

RESEARCH PAPER

# A 125 kDa RNase E/G-like protein is present in plastids and is essential for chloroplast development and autotrophic growth in *Arabidopsis*\*

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Received 4 December 2007; Revised 28 March 2008; Accepted 31 March 2008

## Abstract

Endoribonuclease E (RNase E) is a regulator of global gene expression in *Escherichia coli* and is the best studied member of the RNase E/G ribonuclease family. Homologues are present in other bacteria but the roles of plant RNase E/G-like proteins are not known. *Arabidopsis thaliana* contains a single nuclear gene (At2g04270) encoding a product with the conserved catalytic domain of RNase E/G-like proteins. At2g04270 and the adjacent At2g04280 gene form converging transcription units with a ~40 base overlap at their 3' ends. Several translation products were predicted from the analyses of At2g04270 cDNAs. An antibody raised against a recombinant *A. thaliana* RNase E/G-like protein recognized a 125 kDa protein band in purified chloroplast preparations fractionated by SDS-PAGE. The 125 kDa RNase E/G-like protein was detected in cotyledons, rosette and cauline leaves. T-DNA insertions in exon 6 or intron 11 of At2g04270 result in loss of the 125 kDa band or truncation to a 110 kDa band. Loss of At2g04270 function resulted in the arrest of chloroplast development, loss of autotrophic growth, and reduced plastid ribosomal, *psbA* and *rbcL* RNA levels. Homozygous mutant plants were pale-green, contained smaller plastids with fewer thylakoids and shorter granal stacks than wild-type chloroplasts, and required sucrose at all growth stages following germination right

up to flowering and setting seeds. Recombinant *A. thaliana* RNase E/G-like proteins rescued an *E. coli* RNase E mutant and cleaved an *rbcL* RNA substrate. Expression of At2g04270 was highly correlated with genes encoding plastid polyribonucleotide phosphorylase, S1 RNA-binding, and CRS1/YhbY domain proteins.

Key words: *Arabidopsis*, photosynthesis, polynucleotide phosphorylase, RNase E, ribonuclease.

## Introduction

The conversion of chloroplasts from non-photosynthetic plastids is essential for autotrophic growth and plant development. Chloroplast development requires the co-ordinated expression of genes located in the nucleus and plastids (Lopez-Juez and Pyke, 2005) and is arrested by loss of plastid proteins encoded by the nuclear or plastid genomes. Albino plants arising from arrest of early chloroplast development can result from knockouts of expression-related plastid genes (Allison *et al.*, 1996) or loss of plastid ribosomes (Zubko and Day, 1998, 2002). Deletions and knockouts in photosynthesis-related genes can block chloroplast development at a later stage resulting in pale-green plants in some cases (Klaus *et al.*, 2003; Kode *et al.*, 2006). The vast majority of proteins present in plastids are encoded by the nucleus (Lopez-Juez

\* Sequences deposited in the EMBL database: AJ252122, AJ508604.

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Abbreviations: WT, wild type; aa, amino acids; RNase E, endoribonuclease E; PNPase, polynucleotide phosphorylase; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

and Pyke, 2005). Genetic analyses can be used to distinguish between genes with essential roles from those with minor or redundant functions in chloroplasts. Mutations that give rise to albino or pale green cells have identified nuclear genes with important roles in chloroplast metabolism (Xu *et al.*, 2002; Gutierrez-Nava *et al.*, 2004) and gene expression (Chatterjee *et al.*, 1996; Shirano *et al.*, 2000; Wang *et al.*, 2000; Bisanz *et al.*, 2003; Albrecht *et al.*, 2006; Motohashi *et al.*, 2007).

Advances in chloroplast engineering have provided a new impetus for identifying nuclear genes affecting plastid gene expression. Promoters and ribosome-binding sites are known to be major determinants of transgene expression in transformed plastids (Maliga, 2004). Changes in mRNA stability also appear to modulate RNA levels (Shiina *et al.*, 1998; Eibl *et al.*, 1999; Zou *et al.*, 2003) but the processes involved are poorly understood. Plastids are likely to be descendants of ancient cyanobacteria (Martin *et al.*, 2002). Comparative genome analyses combined with reverse genetics provides a powerful approach for identifying the roles of plant proteins related to well-characterized bacterial proteins. The ribonuclease E/G (RNase E/G) family of proteins play important roles in RNA metabolism in bacteria (Grunberg-Manago, 1999; Callaghan *et al.*, 2005a). Studies on RNase E, which was first identified as an enzyme required for ribosomal RNA processing (Apirion, 1978), underpinned rapid advances in our understanding of mRNA stability in bacteria. *Escherichia coli* RNase E makes the rate-limiting cleavage following 5' pyrophosphate removal that initiates the decay of a large number of mRNA species (Mudd *et al.*, 1990a, b; Babitzke and Kushner, 1991; Celesnik *et al.*, 2007; Deana *et al.*, 2008). RNase E is now known to be an important regulator of global gene expression in *E. coli* (Callaghan *et al.*, 2005a).

The role of RNase E/G-like proteins in eukaryotes is not known. Animals and fungi appear to lack genes encoding RNase E/G-like polypeptides. Genes for RNase E/G-like proteins are found in the nuclear genomes of flowering plants (Lin *et al.*, 1999) and the chloroplast genomes of algae (Reith and Munholland, 1995; Douglas and Penny, 1999; Turmel *et al.*, 1999). These observations are consistent with RNase E/G-like proteins playing a role in angiosperm chloroplasts. However, a number of proteomics studies have failed to detect RNase E/G-like proteins in angiosperm chloroplasts (Ferro *et al.*, 2003; Friso *et al.*, 2004; Kleffmann *et al.*, 2004; Peltier *et al.*, 2006; Pfalz *et al.*, 2006; Baginsky *et al.*, 2007). This work addresses whether RNase E/G-like proteins play a role in plastids using the model plant *Arabidopsis thaliana*. *A. thaliana* contains a single RNase E/G-like gene in its haploid genome (Lin *et al.*, 1999) and is particularly amenable to reverse genetic analysis (Alonso *et al.*, 2003; Rosso *et al.*, 2003). The results in this paper show that the plant RNase E/G-like gene encodes a product present in chloroplasts

that is essential for chloroplast development and autotrophic growth, displays ribonucleolytic activity, affects plastid RNA accumulation, and is co-expressed with nuclear genes involved in plastid RNA metabolism.

## Materials and methods

### Plant propagation and media

T-DNA mutants were generated in the context of the Salk (Alonso *et al.*, 2003) and GABI-KAT programs (Rosso *et al.*, 2003) and provided by the Nottingham Arabidopsis Stock Centre (UK), the Arabidopsis Biological Resource Centre (USA), and Bernd Weishaar (MPI for Plant Breeding Research, Cologne, Germany). *Arabidopsis thaliana* seeds were sterilized in 70% ethanol for 2 min followed by sodium hypochlorite (3% active chlorine) for 5 min, then rinsed with four washes of sterile water before placing on MS medium solidified with 0.7–0.8% agar or 0.25% Phytigel (Sigma-Aldrich, Poole, UK) without or with the following supplements: 2% sucrose; 2% sucrose and 25–50 mg l<sup>-1</sup> kanamycin (Melford, Ipswich, UK); 2% sucrose and 20 mg l<sup>-1</sup> sulphathiazole (Sigma-Aldrich, Poole, UK). Seedlings and plants were propagated on solidified MS medium with 2% sucrose.

### Analyses of T-DNA insertion lines

Supplementary Table 1 lists all primers and can be found at *JXB* online. WT and mutant alleles were identified using the following combination of PCR primers: 15 and gene-specific primers 16 and 17 for SALK\_093546; 15 and gene-specific primers 21 and 22 for SALK\_025999; 15 and gene-specific primers 23 and 24 for SALK\_070705; 20 and gene-specific primers 18 and 19 for GK187E08. The SALK\_093546 and GK187E08 mutations segregate as single recessive loci in progeny seedlings of heterozygous plants grown on media lacking sucrose. The SALK\_093546 *AtRne E/G* mutant was kanamycin sensitive, probably due to silencing of the *nrpII* gene used to select T-DNA insertions. Amongst 199 seedlings germinated on sucrose-free MS media from seeds obtained from selfed heterozygous SALK\_093546 parents, 153 were green and 46 were white. This fits a 3:1 segregation of a single recessive locus. Plating GK187E08 seeds from selfed heterozygote parents on: (i) MS media with 20 mg l<sup>-1</sup> sulphathiazole gave rise to 107 resistant and 35 sensitive seedlings consistent with a single dominant resistance locus; (ii) MS medium lacking sucrose gave rise to 63 green and 23 white seedlings consistent with a single recessive locus. White SALK\_093546 and GK187E08 seedlings were arrested in growth at the cotyledon stage while green seedlings formed true green leaves. White SALK\_093546 and GK187E08 seedlings transferred to MS medium with sucrose gave rise to plants with pale-green leaves on sucrose-medium. Seeds from heterozygous SALK\_093546 and GK187E08 parents germinated directly on MS medium with sucrose (2%) gave rise to approximately a quarter of pale-green plants, which were unable to grow on MS media lacking sucrose. However, the pale-green phenotype was more difficult to score for segregation purposes compared with the white phenotype on sucrose-free medium. All pale-green GK187E08 plants were sulphathiazole resistant, reflecting co-segregation of the mutant phenotype with the T-DNA insert containing *Sul1*. Seeds from a homozygous SALK\_093546 mutant pale-green plant grown *in vitro* only gave rise to pale-green plants on sucrose medium. PCR analyses showed that all pale-green SALK\_093546 and GK187E08 plants tested were homozygous mutants at the At2g04270 locus whereas dark-green plants were heterozygote or WT. DNA blot analysis of SALK\_093546 lines confirmed the homozygote status of pale-green knockout plants (see Supplementary Fig. 1 at *JXB*

online). Homozygous At2g04280-disrupted Salk\_025999 lines were similar in appearance to WT plants and grew in soil. Of 110 seeds from selfed homozygous SALK\_025999 plants germinated on MS medium lacking sucrose, all were green.

### Microscopy

Sections were prepared as described for transmission electron microscopy (Kode *et al.*, 2005) using a FEI Tecnai 12 Biotwin transmission electron microscope (FEI company, Eindhoven, The Netherlands).

### Cloning in *E. coli*

Plasmids were constructed and propagated in *E. coli* strains DH5 $\alpha$ , TOP10, or BL21(DE3)pLysS (Invitrogen, Paisley, UK). Strains with plasmids were propagated in Luria-Bertani (LB) medium containing ampicillin (50  $\mu\text{g ml}^{-1}$ ), kanamycin (40  $\mu\text{g ml}^{-1}$ ), or chloramphenicol (34  $\mu\text{g ml}^{-1}$ ). A cloned 1.35 kbp cDNA probe, amplified with primers 6 and 7 from cDNA prepared using PolyA RNA from *A. thaliana* WT seedlings and oligo dT, was used to isolate a 2.4 kbp cDNA (Fig. 2) from a Lambda Zap II library (Kieber *et al.*, 1993). A 1.7 kbp cDNA was amplified using outer primers 2 and 3, and inner primers 4 and 5 on oligo dT primed (primer 1) cDNA made using poly A-enriched leaf RNA. cDNAs were cloned into pGEM-T Easy (Promega, Southampton, UK). The 1.7 kbp cDNA sequence was cloned between the *NcoI* and *SallI* sites of the pTrc99a expression vector (Pharmacia Biotech) to express the 64 kDa recombinant protein (Fig. 2). Tests for rescue of an *rne* mutation were carried out in *E. coli* KW086 [(MC1061 *recA rne*<sup>ts</sup> *zce-726::Tn10*] (Wang and Cohen, 1994) grown at 43 °C. A 460 bp *NcoI*–*SpeI* fragment from the 1.7 kbp cDNA was joined to a 1621 bp *SpeI*–*NotI* fragment from the 2.4 kbp cDNA and cloned into pET30a (Novagen, Madison, USA). The resulting sequence contains 669 *Atrne* E/G codons and 45 upstream codons from pET30a and encodes a 79 kDa recombinant protein (Fig. 2). *AtRNase E/G* cDNAs isolated in this work have been deposited in the EBI database with accessions: AJ252122, AJ508604.

### Nucleic acid manipulations

Procedures for blot analyses, hybridization probe preparation, phosphorimager analyses of band intensities using Aida software and PCR analyses have been described (Zubko and Day, 2002; Kode *et al.*, 2005). The plastid rRNA probe (Zubko and Day, 2002) contains 1.5 kbp of 16S rRNA and 1.6 kbp of 23S rRNA sequences. Nested PCR primer sets 33 + 34 and 35 + 36 on purified *A. thaliana* chloroplast DNA, and 37 + 38 and 39 + 40 on total *A. thaliana* DNA were used for *psbA* and mt 18S RNA probes, respectively. An *rbcL* probe was PCR amplified using primer set 41 + 42 on cloned *Brassica napus* plastid DNA. RNA for RT-PCR experiments was treated with RNase-free DNase I (Roche, Lewes, UK). The 5' RACE system (Invitrogen, Paisley, UK) was used on polyA RNA from WT leaves using primer 8 for reverse transcription and following C-tailing PCR amplification with primers 9 and 11, followed by 10 and 12. The exon 4 splice variant was detected by RT-PCR with primers 13 and 14. Purified PCR products and plasmids were sequenced with appropriate primers by cycle sequencing using the Big Dye terminator sequencing ready reaction kit (Applied Biosystems, Warrington, UK) and running products on an Applied Biosystems 3100 capillary DNA sequencer. DNA sequences were analysed using the Vector NTI v 9 suite of DNA analysis programs (Invitrogen, Carlsbad, California, USA).

### Protein manipulations

The Vector NTI AlignX program was used to compare RNase E/G-like proteins in pairwise alignments. Protein blot analysis was as

described (Zubko and Day, 2002). Leaves were ground in liquid nitrogen and 300  $\mu\text{l}$  of 1 $\times$  sample buffer (62.5 mM TRIS-HCl, pH 6.8, 10% (v/v) glycerol, 3% (w/v) SDS, 5% (v/v)  $\beta$ -mercaptoethanol, 0.1% (w/v) bromophenol blue) was added per 100 mg of leaf powder. The homogenate was mixed thoroughly, placed in a boiling water bath for 5 min and centrifuged for 2 min (2000 rpm, Eppendorf 5415c microfuge) before loading on pre-cast 10% (w/v) polyacrylamide ready gels with 4% stacking gels (Bio-Rad Laboratories, Hemel Hempsted, UK) for SDS-PAGE in a Bio-Rad MiniProtein II system. The over-expressed 714 aa *AtRNase E/G*-like recombinant polypeptide was purified by fractionation on preparative SDS–polyacrylamide gels, electroeluted from Coomassie-stained gel slices, or eluted from sonicated potassium-chloride-stained gel slices by diffusion at 4 °C. After concentration using Microcon centrifuge filter devices (Millipore UK Ltd, Watford, UK), approximately 400  $\mu\text{g}$  of the purified polypeptide was used to make antibodies in sheep (Diagnostics Scotland, Edinburgh, UK). Prior to use against western blots, the antibodies were affinity-purified using overexpressed *AtRNase E/G*-like protein bound to nitrocellulose strips. The nitrocellulose strips were washed and affinity purified antibodies eluted as described (Mudd and Higgins, 1993). Proteins from SDS-PAGE gels were transferred to Hybond-ECL nitrocellulose (Amersham, Little Chalfont, UK) by electroblotting in western transfer buffer (25 mM TRIS, 192 mM glycine, 20% v/v methanol, pH 8.3) in a Bio-Rad mini transblot cell (50 min at 100 V). After staining with Ponceau red to ensure even transfer, blotted nitrocellulose sheets were incubated with primary antibodies against *AtRNase E/G*, LS RuBisCO or  $\alpha$ -tubulin (Zubko and Day, 2002). Binding of the primary antibody was detected using secondary antibodies linked to alkaline phosphatase (Sigma-Aldrich, Poole, UK) and staining with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) liquid substrate (Sigma-Aldrich, Poole, UK). Highly purified intact chloroplasts were prepared on Percoll (Amersham, Little Chalfont, UK) gradients (lower layer 85% Percoll mix; upper layer 40% Percoll mix) as described by Gruissem *et al.* (1986).

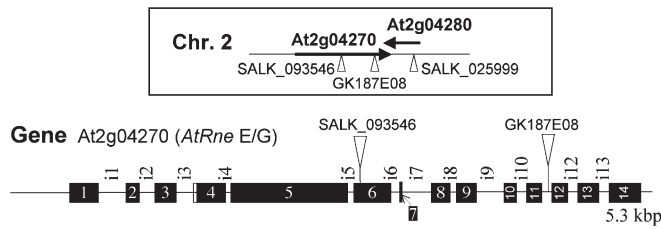
### In vitro cleavage assays

The template for RNA synthesis was made by PCR with oligonucleotides 31 and 32 on a cloned *Brassica napus* plastid DNA sequence (A Day and K van Zuilen, unpublished data) containing the *rbcL* gene. These primers would amplify the region between co-ordinates 54 778 and 56 778 in the *A. thaliana* plastid genome (Sato *et al.*, 1999). The inclusion of the T7 promoter region in primer 31 allows the purified PCR product to be used as a template to synthesize RNA using T7 RNA polymerase (Milligan and Uhlenbeck, 1989). Preparation of radiolabelled RNA with T7 RNA polymerase (Roche, Lewes, UK) and 400 Ci  $\text{mmol}^{-1}$  [ $\alpha$ -<sup>32</sup>P]-UTP (GE-Healthcare, Little Chalfont, UK) and conditions for induction of expression, preparation of cell extracts, *in vitro* assays, and fractionation of products on 6% urea-polyacrylamide gels have been described (Mudd and Higgins, 1993).

## Results

### Structure and transcription products of the *Atrne E/G* gene

An RNase E/G-like protein is encoded by gene At2g04270 on *A. thaliana* chromosome 2 (Lin *et al.*, 1999) adjacent to a gene (At2g04280) of unknown function (Fig. 1). Transcripts from both genes overlap by ~40 bases in their 3' untranslated regions, which might

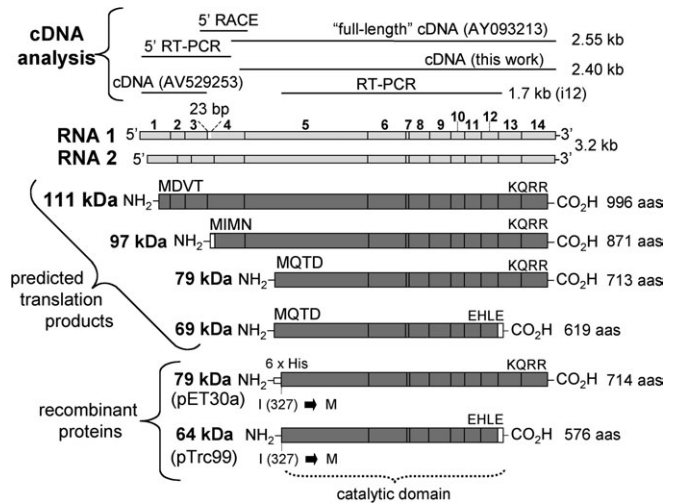


**Fig. 1.** Organization of the *AtRne* E/G-like (At2g04270) gene. Box shows adjacent At2g04270 and At2g04280 genes. Salk\_093546, Salk\_025999, and GK187E08 T-DNA insertion sites are shown.

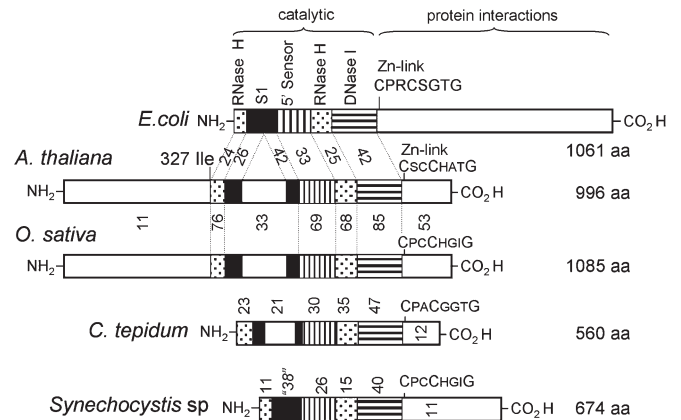
influence the polyadenylation site (Jen *et al.*, 2005; Wang *et al.*, 2005). The At2g04270 RNase E/G-like gene (*AtRNase* E/G-like) was poorly expressed, hindering the analysis of transcription products. Analyses of cDNAs predict four polypeptides of 111, 97, 79, and 69 kDa (Fig. 2). A 2.55 kbp cDNA (AY093213) from a full-length cDNA collection (Seki *et al.*, 2002) would encode a 79 kDa protein starting with the amino acids MQTD. Overlap of the 5' ends of RACE products with EST AV529253 gives a contig (RNA 2 in Fig. 2) encoding a 97 kDa protein with amino acids MIMN at the amino terminus. A splice variant extending exon 4 was detected by RT-PCR using a primer located at the start of Est AV529253. The frame shift resulting from the 23 base insertion predicts an alternative start codon located in exon 1 (RNA 1 in Fig. 2) which would give rise to a 111 kDa polypeptide starting with amino acids MDVT. This 111 kDa polypeptide was co-linear with sequence accession AF450479 (M Walter and J Kudla, unpublished results). RT-PCR using primers located in intron 12 and exon 5 amplified a 1.7 kb cDNA product. This truncates the C-terminus and would shorten each protein; the 79 kDa protein would be reduced to 69 kDa. The presence of intron 12 in a cDNA might reflect alternative splicing or amplification of a partially processed pre-mRNA species. Targeting programs predict a plastid location for the 111 kDa protein and a cytosolic location for the 79 kDa protein (Emanuelsson *et al.*, 2000; Small *et al.*, 2004; Hoglund *et al.*, 2006). The 97 kDa was predicted to be targeted to mitochondria (Emanuelsson *et al.*, 2000; Small *et al.*, 2004) or the nucleus (Hoglund *et al.*, 2006). Protein analyses (see below) are required to confirm the presence of one or more of these predicted translation products.

### Structure of the *AtRNase* E/G-like protein

The domains of *E. coli* RNase E have been located in the 2.9 Å crystal structure (Callaghan *et al.*, 2005a). Only the N-terminal catalytic domain of *E. coli* RNase E aligns with residues 327–839 of the 996 aa *AtRNase* E/G-like protein (Fig. 3). The highest identity (42%) was found in the DNase I-like domain. Large N-terminal extensions preceding the catalytic domain were found in the plant



**Fig. 2.** RNA and predicted protein products of the *AtRne* E/G-like gene. RNA 1 and 2 differ by alternative splicing of exon 4 and represent contigs of overlapping cDNAs, 5' RT-PCR, and 5' RACE products. Exons are located on RNA 1 and 2. Predicted polypeptides encoded by RNA 1, RNA 2 and the longest 'full-length' cDNA (Seki *et al.*, 2002) and recombinant proteins expressed in *E. coli* are shown. Terminal amino acids are shown.



**Fig. 3.** Conservation of the catalytic domain in the *AtRNase* E/G-like protein. Percentage identities of RNase H, S1, 5' sensor, and DNase I domains in pair-wise alignments with the *A. thaliana* protein are shown. Accession nos: P21513 (*E. coli*), BAD03665 (*Oryza sativa*), Q8KAA6 (*Chlorobium tepidum*), P72656 (*Synechocystis* sp. PCC 6803). The Zn-link motif co-ordinates a zinc ion involved in quaternary structure (Callaghan *et al.*, 2005a), S1 domains of *A. thaliana* and *Synechocystis* sp. were compared without the plant-specific insert shown in white.

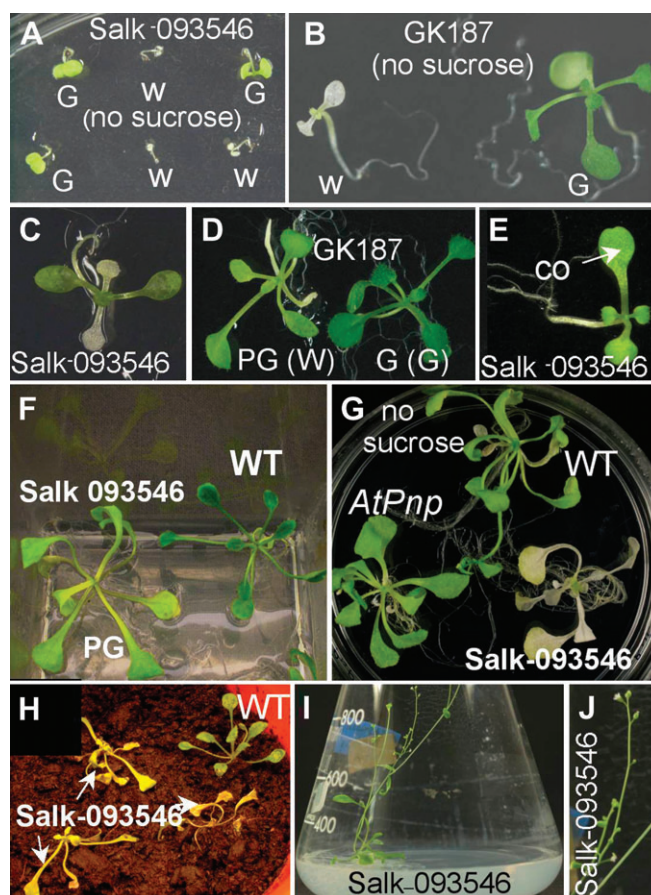
proteins. The C-terminal regions of the *A. thaliana* and rice proteins exhibit greater sequence identity (53%) than the N-terminal extensions (11%). Unlike the *E. coli* and *Synechocystis* proteins, the *Chlorobium tepidum* and plant RNase E/G-like proteins contain an insertion in the region corresponding to the S1 domain (Fig. 3), which might affect interactions with RNA substrates (Lee and Cohen, 2003). A variant of the Zn-link motif present in bacterial RNase E/G proteins (Callaghan *et al.*, 2005b) with consensus CXCCH(A/G) (I/T)G was present in plant

RNase E/G-like proteins (Fig. 3). The motif is required for co-ordinating a zinc ion in the quaternary structure of the catalytic domain (Callaghan *et al.*, 2005a).

*The AtRneE/G-like gene is required for chloroplast development and autotrophic growth*

Heterozygous plants from the Salk (Alonso *et al.*, 2003) and GABI-KAT (Rosso *et al.*, 2003) collections with T-DNA insertions in exon 6 (SALK\_093546) or intron 11 (GK187E08) (Fig. 1) were identified by PCR and DNA blot analysis. When seedlings from these heterozygous plants were grown on media lacking sucrose they segregated to give a 3:1 ratio of green to white seedlings. White SALK\_093546 and GK187E08 seedlings were arrested at the cotyledon stage (Fig. 4A, B, labelled W) and easily distinguished from green WT and heterozygous seedlings on sucrose-free medium (Fig. 4A, B, labelled G). The white phenotype could be rescued by transferring mutant seedlings to media containing sucrose whereupon new pale-green leaves emerged between the white cotyledons (Fig. 4C, D, left plant; F, left plant). All the resulting pale-green plants tested by PCR and DNA blot analyses were homozygote for the mutant At2g04270 allele whereas normal green plants were either homozygote or heterozygote for the WT At2g04270 allele. RT-PCR with primers located in exons 12 and 14 indicated *AtRne E/G* transcript levels downstream of the T-DNA insertions were reduced in SALK\_09346 and elevated in GK187E08 mutants (not shown). The 3:1 segregation of green to white seedlings on sucrose-free media for both At2g04270 disrupted lines (Salk\_O93546 and GK187E08) was consistent with this phenotype resulting from a mutation at a single locus. The observation that the white (on sucrose-free medium) and pale-green (on sucrose medium) phenotypes co-segregate with the disrupted At2g04270 alleles in homozygote mutants in two independent lines (Salk\_093546 and GK187E08) strongly suggests these phenotypes result from loss-of-function of the At2g04270 gene rather than a second T-DNA insertion in an unrelated gene. Homozygous plants with an insertion in the adjacent At2g04280 gene (SALK\_025999) grew on media lacking sucrose and were similar in appearance to WT plants (not shown).

Transfer of pale-green mutant Salk\_093546 or GK187E08 (not shown) plants to media lacking sucrose (Fig. 4G) or to soil (Fig. 4H) arrested growth and resulted in death whereas WT and a T-DNA insertion in an intron of gene At3g03710 (Salk\_070705) encoding a polynucleotide phosphorylase-like (AtPNP) protein were able to grow without sucrose (Fig. 4G). These results (Fig. 4A–H), based on two independent T-DNA insertions, suggest that homozygous At2g04270 mutant plants are unable to grow autotrophically and require sucrose at all stages of growth. Homozygous SALK\_093546 mutant plants flowered



**Fig. 4.** Phenotype of plants with T-DNA insertions in the *AtRne-E/G*-like gene. White (W) and green (G) seedlings from heterozygote (A) Salk\_093546, and (B) GK187E08 parents germinated on sucrose-free media. (C, D, F) White (W) seedlings from (A, B) when rescued on sucrose media produce plants with pale-green (PG) leaves. (E) Pale-green cotyledons (CO) of seedling from homozygote Salk\_093546 mutant parent germinated on sucrose media. Transfer of mature *in vitro* Salk\_093546 mutant plants to (G) sucrose-free media, or (H) soil for 10 d results in death. (I, J) Homozygote Salk\_093546 mutant plant flowering *in vitro*.

*in vitro* on sucrose medium allowing the collection of seeds (Fig. 4I, J). Seeds from self-fertilized SALK\_093546 homozygous mutant plants germinated on sucrose medium gave rise to seedlings with pale-green cotyledons (Fig. 4E, labelled CO) and pale-green leaves.

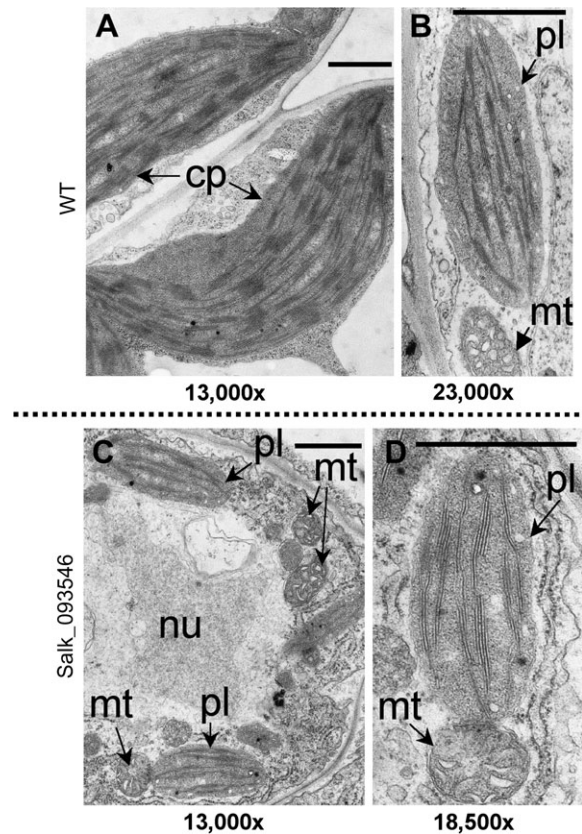
Whilst some pale-green mutants are viable in soil (Jarvis *et al.*, 1998) sucrose-dependent growth combined with pale green leaves is a phenotype associated with photosynthetic mutants unable to fix carbon dioxide (Kode *et al.*, 2006). To examine plastid ultrastructure in Salk\_093546 mutants, sections were prepared from cauline leaves of plants grown on sucrose medium for electron microscopy. Chloroplasts packed with thylakoids and large granal stacks were predominant in WT leaf cells (Fig. 5A, labelled cp). Small less-developed plastids were also found in WT leaves (Fig. 5B) but were less common. The most common plastids in mutant leaves were smaller

than WT chloroplasts with fewer thylakoids and short granal stacks (Fig. 5C, D) indicating an arrest in chloroplast development. Fully developed chloroplasts were not observed in mutant leaf sections. The morphology of mitochondria appeared similar in WT and mutant plants (Fig. 5B, C, D; labelled mt). These results suggest that the *AtRne* E/G-like gene is required for converting small non-photosynthetic plastids to chloroplasts allowing photosynthesis and sucrose-independent growth.

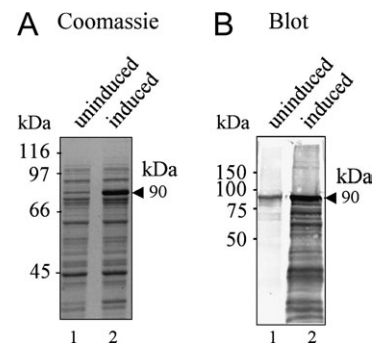
#### A 125 kDa *AtRNase* E/G-like protein is present in chloroplasts

To express the *A. thaliana* RNase E-like protein in *E. coli*, the N-terminus was removed and isoleucine at position 327 changed to the N-terminal methionine of the recombinant protein. The N-terminus of the truncated protein aligns with the start of *E. coli* RNase E (Fig. 2). The modified *AtRne* E/G-like cDNA cloned in pET30a encodes a 79 kDa protein containing the catalytic domain and C-terminus (Fig. 2). Induction of pET30a expression in *E. coli* BL21(DE3)pLysS cells gave rise to a 90 kDa band on SDS-PAGE gels (Fig. 6A, lane 2) that was not detected in extracts from uninduced cells (Fig. 6A, lane 1). This slower-than-expected electrophoretic mobility of the recombinant *AtRNase* E/G-like protein is also a property of *E. coli* RNase E (Mudd and Higgins, 1993). The specificity of a polyclonal antibody raised against the recombinant 79 kDa *AtRNase* E/G-like protein was tested by western blot analysis using uninduced and induced *E. coli* extracts. The antibody bound strongly to the induced 90 kDa protein and smaller polypeptides, which were likely to be degraded or prematurely terminated translation products of the *AtRne* E/G-like cDNA expressed in pET30a (Fig. 6B, lane 2). Leakiness of the pET30a vector gave rise to weak bands in the uninduced lane (Fig. 6B, lane 1).

The antibody raised against the recombinant *AtRNase* E/G-like protein recognized a 125 kDa band in total soluble protein from wild-type plants fractionated by SDS-PAGE (Fig. 7A, lane 7). If this 125 kDa protein is the product of the *AtRne* E/G gene it should be altered in T-DNA insertion mutants. Two homozygous mutant plants were used for the SALK\_093546 and GK187E08 T-DNA insertion lines (T-DNA insertion sites shown in Fig. 1). The 125 kDa band was not detected in SALK\_093546 lines (Fig. 7A, lanes 2, 3) and was replaced by a smaller band of 110 kDa in GK187E08 lines (Fig. 7A, lanes 4, 5). A green heterozygote plant with the WT and mutant GK187E08 alleles accumulated both 110 and 125 kDa proteins (Fig. 7A, lane 6). The 110 kDa band can be explained by termination of translation at a stop codon located at the start of intron 11, which would remove the C-terminal 155 aa of the *AtRNase* E/G-like protein in T-DNA insertion line GK187E08 and result in

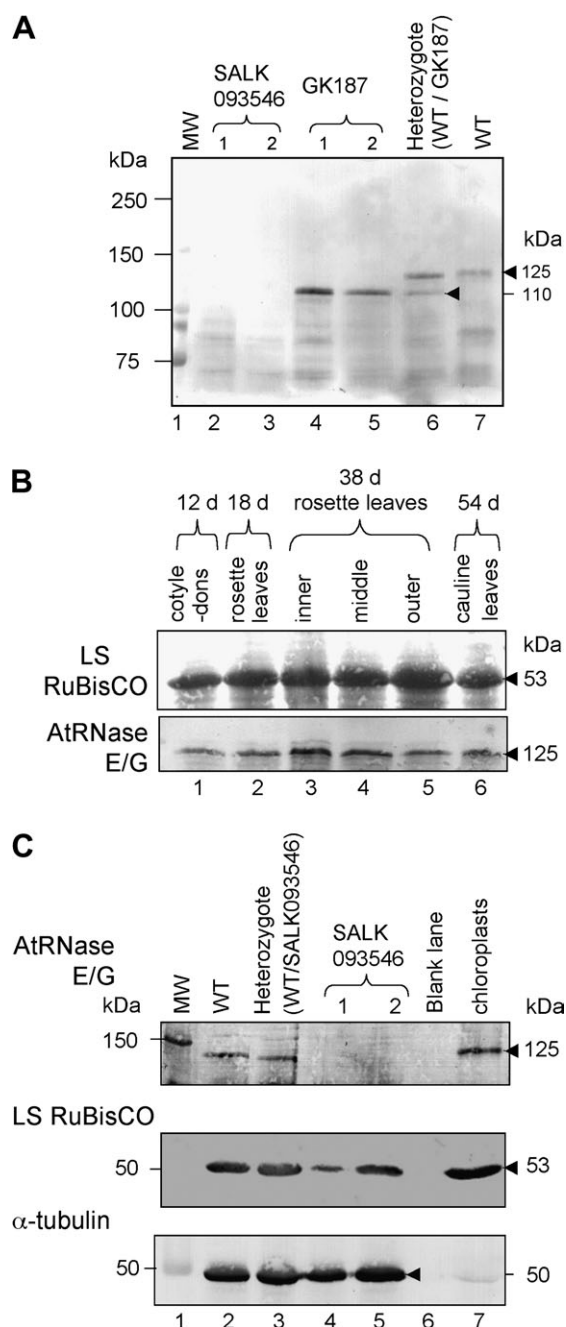


**Fig. 5.** Ultrastructure of WT and SALK\_093546 mutant cells. (A) WT chloroplasts (cp), (B) mitochondrion (mt) and immature plastid (pl) in WT leaves, (C) plastids, mitochondria, and nucleus (nu) in SALK\_093546 mutant, and (D) magnified SALK\_093546 mutant plastid. Scale bar 1  $\mu$ m.



**Fig. 6.** pET30a based overexpression of a 714 aa *AtRNase* E/G-like recombinant protein in *E. coli*. (A) SDS-PAGE fractionated total soluble proteins from uninduced and induced *E. coli* cells. The induced 90 kDa RNase E/G-like protein is arrowed. (B) Western blot analysis using antibodies raised against the purified recombinant 90 kDa RNase E/G-like protein.

a reduction of 18 kDa. In summary, the results indicate that the major protein product of the *AtRne* E/G-like gene migrated as a 125 kDa protein band on SDS/PAGE gels, and was not detectable or was altered in size in T-DNA insertion lines. Only one of the proteins predicted from the



**Fig. 7.** Protein blot analyses of the AtRNase E/G-like protein in plants. (A) AtRNase E/G-like polypeptides in WT, mutant, and heterozygote plants. (B) LSRuBisCo and AtRNase E/G-like polypeptide levels in WT leaves of different ages in days (d). (C) AtRNase E/G-like polypeptides in total protein from WT, heterozygote and mutant plants and purified WT chloroplasts. Control blots with LSRuBisCO and  $\alpha$ -tubulin-specific antibodies are shown. Size standards and estimated band sizes are indicated.

analysis of transcripts (Fig. 2) accumulates to detectable levels in WT plants. The larger than expected size of 125 kDa might reflect low mobility on SDS-PAGE gels. However, the possibility that the protein is larger than predicted from cDNA analyses cannot be ruled out. In

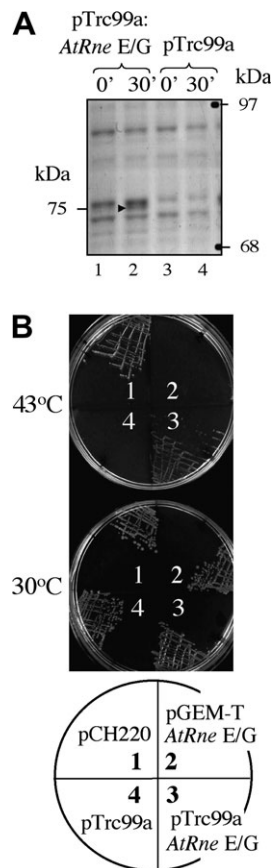
agreement with the findings that the *AtRne* E/G-like gene product was required for sucrose-independent growth right up to flowering and setting seeds, the 125 kDa RNase E/G-like protein was detected in cotyledons (Fig. 7B, lower panel, lane 1), rosette leaves of different ages (Fig. 7B, lanes 2–5), and cauline leaves (Fig. 7B, lane 6). The levels of the large subunit of ribulose biphosphate carboxylase/oxygenase (LS RuBisCO) are shown in Fig. 7B (upper panel) for comparison.

The AtRNase E/G-specific antibody detected a 125 kDa band in solubilized proteins from purified WT chloroplasts (Fig. 7C, top panel, lane 7). Importantly, the band co-migrates with the bands detected in total soluble protein samples from wild-type and heterozygous SALK\_093546 plants (Fig. 7C, top panel, lanes 2, 3). Moreover, no co-migrating band was present in total soluble protein from two SALK\_093546 mutant plants, which lack the AtRNase E/G-like protein (Fig. 7C, lanes 4, 5). These results strongly suggest that the 125 kDa RNase E/G band detected in total protein extracts was also present in purified chloroplasts. An antibody raised against the large subunit of ribulose biphosphate carboxylase/oxygenase (LS RuBisCO) showed this plastid-localized photosynthetic protein was present in WT, heterozygote and SALK\_093546 mutant plants (Fig. 7C, lanes 2–5) and purified chloroplasts (lane 7). The levels of LS RuBisCO appeared to be reduced in SALK\_093546 mutant plants. High levels of  $\alpha$ -tubulin were found in total soluble protein samples from WT, heterozygote, and SALK\_093546 mutant plants (Fig. 7C, lanes 2–5) but not in purified WT chloroplasts (Fig. 7C, lane 7) indicating successful purification of chloroplasts from cytosolic proteins.

#### Rescue of a temperature-sensitive RNase E mutation

A cDNA encoding a 576 aa recombinant protein (Fig. 2) containing the entire catalytic domain was cloned into the Trc99a expression vector. Thirty min of IPTG induction of pTrc99a-AtRne E/G gave rise to a polypeptide of 75 kDa (Fig. 8A, lane 2) that was not detected in extracts from uninduced cells (Fig. 8A, lane 1) or induced cells containing the pTrc99a vector without an insert (Fig. 8A, lane 4). The 75 kDa band exhibits a lower-than-expected mobility for this 64 kDa recombinant protein, which was consistent with the lower-than-expected electrophoretic mobilities of other AtRNase E/G-like polypeptides (see above).

Four plasmids were used in rescue experiments with the temperature-sensitive RNase E mutant strain *E. coli* KW086 (Wang and Cohen, 1994): (i) pCH220, (ii) pGEM-T-*AtRne* E/G, (iii) pTrc99a, (iv) pTrc99a-*AtRne* E/G. Plasmid pCH220 contains a truncated 830 aa long *E. coli* RNase E protein, which was known to rescue the *rne*<sup>ts</sup> mutation and provided a positive control (Mudd and



**Fig. 8.** Rescue of an *E. coli* *rne*<sup>ts</sup> mutant with the catalytic domain of the AtRNase E-like protein. (A) Cell lysates from *E. coli* strains containing plasmid Trc99a-*AtRne* E/G coding for a 576 aa polypeptide or Trc99a vector (control), at time 0 and 30 min after addition of 1 mM IPTG, were fractionated on a 7% (w/v) SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue. Molecular weight markers are indicated on the right. The induced 75 kDa RNase E/G-like polypeptide is arrowed. (B) Four *E. coli* KW086 strains containing the temperature-sensitive RNase E mutation and plasmids (1) pCH220 (positive control), (2) pGEM-T *AtRne* E/G (negative control), (3) pTrc99a *AtRne* E/G, (4) pTrc99a (negative control). Cells were streaked out on LB plates containing ampicillin and IPTG and incubated at 30 °C (permissive temperature) or 43 °C (non-permissive temperature).

Higgins, 1993). pGEM-T-*AtRne* E/G contains the cDNA encoding the 576 aa *A. thaliana* RNase E/G-like polypeptide but does not express the plant protein in *E. coli*. The cDNA was expressed when cloned into the pTrc99a vector (Fig. 8A, lane 2). The strains were grown on ampicillin plates containing IPTG to induce expression of the promoter in the pTrc99a expression vector. Plates were incubated at the permissive 30 °C or the non-permissive 43 °C temperature. All strains grew at 30 °C (Fig. 8B, lower plate). At the non-permissive temperature only the plasmids expressing *E. coli* RNase E (pCH220) and the plant RNase E-like protein (pTrc99a-*AtRne* E/G) rescued the mutation thereby allowing growth of the bacteria. The vector alone (Trc99a) or plasmid that does not express the plant protein (pGEM-T-*AtRne* E/G) do not

allow growth at 43 °C (Fig. 8B). Six randomly-picked transformants from two independent transformations of the KW086 strain with plasmid pTrc99a-*AtRne* E/G restored growth at the non-permissive temperature. Plasmid preparations from these transformants and restriction enzyme analysis of the plasmid DNA confirmed that they all contained the expected plasmid pTrc99a-*AtRne* E/G (data not shown). The specific ability of Trc99a-*AtRne* E/G but not pGEM-T-*AtRne* E/G or pTrc99a to rescue growth, rules out reconstitution of functional *E. coli* RNase E, by recombination or reversion of the *rne*<sup>ts</sup> mutation, in Trc99a-*AtRne* E/G containing KW086 strains. This rescue of the *rne*<sup>ts</sup> mutation by the recombinant plant AtRNase E/G-like protein supports conservation of function.

#### *Ribonuclease activity of a recombinant 79 kDa AtRNase-E/G protein*

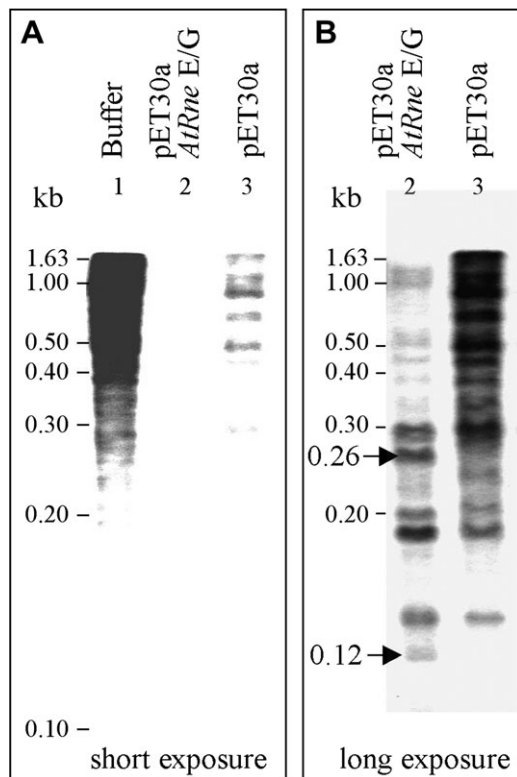
*In vitro* studies have shown bacterial RNase E/G homologues containing the conserved ribonucleolytic catalytic domain to be ribonucleases (Jager *et al.*, 2001; Lee and Cohen, 2003). Protein extracts were prepared from *E. coli* strains either containing (pET30a with *AtRne*-E/G coding sequence) or lacking (pET30a vector without insert) the overexpressed recombinant 79 kDa AtRNase E/G-like protein (Fig. 2). If the AtRNase E/G is a ribonuclease it should give rise to RNA cleavage products that are not detected in the pET30a vector-only *E. coli* extracts. Moreover, overexpression of a ribonuclease should result in more rapid degradation of the RNA substrate. This overexpression approach was used successfully to identify cleavage products of *E. coli* RNase E (Mudd and Higgins, 1993). The use of *E. coli* strains with and without recombinant plant enzymes overcomes the technical problems of excluding contaminating RNase activities present in purified plant proteins. Equal amounts of a radiolabelled RNA substrate transcribed from a 2 kbp plastid region (map coordinates 54 778–56 788) containing the *rbcL* gene (Sato *et al.*, 1999) were incubated with buffer alone, or protein preparations either containing or lacking the overexpressed recombinant AtRNase E/G-like protein. RNA samples incubated with buffer or cell extracts were fractionated by denaturing gel electrophoresis and visualized by autoradiography. The high radioactivity of the substrate RNA incubated in buffer alone resulted in saturation and blackening of the film by large >1.63 kb RNA molecules and smaller less abundant molecules; the latter probably representing incomplete transcription products (Fig. 9A, lane 1). Ribonuclease activity in the *E. coli* extracts degraded most of the substrate RNA (Fig. 9A, lanes 2, 3). At longer autoradiographic exposures, bands were visible in the RNA samples treated with *E. coli* extracts. A reduction in the sizes and intensities of the high MW RNA molecules at



the top of the gel suggest the extract containing the overexpressed AtRNase E/G-like protein (Fig. 9B, lane 2) degrades the RNA substrate more effectively than the extract lacking this overexpressed protein (Fig. 9B, lane 3). Unique RNA bands of 0.26 and 0.12 nucleotides accumulate in the lane corresponding to RNA substrate treated with the AtRNase E/G-like protein (Fig. 9, lane 1) that were not found in the pET30a vector only lane. The overexpressed AtRNase E/G-like protein was likely to be responsible for these ribonucleolytic cleavage events, which were not detected in the RNA substrate treated with *E. coli* extracts lacking the recombinant enzyme. The increased ribonucleolytic activity and unique cleavage events associated with the overexpressed AtRNase E/G-like protein suggest it is a ribonuclease.

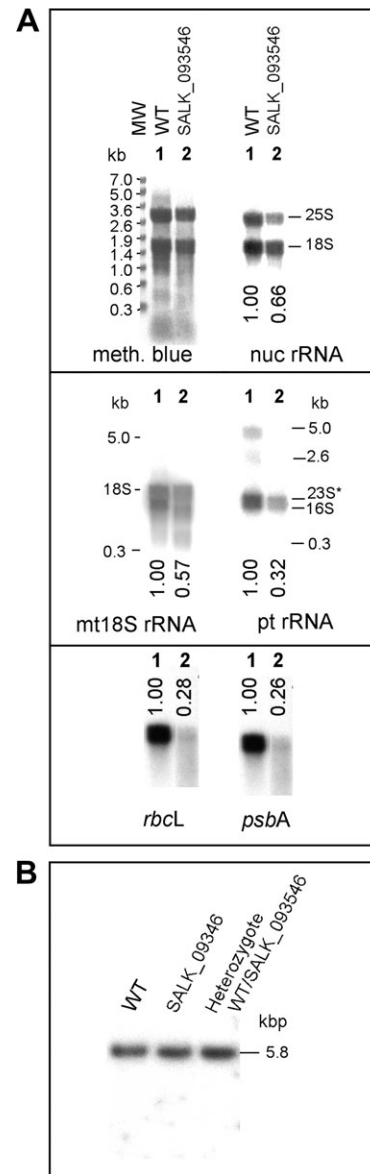
#### Loss-of-function of the AtRNase E/G-like protein influences plastid RNA levels

The SALK\_093546 mutant, which contains no detectable AtRNase E/G-like protein, was used to examine the effects of the *AtRne* E/G-like gene on plastid RNA



**Fig. 9.** Ribonuclease activity of the AtRNase E/G-like protein. (A) A control buffer only sample and extracts from *E. coli* cells overexpressing the 714 aa recombinant AtRNase E/G-like polypeptide (pET30a-*AtRne* E/G) or lacking the protein (pET30a) were incubated with a radioactive *rbcL* substrate and fractionated on a 7% urea/polyacrylamide gel. The image of the gel visualized by autoradiography is shown. (B) Longer exposure of lanes 2 and 3. Unique bands arising from the substrate treated with the AtRNase-E/G-like polypeptide are arrowed. Location of MW size standards are shown.

accumulation (Fig. 10A). Total RNA from above-ground parts of WT and mutant plants grown *in vitro* was used for blot analyses (Fig. 10A). Band intensities were standardized using hybridization signals of cytosolic 25S and 18S ribosomal RNAs. Quantitation of the 25S and 18S band intensities (Fig. 10A, nuc rRNA) show the SALK\_093546 lane was loaded with less RNA (0.66) than the WT lane (1.00). Limited variation of the 18S mitochondrial rRNA (mt18S rRNA) band intensities was



**Fig. 10.** RNA levels in WT and homozygous, pale green SALK\_093546 *AtRne* E/G mutant plants. (A) Blot analyses of total RNA fractionated on denaturing formaldehyde agarose gels. Methylene (meth.) blue-stained total RNA blot from WT (lane 1) and SALK\_093546 mutant (lane 2). Hybridization profiles: nucleus-encoded ribosomal RNA (nuc rRNA); mitochondrial 18S ribosomal RNA (mt 18S rRNA); plastid 23S and 16S ribosomal RNAs (pt rRNA); *rbcL* mRNA; *psbA* mRNA. Relative band intensities are indicated. (B) Southern blot using *rbcL* probe against *Hind*III digested total DNA.

found between WT and mutant plants. In contrast, taking into account the reduced 0.66× loadings of the SALK\_093546 lanes, the levels of plastid-encoded RNAs tested were reduced overall by greater than 2-fold in mutant relative to WT plants. These significantly reduced hybridization signals were observed for the plastid encoded ribosomal RNAs (pt rRNA), *rbcL* and *psbA* mRNAs (Fig. 10A). A prominent smear was visible below the *rbcL* and *psbA* main bands in mutant lanes indicating elevated levels of incomplete or degraded transcripts. Processing of an unstable bacteriophage T<sub>4</sub> gene 32 mRNA into a stable species requires *E. coli* ribonuclease E (Mudd *et al.*, 1988). Similarly, the reduction in levels of mature plastid RNA transcripts can be explained if the AtRNase E/G-like protein is required to process primary transcripts to stable forms. Reduced *rbcL* mRNA levels correlate with a reduction in LS RuBisCO protein levels (Fig. 7C). Differences in plastid RNA levels were not due to template abundance. DNA blot analyses with a plastid *rbcL* probe indicated similar levels of plastid DNA in WT and mutant plants (Fig. 10B). Lower plastid RNA levels correlate with the reduced plastid volume per cell (Fig. 5) resulting from the arrest of chloroplast development in the SALK\_093546 mutant.

#### Analysis of genes co-expressed with the AtRne E/G-like gene

Co-expression of genes provides a powerful tool to identify genes involved in common cell and developmental pathways (Brown *et al.*, 2005). The *Arabidopsis* co-expression tool allows the identification of genes with correlated expression patterns amongst a genome set of 22 000 genes (Jen *et al.*, 2006). Correlation coefficients for co-expression (*r*) values above 0.7 are of interest. Table 1 shows that three of the top four genes co-expressed with the AtRne E/G-like gene are plastid proteins and are likely to play a role in RNA metabolism. Genes co-expressed with the AtRNase E/G-like protein include a CRS1/YhbY domain protein implicated in group II intron splicing in plastids (Till *et al.*, 2001), an unknown protein with an S1 RNA binding domain, and polynucleotide phosphorylase. RNase E and polynucleotide phosphorylase are found in an RNA degrading multi-protein complex in *E. coli* and act in concert to degrade RNA (Py *et al.*, 1994; Grunberg-Manago, 1999). Co-

expression of the plant AtRne E/G-like and plant polynucleotide phosphorylase (Hayes *et al.*, 1996; Li *et al.*, 1998) genes provides a possible mechanism for coordinating the activities of their products in plastids.

## Discussion

The AtRne E/G gene (At2g04270) in *A. thaliana* encodes an RNase E/G-like protein present in chloroplasts that is required for plastid RNA accumulation, plastid development, and autotrophic growth. Recombinant plant RNase E/G-like proteins containing the evolutionary-conserved catalytic domain rescue an *E. coli* RNase E mutation and possess ribonucleolytic cleavage activity. Loss-of-function of the At2g04270 gene results in plants with pale-green cotyledons and pale-green true leaves, in the presence of sucrose, and white cotyledons and white true leaves in the absence of sucrose. The similar phenotypes of cotyledons and leaves in At2g04270 mutants contrasts with mutations in genes that have a more marked effect on the pigmentation of cotyledons relative to true leaves (Yamamoto *et al.*, 2000; Albrecht *et al.*, 2006). Pale-green leaves of mutant plants contained small plastids with a reduced number of thylakoids and short granal stacks compared to chloroplasts. These results support an important role for the AtRNase E/G-like protein in chloroplast function and development. The observation that loss-of-function of the AtRne E/G gene was not lethal in homozygous mutant plants provided they were grown on sucrose might indicate that absence of the AtRNase E/G-like protein has a limited impact on extra-plastidic processes.

Specific antibodies against a recombinant AtRNase E/G-like protein recognized a 125 kDa protein in total soluble protein from above-ground parts at all developmental stages tested from cotyledons to cauline leaves. The 125 kDa protein was not detected in soluble protein from SALK\_093546 mutants with a T-DNA insertion in exon 6. A truncated 110 kDa AtRNase E/G protein was found in GK187E08 mutants with a T-DNA insertion in intron 11. This suggests that the activities of the 125 kDa AtRNase E/G protein required for chloroplast development and autotrophic growth are not functional in the 110 kDa mutant protein. These results confirm the presence of only one AtRNase E/G-polypeptide in plants and its size (125 kDa) does not correspond to any of the four 111 kDa,

**Table 1.** Top four genes co-expressed with the AtRne-E/G like gene

Analysis using ACT (Jen *et al.*, 2006) on public microarray datasets.

	Gene	Function	Predicted location	<i>r</i>	References
1	At3g18390	CRS1/YhbY domain possible RNA binding	Plastid	0.89	Till <i>et al.</i> , 2001
2	At1g02150	Pentatricopeptide repeat protein	Mitochondrion	0.88	Cheuk <i>et al.</i> , unpublished data
3	At1g12800	S1 RNA-binding domain-containing protein	Plastid	0.87	Rhee <i>et al.</i> , 2003
4	At3g03710	Polyribonucleotide phosphorylase (PNPase)	Plastid	0.87	Hayes <i>et al.</i> , 1996

97 kDa, 79 kDa and 69 kDa polypeptides predicted from the analysis of *AtRne* E/G transcription products (Fig. 2). The larger than expected size of the 125 kDa RNase E/G protein might reflect aberrant electrophoretic mobility on SDS/PAGE gels. Recombinant 64 kDa and 79 kDa AtRNase E/G-like polypeptides migrated as larger-than-expected bands on SDS/PAGE gels and this lower-than-expected electrophoretic mobility might be a property of the protein. The 118 kDa *E. coli* RNase E protein migrates as a 180 kDa band on SDS/PAGE gels (Mudd and Higgins, 1993). Mapping the 5' end of the full-length message was made difficult by the relatively low abundance of *AtRne* E/G RNA products. Accumulation of unspliced pre-mRNA, differential splicing (Alexandrov *et al.*, 2006), non-AUG start codons (Hedtke *et al.*, 2002; Christensen *et al.*, 2005) or multiple AUG start sites (Chabregas *et al.*, 2003; Hanfrey *et al.*, 2005; Sunderland *et al.*, 2006) make accurate predictions of the N-termini of proteins from genomic and cDNA sequences more difficult and the possibility cannot be excluded that the N-terminus of the 125 kDa protein lies upstream of the predicted start site in exon 1.

The predicted 111 kDa protein contains a plastid targeting sequence which was predicted by the ChloroP program (Emanuelsson *et al.*, 2000) to be cleaved to a 104 kDa mature protein following import into plastids. Western blot analysis with an AtRNase E/G-specific antibody demonstrated the presence of the 125 kDa protein in purified chloroplasts. These results do not exclude the presence of *AtRne* E/G products at other locations within the cell. Low abundance and intraplastidic location might explain the failure of a number of proteomics studies to identify the 125 kDa RNase E/G-like protein in chloroplasts (Ferro *et al.*, 2003; Friso *et al.*, 2004; Kleffmann *et al.*, 2004; Peltier *et al.*, 2006; Baginsky *et al.*, 2007). Targeting studies involving fusions to GFP provide an alternative to immunolocalization to identify the subcellular location of the AtRNase E/G-like protein. However, this approach can give rise to contradictory results (Christensen *et al.*, 2005) and at present was not suitable for the AtRNase E/G-like protein. This was because of the discrepancy in sizes of the proteins predicted from cDNA sequences and the 125 kDa protein found in chloroplasts, which raises the possibility that the true N-terminus has not been identified. A plastid location for RNase E/G-like proteins was supported by partial sequencing of a 126 kDa protein present in a Triton-insoluble fraction from pea chloroplasts (Phinney and Thelen, 2005). The sequenced peptides from the 126 kDa pea protein share high identity with the *A. thaliana* RNase E/G-like protein (Phinney and Thelen, 2005) translated from deposited cDNA sequence AJ252122 (this work).

Loss-of-function of the AtRNase E/G-like protein was associated with reduced accumulation of plastid rRNA, *psbA* and *rbcL* mRNA. This could result from a direct

effect of the AtRNase E/G-like protein on plastid RNA metabolism, or indirectly, for example as a consequence of arrested plastid development. Reduced plastid RNA levels were also found in yellow *DAL* (Bisanz *et al.*, 2003) and pale-green, *pale cress* (Meurer *et al.*, 1998) mutants of *A. thaliana* in which chloroplast development was blocked. In pale-green *A. thaliana* *rnr1* mutants, *rbcL* and *psbA* levels accumulate to WT levels but ribosomal RNA processing was impaired (Kishine *et al.*, 2004). Support for a direct role of the AtRNase E/G-like in RNA metabolism is indicated by the presence of the conserved RNase E/G ribonucleolytic domain and the ribonucleolytic activity of the recombinant protein. Further work is required to determine whether the phenotypic consequences of loss-of-function of the AtRNase E/G-like protein results from a global effect on RNA metabolism (many targets) or lack of processing of a limited number of targets, which remain to be identified. In this regard, it is interesting to note that the lesion(s) underlying the non-viability of *E. coli* *rne* mutants remain(s) unclear (Takada *et al.*, 2005) despite 20 years of productive research on the role of *E. coli* RNase E in mRNA decay (Mudd *et al.*, 1988, 1990b; Callaghan *et al.*, 2005a). It remains possible that the essential nature of the *E. coli* RNase E and plant RNase E/G-like proteins might be due to functions (Taghbalout and Rothfield, 2007) unrelated to RNA metabolism. The finding that the *AtRne* E/G-like gene was co-expressed with genes encoding a plastid-targeted polynucleotide phosphorylase and plastid proteins with S1 RNA binding and CRS1/YhbY domains raises the possibility that these genes are involved in common pathways in plastids.

## Supplementary data

Supplementary data can be found at *JXB* online.

All oligonucleotides are listed in Supplementary Table 1. The figure shows the location of the primers on the At2g04270 sequence. DNA blot analyses of the Salk\_093546 mutant is shown in Supplementary Figure 1.

## Acknowledgements

We thank: the Salk Institute Genomic Analysis Laboratory and Bernd Weissbar (MPI for Plant Breeding Research, Cologne) for providing sequence-indexed *Arabidopsis* T-DNA insertion mutants; the ABRC (Columbus) and NASC (Nottingham) for distributing these materials; Professor SN Cohen (Stanford) for *E. coli* KW086; Professor CF Higgins (Durham) for supporting the project; the Institute for Genomic Research (USA) and Sol Genomics Network (Cornell) for sequence data; Roger Meadows for sectioning; The University of Manchester EM and DNA sequencing facilities, and the constructive criticism of two anonymous reviewers. Supported by the Biotechnology and Biological Sciences Research Council (UK) and Agricultural Plant Stress Research Center (R11-2001-092-04003-0) of KOSEF (South Korea).

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