1989; Piñol-Roma and Dreyfuss 1992).

More recently, a homologue of the MA16 protein, the glycine-rich RNA-binding protein 7 from Arabidopsis (AtGRP7) has been found to shuttle between the nucleus and cytoplasm (Ziemienowicz et al. 2003), and be required for the export of mRNA from the nucleus to the cytoplasm under cold stress (Kim et al. 2008).

Immunoelectron microscopy (Alba et al. 1994) has revealed that the MA16 protein is present in the cytoplasm and accumulated in the dense fibrillar component at the nucleolus, where rRNA processing takes place. Fluorescence microscopy of transient expression of the MA16 protein fused to green fluorescent protein in epidermal onion cells has shown that the GAR domain is necessary and sufficient for both nuclear and nucleolar location (Gendra et al. 2004).

MA16 interacts with a DEAD box helicase, a protein able to bind and unwind double-stranded RNA structures. Both proteins co-localise in the nucleus and nucleolus; and in turn the helicase has been shown to bind to fibrillarin, (Gendra et al. 2004) which is involved in rRNA processing, rRNA methylation and ribosome assembly.

In addition to the RNA-binding activities, the characterisation of the MA16 protein by in vivo labelling has shown that it has an associated [^{32}P]phosphate label that is removed by alkaline phosphatase. On the basis of these observations, it was suggested that the protein may be post-translationally modified by phosphorylation (Freire and Pages 1995), but further analysis showed that the [^{32}P]phosphate label is sensitive to different treatments, indicating that modification distinct from protein phosphorylation might occur. Considering that MA16 protein is an RNA-binding protein, these findings prompted us to examine the nature of the [^{32}P]phosphate label associated with the MA16 protein in more detail.

Materials and methods

Plant material

Mature embryos (60 days after pollination) of *Zea mays* L. pure inbred line W64A were dissected manually.

In vivo labelling extracts

Isolated maize embryos were labelled in vivo for 6 h. For each assay, three isolated embryos were incubated with 200 µl of water containing the labelling mix L-[^{35}S]methionine and L-[^{35}S]cysteine (Perkin Elmer) at 1–3 × 10^7 Bq/ml. Phosphate labelling was with 2–6 × 10^7 Bq/ml [^{32}P]phosphate pH (5–7) (Perkin Elmer).

Total extracts were prepared from each group of embryos, ground with quartz powder in 400 µl of extraction buffer: 10 mM Tris HCl (pH 8); 50 mM NaCl; 0.05% Nonidet P-40 containing 1 mg/ml leupeptin (Sigma); and 0.5% aprotinin (Sigma). After centrifugation for 2 min at 13,000 rpm, the supernatant was collected and used immediately. Aliquots of supernatant were incubated with micrococcal nuclease (100 U/ml final concentration) for 30 min at 30°C in presence of 1 mM $CaCl_2$. The reaction was stopped by the addition of EGTA to a final concentration of 5 mM, or with RNase-A (0.01 µg/µl final concentration), RNase T1 (0.3 and 3 U/µl) for 20 min at 37°C, or with proteinase K (0.2 µg/µl final concentration) for 20 min, supplemented with 1% SDS, followed by phenol/chloroform extraction.

Immunopurification

Immunopurification was basically as described by Freire and Pages (1995). All steps were carried out at 8°C.

The protein A-Sepharose (25 µl), pre-equilibrated in the extraction buffer, was incubated by orbital rotation with MA16 antiserum (1:75) in 400 µl of extraction buffer. After 2 h, the resin was washed four times with the same volume of buffer followed by brief centrifugation at 13,000 rpm. The antibody-protein A-Sepharose complexes were incubated for 30 min with the differently treated extracts. After incubation, the resin was washed 5 times with the extraction buffer and eluted with Laemmli sample buffer (Laemmli 1970). The eluted products were resolved on 15% SDS-PAGE and the presence of [^{35}S]methionine–cysteine and [^{32}P]phosphate labels associated with the MA16 protein was revealed by autoradiography. Dual [^{35}S]methionine–cysteine and [^{32}P]phosphate autoradiograms were obtained from the same gels. The dry gels were exposed to the film at room temperature, directly in contact with the film to favour the [^{35}S] radiation. To enhance the [^{32}P] signal, the gels were covered with aluminium to prevent [^{35}S] radiation and exposed with an intensifying screen at −80°C.

Fractionation using glycerol gradients

Total extracts were prepared as described above. Aliquots of 200 µl were loaded onto a 3.8 ml 5–20% glycerol density gradient made in the extraction buffer. Centrifugation was for 16 h at 25,000 rpm in a Beckman SW60 rotor. Aliquots of all fractions (200 µl) were resolved by SDS-PAGE and assayed for MA16 protein by immunoblotting. The protein standards (human Ig-G, 7S; bovine catalase, 11.3S) were processed in parallel and visualised by Coomasie-blue staining.

Results

MA16 protein is linked to nucleic acid

On the basis of in vivo labelling, where a [^{32}P]phosphate label linked to the MA16 protein was removed by alkaline phosphatase, it was suggested that the protein may be post-translationally modified by phosphorylation (Freire and Pages 1995). However, further analysis showed that the [^{32}P]phosphate label was labile to nuclease treatments, indicating that it may correspond to a nucleic-acid, as alkaline phosphatase also catalyses the release of terminal phosphate groups from DNA and RNA. Subsequently this release could trigger a rapid degradation of the nucleic-acid by the nucleases present in the extract.

As MA16 is an RNA-binding protein, these observations led us to consider that the [^{32}P]phosphate label may correspond to a linked nucleic acid.

To investigate the nature of the [^{32}P]phosphate label associated with the MA16 protein, total extracts were obtained from simultaneous in vivo labelling with [^{32}P]phosphate and [^{35}S]methionine–cysteine. The protein extracts were treated with micrococcal nuclease and RNase A and the MA16 protein immunoprecipitated with a specific antiserum. The immunoprecipitated products were eluted with SDS-loading buffer and resolved by SDS-PAGE. The presence of [^{35}S]methionine–cysteine and [^{32}P]phosphate labels associated with the protein was revealed by autoradiography as shown in Fig. 1. Both autoradiograms come from the same gel. In panel a, the dry gel was exposed directly to favour signal from the [^{35}S] radiation, while panel b shows the signal from [^{32}P] radiation, enhanced using an intensifying screen. The results showed that the [^{35}S] label associated with MA16 protein

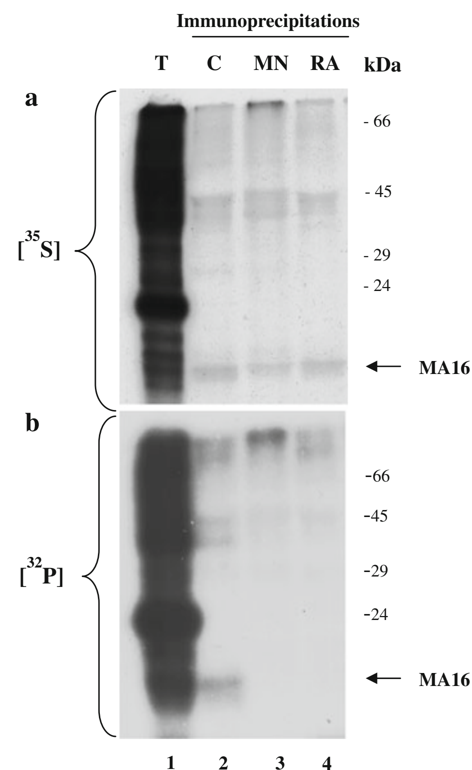

Fig. 1 MA16 protein is linked to nucleic acid. Maize embryo total extracts from in vivo double labelling with [^{32}P]phosphate and [^{35}S]methionine–cysteine were treated with micrococcal nuclease and RNase A. MA16 protein was immunoprecipitated with a specific antiserum. The immunoprecipitated products were resolved by SDS-PAGE and revealed by autoradiography. *Panels a* and *b* are from the same gel. *Panel a* [^{35}S]methionine–cysteine label; *panel b* [^{32}P]phosphate label. *Arrow* indicates the MA16 protein. *Lanes* (*1*), an aliquot of the total protein extract (*T*); *lanes* (*2*), immunoprecipitation from an untreated protein extract (control, *C*); *lanes* (*3*), immunoprecipitation from protein extracts treated with micrococcal nuclease (*MN*); and *lanes* (*4*), immunoprecipitation from protein extracts treated with RNase A (*RA*)

was detected in all treatments (panel a, lanes 2–4), confirming that the protein was not degraded during incubation with the nucleases. In contrast, the [^{32}P]phosphate label was only detected under untreated conditions (panel b, lane 2), completely depleted after digestion with micrococcal nuclease (MN) and RNase A (RA) (panel b, lanes 3 and 4). This sensitivity to micrococcal nuclease digestion suggests that the [^{32}P]phosphate label associated to MA16 protein may be a single-strand nucleic acid, and the RNase A degradation suggests that the nucleic acid contains ribonucleotides.

Within the limit of resolution of these experiments, as the mobility of [^{35}S] labelled MA16 protein on SDS-PAGE did not significantly changed after the nuclease treatments, it suggests that the [^{32}P]phosphate label associated to MA16 protein could be a ribonucleotide or a very short ribonucleotide chain.

MA16 protein is indeed linked to a ribonucleotide(s)

To further examine the molecular nature of the [^{32}P]phosphate label associated to MA16 protein, aliquots of in vivo [^{32}P]phosphate labelled extract were treated with RNase T1 at two concentrations and followed by immunoprecipitation with MA16 antiserum. The immunoprecipitates were resolved by SDS-PAGE.

The results (Fig. 2) show that the [^{32}P]phosphate signal was reduced after treatment with RNase T1 at 0.3 U/μl (lane 2), and was completely absent after digestion at 3 U/μl (lane 3). The correlation between the disappearance of the [^{32}P]phosphate label and the RNAse T1 concentrations used in the assay indicates that degradation of the [^{32}P]phosphate label was specifically due to the ribonuclease activity, identifying it as a ribonucleotide component.

MA16 protein is stably linked to a ribonucleotide(s)

To analyse the components of the putative MA16 protein-ribonucleotide(s) adduct, the embryo protein extracts from in vivo [^{32}P]phosphate labelling were immunoprecipitated with MA16 antiserum. The immunoprecipitated complexes were subjected to proteolytic digestion with proteinase K followed by phenol/chloroform extraction, and the phenol and aqueous phases precipitated and resolved by SDS-PAGE. The precipitated phenol phase was expected to retain the majority of proteinaceous components while the precipitated aqueous phase the nucleic acids.

As shown in Fig. 3, the bands of [^{32}P]phosphate label appeared in the control immunoprecipitation (lane 1) and in the phenol phase obtained from the untreated immunoprecipitation of MA16 protein (lane 2). The labelled band in the phenol phase was not detected after proteinase K digestion (lane 4) while a labelled smear of [^{32}P]phosphate was observed in the aqueous phase after proteinase K digestion (lane 5). However, no [^{32}P]phosphate labelled band of the same size as the MA16 protein was detected. Moreover, in control experiments with MA16 antiserum of deproteinated in vivo [^{32}P]phosphate-labelled RNA, no product was immunoprecipitated (not shown).

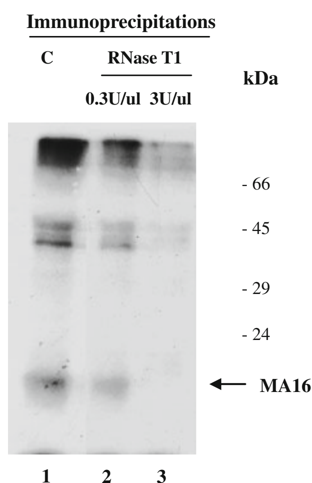

Fig. 2 MA16 protein is linked to a ribonucleotide(s). Total protein extracts from in vivo labelling with [^{32}P]phosphate were treated with RNase T1 at two concentrations followed by immunoprecipitation with MA16 antiserum. The immunoprecipitated products were separated on SDS-PAGE and revealed by autoradiography. *Lane* (*1*), immunoprecipitation of MA16 protein from untreated extracts (control, *C*); *lane* (*2*) and *lane* (*3*), immunoprecipitations from extracts treated with 0.3 and 3 U/ul of RNase T1 respectively

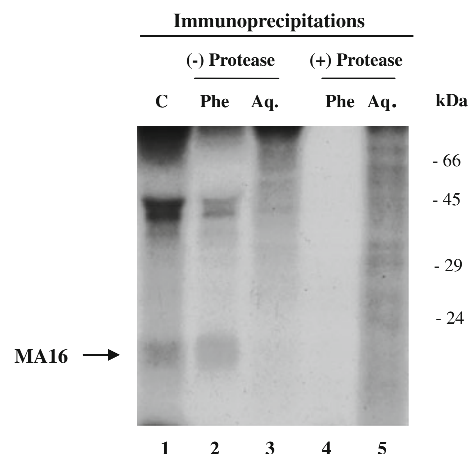

Fig. 3 MA16 protein is stably linked to a ribonucleotide(s). Total extracts from in vivo labelling with [^{32}P]phosphate were immunoprecipitated with the MA16 antiserum. The immunoprecipitate complexes were digested with proteinase K followed by phenol/chloroform extraction. The precipitated products, from the phenol and aqueous phases, were separated on SDS-PAGE. *Lane* (*1*), control immmunoprecipitation (*C*); *lane* (*2*) and *lane* (*3*), products from an untreated immunoprecipitation, present in the phenol phase (*Phe*) and in the aqueous phase (*Aq.*). *Lanes* (*4*) and (*5*) show the products in the phenol and the aqueous phase, respectively, from proteolysed immunoprecipitations

The [^{32}P]phosphate label may be intrinsically attached to MA16 protein by a stable linkage, based on the resistance to phenol extraction and SDS-PAGE electrophoresis but sensitivity to proteinase K digestion.

The [^{32}P]phosphate label linked to MA16 is removed by a phosphorolytic activity

Throughout the characterisation studies of the MA16 protein from in vivo labelling, it was observed that the [^{32}P]phosphate MA16 label was sensitive to inorganic phosphate. The results of these observations are shown in Fig. 4. Panels a and b come from the same gel as described above. Under standard conditions, immunoprecipitation showed both [^{35}S]methionine–cysteine and [^{32}P]phosphate labels associated with the MA16 protein (panels a and b, lanes 2). However, after washing the immunoprecipitation complexes with phosphate buffer, the [^{32}P]phosphate label disappeared (panel b, lane 3), while the [^{35}S] labelling was still visible (panel a, lane 3). The crucial factor in these experiments was the use of sodium phosphate washing buffers (50 mM sodium phosphate, pH 8).

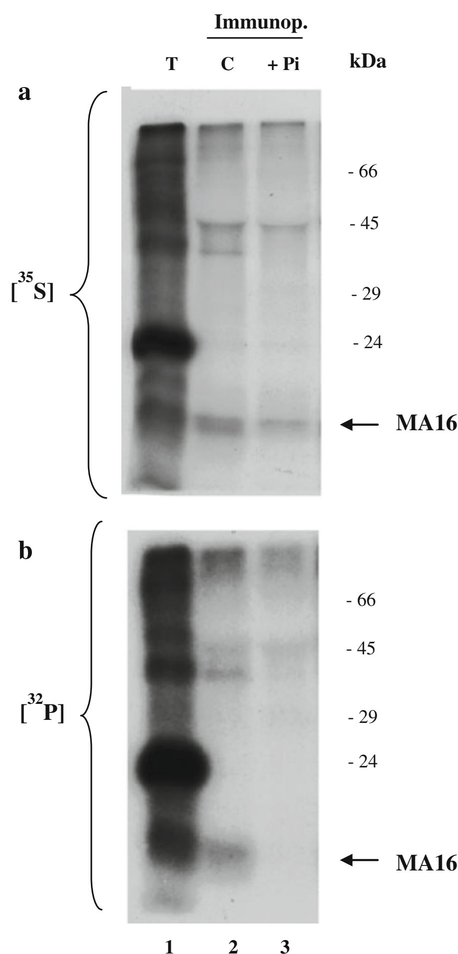

Fig. 4 The [^{32}P]phosphate label linked to MA16 is removed by a phosphorolytic activity. Total protein extracts from in vivo labelling with [^{32}P]phosphate and [^{35}S]methionine–cysteine were immunoprecipitated with MA16 antiserum in standard conditions and followed by phosphate buffer (50 mM, pH 8) washes. *Panel a* and *b* are from the same gel. *Panel a*, the [^{35}S]methionine–cysteine label and *panel b*, the [^{32}P]phosphate label. *Lane* (*1*), total extract (*T*); *lane* (*2*), immunoprecipitation in standard conditions (*C*); *lane* (*3*), immunoprecipitates washed with phosphate buffer (*Pi*)

If the MA16 protein is stably bound to a ribonucleotide(s), it is possible to infer that it might be degraded by phosphorolysis catalysed by an RNA phosphorylase. Among the plethora of RNases, phosphorolytic activity is a distinguishable feature of the deadenylyl removase activity of the bifunctional enzyme glutamine synthetase adenylyl transferase (GS-AT) (Itzen et al. 2011), and the phosphate-dependent exoribonuclease PDX family members (Zuo and Deutscher 2001) which are part of the eukaryotic exosome and bacterial degradosome complexes involved in several pathways of RNA metabolism (Parker and Song 2004; Rauhut and Klug 1999).

MA16 protein is not incorporated into high molecular weight complexes

The MA16 protein has been found to bind a wide spectrum of RNAs with high affinity to particular RNAs, comprising rRNA and mRNA sequences (Freire and Pages 1995). Moreover, immunoprecipitation revealed that the protein is associated with other proteins, suggesting that it may be part of a ribonucleoprotein particle (Freire and Pages 1995), and it was shown that the ribonucleotide(s) linked to MA16 protein was degraded by phosphorolytic activity probably catalysed by an RNA phosphorylase. Therefore, the next step was to examine if MA16 protein was part of a molecular complex.

Total extracts were separated by centrifuging through a 5–20% glycerol gradient (Fig. 5). The majority of MA16 protein sedimented in the first fractions consisted of a free protein or a small complex of density less than 7S. The MA16 protein was not found stably incorporated into high molecular complexes.

The exosome complexes have been previously reported in plants to be higher than 14S. In Arabidopsis, the exosome complex is about 500 kDa (Chekanova et al. 2000), and the chloroplastic degradosome, composed exclusively of polynucleotide phosphorylases, is approximately 600 kDa (Baginsky et al. 2001).

Discussion

The studies using in vivo labelling indicated that the glycine-rich RNA-binding MA16 protein from maize has a [^{32}P]phosphate label associated. Biochemical characterisation

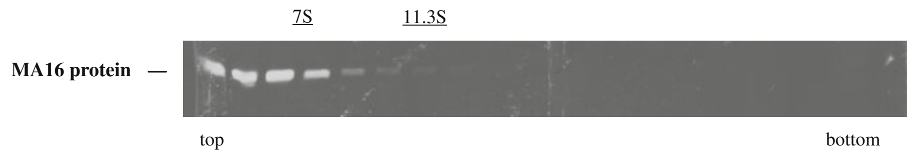

Fig. 5 MA16 protein is not stably incorporated into high molecular complexes. Total protein extracts from maize embryo were resolved by sedimentation through a 5–20% glycerol gradient. All fractions, 20 aliquots of 200 µl, were precipitated and run on SDS-PAGE and assayed for MA16 protein by immunoblotting. Standards (human Ig-G, 7S; bovine catalase, 11.3S) separated in parallel are indicated

of the associated label showed that it was resistant to phenol extraction and to SDS-PAGE but was hydrolysed by various ribonucleases. These results suggest that the [^{32}P]phosphate label is a consequence of a stable linkage between the MA16 protein and a ribonucleotide(s) forming a putative natural covalent complex.

The MA16 protein sequence contains several putative phosphorylation sites for protein kinase II: if this phosphorylation occurs, it could be a prerequisite or an intermediate stage, developmentally regulated in response to environmental conditions, for the ribonucleotide(s) linked to the MA16 protein.

Even though protein phosphorylation on MA16 cannot be excluded, the present work leads us to infer that the [^{32}P]phosphate label associated with the MA16 protein is a putative ribonucleotide structure with the following features: it should be a ribonucleotide or a very short chain of ribonucleotides as the electrophoretic mobility of the MA16 protein was not highly perturbed on SDS-PAGE, and it may contain cytosine or uridine, and guanosine as it was sensitive to RNase A that cleaves at pyrimidines (Blackburn and Moore 1982) and to ribonuclease T1, a guanylo-endoribonuclease, which cleaves at guanosine residues (Takahashi and Moore 1982).

There are precedents for this biological evidence for proteins covalently bound to nucleotide chain. Stable association between a protein and a nucleic acid by covalent linkage has previously been described and native covalent linkages have been identified between viral proteins and their corresponding DNA or RNA genomes. Examples of these are the bacteriophage phi X A-protein (Roth et al. 1984), the adenovirus terminal protein (Challberg et al. 1980), and the viral VPg protein to poliovirus RNA genome (Lee et al. 1977; Flanegan et al. 1977) and to plant potyvirus RNA genome (Murphy et al. 1991). Among cellular proteins, the tumour suppressor p53 protein (Samad et al. 1986) and the prothymosin μ (Vartapetian et al. 1988) have been found covalently linked to RNA.

Other processes of covalent attachment of RNA structures to protein side chains are nucleotidylylation and ADP-ribosylation.

Nucleotidylylation is a post-translational protein modification which reaction is catalysed by adenylyl and uridylyl transferases that yields ribonucleotidylyl monomer bound to protein substrates (Itzen et al. 2011). In contrast, the [^{32}P]phosphate label associated to the MA16 protein could be a short heterogenous ribonucleotide chain containing pyrimidine and guanosine residues.

On the other hand, it has been shown that the glycine-rich RNA-binding protein AtGRP7 from Arabidopsis, a homologue of the MA16 protein, is ADP-ribosylated upon bacterial infection by a mono-ADP-ribosyltransferase, the injected effector HopU1 protein (Fu et al. 2007). The authors suggest that the ADP-ribosylation quells host immunity, interfering with the ability of AtGRP7 to bind RNA. However, the [^{32}P]phosphate label associated to the MA16 protein seems to be an RNA structure distinct from post-translational ADP-ribosylation, since the [^{32}P]phosphate label was removed by RNase A, RNase T1, and micrococcal nuclease. This is not the case for ADP-ribosylation, which is resistant to these treatments (Nishizuka et al. 1967).

The first idea to be drawn from the depletion of the [^{32}P] MA16 phosphate label (Fig. 4) is that the [^{32}P] label contains ribonucleotide components, since it was removed by phosphorolysis. In contrast, protein phosphorylation is not sensitive to phosphorolysis**.** Secondly, these findings raise the possibility that the ribonucleotide(s) associated to the MA16 protein might be degraded by a phosphate-dependent ribonuclease.

Among the plethora of RNases, phosphorolytic activity is a distinguishable feature of at least two types of enzyme: the deadenylyl removase activity of the bifunctional enzyme glutamine synthetase adenylyl transferase (GS-AT) (Itzen et al. 2011) and the phosphate-dependent exoribonuclease PDX family (Zuo and Deutscher 2001).

Glutamine synthetase adenylyl transferase employs inorganic phosphate to phosphorolyse the AMP group from glutamine synthetase, while PDX phosphorylases require inorganic phosphate as the attacking group to degrade RNA from the 3′ end. PDX are also nucleotidylyl transferases capable of randomly non-template dependent 5′–3′ RNA polymerisation (Zuo and Deutscher 2001). However, it is unknown if PDX enzymes are also able to catalyse both nucleotidylylation and denucleotidylylation on protein substrates.

The phosphate-dependent exoribonuclease have been found in eukaryotes as part of the exosome complexes

involved in several pathways of RNA metabolism both in the cytoplasm and in the nucleus. The cytoplasmic complex participates in mRNA decay, mRNA turnover and mRNA surveillance (Houseley et al. 2006), while the nuclear complex also processes rRNA and a variety of small nuclear and nucleolar RNAs. It is well known that the nuclear exosome plays a primary role in the conversion of 7S pre-rRNA to 5.8S rRNA (Butler 2002; Mitchell and Tollervey 2000; Parker and Song 2004; Raijmakers et al. 2004).

Both exosome complexes contain enzymes that are similar to the bacterial polynucleotide phosphorylases (PNPases). These enzymes remain associated with RNA until complete degradation, probably assisted by accessory RNA-binding elements that allow positioning of the complex. In vitro, PNPases are inhibited by stem-loop structures. This could explain the conserved association of RNA helicases with phosphorolytic exoribonuclease complexes. It has been postulated that the RNA helicases might assist the nuclear exosome in rRNA maturation as well in the processing of snRNAs and snoRNAs, and that exosomes require additional factors that are not stably associated with the complex (Symmons et al. 2002).

Consistent with this model, the MA16 protein, not incorporated into a high molecular complex, may act as an additional factor or target of the nuclear exosome. The protein showed high affinity for rRNA (Freire and Pages 1995), and is located in the dense fibrillar component (Alba et al. 1994) within which pre-rRNA is processed to yield mature 18S, 28S and 5.8S RNAs. The MA16 protein has also been shown to interact with a nuclear/nucleolar DEAD box RNA-helicase, which in turn binds to fibrillarin (Gendra et al. 2004) that is an essential component in pre-rRNA processing and modification**.**

The protein may be involved in rRNA processing with the nuclear/nucleolar DEAD box RNA-helicase and a putative phosphate-dependent ribonuclease. According to the observed pattern of expression of the MA16 protein, the RNA processing activity would be developmentally regulated and involved in environmental stress responses.

Proteins with RNA chaperone activity have a high level of intrinsically disordered regions with a net charge and enriched in disorder-promoting amino acids such as glycine, arginine, glutamic and lysine. These proteins bind RNAs transiently, with weak affinity and low specificity, through electrostatic interactions (Rajkowitsch et al. 2007). The carboxy-terminal region of MA16 was predicted to be intrinsically disordered (Supplementary information) and with sequences similar to the RGG boxes and GAR domain of RNA chaperones hnRNP A1 (Munroe and Dong 1992) and nucleolin (Ghisolfi et al. 1992).

Experimentally, MA16 has been found to have a wide spectrum of RNA-binding activities for several RNAs due to nonspecific electrostatic interactions (Freire and Pages 1995). In addition, the MA16 protein has been shown to interact with an RNA helicase (Gendra et al. 2004) probably involved in RNA restructuring.

Essential functions in the recognition of specific sequences contained in particular RNA targets could be initiated through the ribonucleotide(s) linked to the MA16 protein. Many RNA regulatory mechanisms catalysed by ribonucleoprotein complexes might be the consequence of the activity of protein-ribonucleotidylyl covalent adducts where the ribonucleotide(s) bound to the protein(s) could specifically guide the process.

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