

# mRNA biogenesis-related helicase eIF4AIII from *Arabidopsis thaliana* is an important factor for abiotic stress adaptation

Cecilia Pascuan<sup>1,2</sup> · Romina Frare<sup>1</sup> · Karina Alleva<sup>1</sup> · Nicolás Daniel Ayub<sup>1,2</sup> · Gabriela Soto<sup>1,2</sup> 

Received: 1 December 2015 / Accepted: 2 February 2016 / Published online: 16 February 2016  
© Springer-Verlag Berlin Heidelberg 2016

**Abstract** Similar to other plant species, *Arabidopsis* has a huge repertoire of predicted helicases, including the eIF4AIII factor, a putative component of the exon junction complex related to mRNA biogenesis. In this article, we integrated evolutionary and functional approaches to have a better understanding of eIF4AIII function in plants. Phylogenetic analysis showed that the mRNA biogenesis-related helicase eIF4AIII is the ortholog of the stress-related helicases PDH45 from *Pisum sativum* and MH1 from *Medicago sativa*, suggesting evolutionary and probably functional equivalences between mRNA biogenesis and stress-related plant helicases. Molecular and genetic analyses confirmed the relevance of eIF4AIII during abiotic stress adaptation in *Arabidopsis*. Therefore, in addition to its function in mRNA biogenesis, eIF4AIII can play a role in abiotic stress adaptation.

**Keywords** *Arabidopsis* · Helicases · mRNA biogenesis · Abiotic stress · Evolution

Plant genomes encode the largest eukaryotic repertoires of putative functional helicases. *Arabidopsis thaliana*, the

most important plant model species, contains 113 hypothetical genes exhibiting conserved helicase signature motifs (Umate et al. 2010). Despite the functional characterization of some helicases in different plant species, the gene redundancy has complicated the integration of experimental data for an in-depth understanding of their functions. In this context, studies based on the mRNA biogenesis-related helicase eIF4AIII from *Arabidopsis thaliana* (Koroleva et al. 2009) and the stress-related helicases PDH45 from *Pisum sativum* (Pham et al. 2000) and MH1 from *Medicago sativa* (Luo et al. 2009) have been regarded as two distant and unrelated groups. PDH45 contains ATP-dependent DNA and RNA helicase, DNA-dependent ATPase, and ATP-binding activities (Pham et al. 2000). PDH45 expression is induced by salinity, dehydration, wounding and low temperature, thus suggesting that PDH45 is a general factor involved in abiotic stress adaptation (Sanan-Mishra et al. 2005). Consistent with this, overexpression of PDH45 in tobacco and rice enhances salinity tolerance (Amin et al. 2011; Sanan-Mishra et al. 2005). MH1 has not been previously characterized in terms of its enzymatic activity but, similar to PDH45, MH1 overexpression increases tolerance to abiotic stress (Luo et al. 2009). Biochemical characterization of transgenic plants has also suggested that MH1 could enhance abiotic stress tolerance via the antioxidant response (Luo et al. 2009). Subcellular localization studies of eIF4AIII (AT3G19760) indicate that this specific *Arabidopsis* helicase is a component of the exon junction complex, which is involved in different aspects of mRNA biogenesis, including transcription, splicing and translation (Koroleva et al. 2009). While its function at subcellular level has been characterized, the relevance of eIF4AIII in major plant processes such as stress adaptation has not yet been explored. In this focus article, we integrated

---

Communicated by N. Stewart.

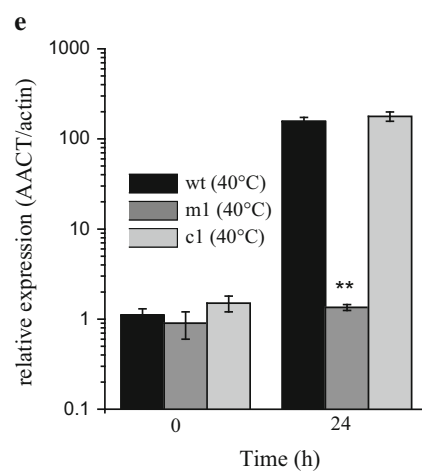
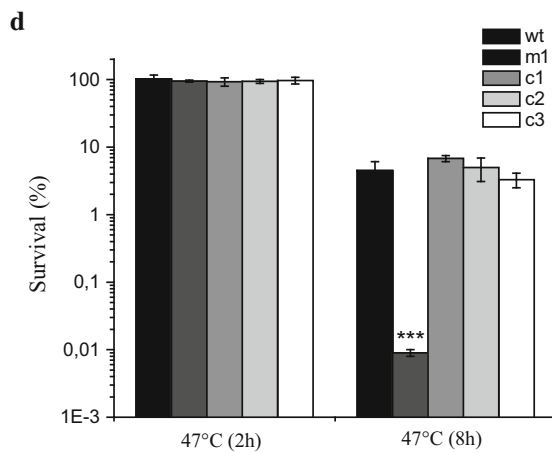
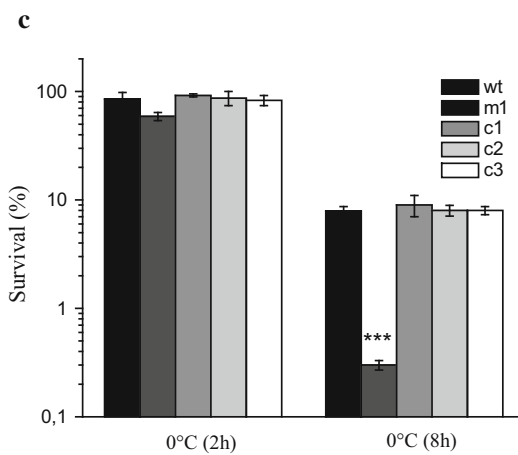
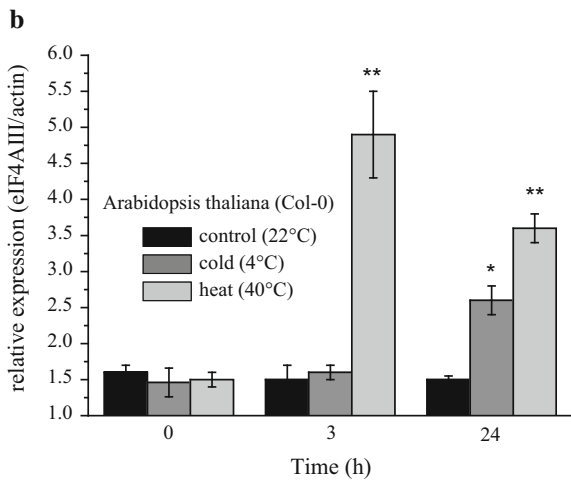
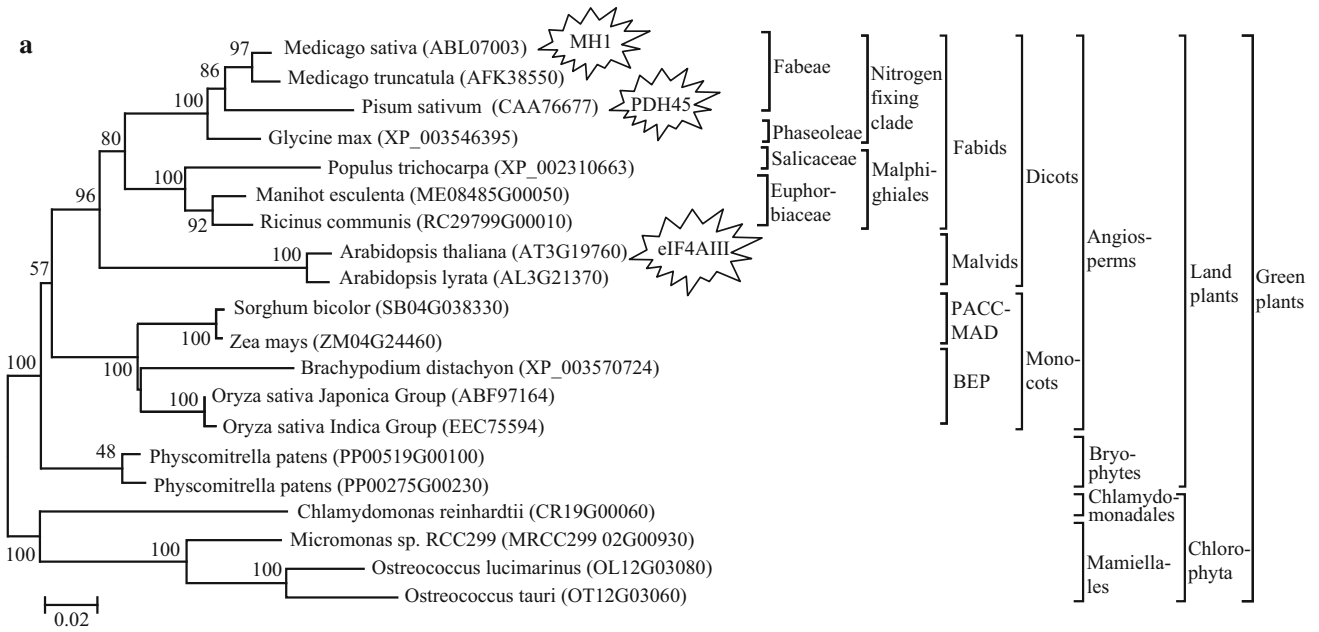
**Electronic supplementary material** The online version of this article (doi:10.1007/s00299-016-1947-5) contains supplementary material, which is available to authorized users.

---

✉ Gabriela Soto  
soto.gabrielacinthia@inta.gov.ar; gabysoto80@gmail.com

<sup>1</sup> CONICET, Avda. Rivadavia 1917, C1033AAJ CABA, Buenos Aires, Argentina

<sup>2</sup> IGEAF- INTA, De los reseros S/N, Castelar C25 (1712), Buenos Aires, Argentina



**Fig. 1 a** Phylogenetic analysis of Arabidopsis eIF4AIII using the NJ method. mRNA biogenesis (eIF4AIII) and stress-related (PDH45 and MH1) plant helicases described in previous studies are boxed. The current classification of green plants is found on the right. Genetic distances computed using Poisson correction model by using the following parameters: substitutions to include = all, gaps/missing data = pair-wise deletion, phylogeny test = bootstrap 500 replicates and root on midpoint according to Soto et al. (2012). Bootstrap percentages are indicated at the branch points. Only as an example of pre-selection of putative orthologous genes in each plant genome, we show preliminary phylogenetic analysis of Arabidopsis helicases where PDH45 seems to be the orthologous protein of eIF4AIII (AT3G19760) (Fig. S1). **b** Quantitative analysis of the expression of the eIF4AIII gene in wild-type Arabidopsis plants under cold and heat stress conditions. Expression profiles of eIF4AIII are relative to the actin gene (Soto et al. 2010). Control, 22 °C (black bar); cold, 4 °C (dark grey bar); heat, 40 °C (light grey bar). Values represent mean + SEM,  $n = 3$  independent experiments. Thermotolerance phenotype of eIF4AIII mutant showing **(c)** cold and **(d)** heat sensitivity as 7-day-old seedlings. Percentage of survival of plants was determined 7 days after abiotic stress. *Arabidopsis thaliana* (Col-0) plant material was used as wild-type (wt). Seeds of the eIF4AIII T-DNA insertion line GABI\_955D10 were supplied by the Max Planck Institute for Plant Breeding Research (<http://www.gabi-kat.de>). eIF4AIII (c1), MH1 (c2) and AACT (c3) were amplified by PCR and cloned in pBI121 (AF485783) to transform the mutant line eIF4AIII homozygous mutant (m1) by floral dipping as described by Zhang et al. (2006). Wild-type (black bar); m1 (dark grey bar); c1 (grey bar); c2 (light grey bar); c3 (white bar). Values represent mean + SEM,  $n = 10$  independent experiments. **e** Quantitative analysis of the expression of the AACT gene in wild-type and eIF4AIII mutant Arabidopsis plants under heat stress conditions. Expression profiles of AACT are relative to the actin gene (Soto et al. 2010). Wild-type (black bar); m1 (dark grey bar); c1 (light grey bar). Values represent mean + SEM,  $n = 3$  independent experiments. Asterisks indicate a statistically significant difference (Tukey: \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ )

evolutionary and functional approaches to elucidate possible functions of eIF4AIII in abiotic stress adaptation.

Orthologous genes in different species are evolutionarily closely related and are therefore expected to have similar biochemical and biological functions (Phillips 2006). The robust method to identify orthologs is derived from the analysis of phylogenetic trees. For orthologous assignment, the gene tree has to be congruent (i.e., the same topology) with the species tree (Perez Di Giorgio et al. 2014). These patterns of congruence are very hard to achieve when protein families contain tens or even hundreds of genes per genome. However, we have previously found that when the phylogenetic studies of thiolase and aquaporin superfamilies are restricted to proteins with high amino acid identity, there is a shift from incongruence to congruence (Fox et al. 2014; Perez Di Giorgio et al. 2014; Soto et al. 2010, 2011, 2012). Considering previous experiences, and given that helicases constitute a broadly diversified family of genes, the phylogenetic study of eIF4AIII was restricted to proteins with high amino acid identity (>70 %). Using this stringent criterion, we observed complete congruence between plant helicases and organismal trees (Fig. 1a).

Since a congruent pattern is only one topology, the probability that a congruent pattern occurs by chance is virtually null (Perez Di Giorgio et al. 2014). Thus, our evolutionary analysis constitutes strong evidence supporting the hypothesis that the stress-related helicases PDH45 from *Pisum sativum* (CAA76677) and MH1 from *Medicago sativa* (ABL07003) are the orthologs of the mRNA biogenesis-related helicase eIF4AIII from *Arabidopsis thaliana* (AT3G19760) (Fig. 1a). In contrast to paralogous genes, which normally exhibit divergent functions, orthologous genes in different organisms play similar functions (Perez Di Giorgio et al. 2014). Therefore, in addition to their evolutionary equivalence, we can speculate that PDH45, MH1 and eIF4AIII have similar roles related to both mRNA biogenesis and abiotic stress adaptation. In this context, we empirically explored the role of eIF4AIII in abiotic stress adaptation.

We studied the induction of eIF4AIII expression under abiotic stress and found that eIF4AIII transcript levels were significantly higher in both cold and heat environments than in control conditions (Fig. 1b). In addition, we observed that homozygous mutant plants carrying a T-DNA insertion mutation in eIF4AIII were more sensitive to both cold (Fig. 1c) and heat (Fig. 1d) stresses than wild-type plants, and that complementation of mutant plants with eIF4AIII restored abiotic stress resistance (Fig. 1c, d). Thus, these results showed that eIF4AIII is a stress-related plant helicase like its orthologous proteins PDH45 and MH1. Moreover, a strategy to investigate ortholog functionality is to perform a cross-species complementation analysis. Here, we showed that MH1 can complement the defective stress phenotype of Arabidopsis eIF4AIII mutant (Fig. 1c, d). This analysis provides evidence of conserved function between eIF4AIII and MH1 orthologs. Additionally, we showed that Arabidopsis eIF4AIII mutant had lower expression of acetoacetyl-CoA thiolase (AACT), also called thiolase II, than its parental wild-type line under heat stress conditions (Fig. 1e). AACT catalyzes the rate-limiting step in the mevalonate pathway during abiotic stress adaptation in plants (Soto et al. 2011), and its expression level is crucial to the production of reduced compounds involved in the mitigation of abiotic stress in eukaryotes and bacteria (Fox et al. 2014). Interestingly, we showed rescue of Arabidopsis eIF4AIII mutant sensitive phenotype by expressing AACT (Fig. 1c, d), suggesting that eIF4AIII can regulate abiotic stress resistance, at least partially, via the control of AACT expression.

The roles of helicases in essential functions and abiotic stress adaptation have been analyzed in several organisms, including plants, but the relationship between these types of helicases had not yet been analyzed in depth. Here, we presented evolutionary and functional evidences of equivalence between stress-related and mRNA biogenesis

helicases. Therefore, this focus article offers a starting point to integrate experimental data on plant helicases to a better understanding of their function in major plant processes.

**Author contribution statement** Conceived and designed the experiments: GS. Performed the experiments: CP RF KA. Analyzed the data: NDA CP GS. Wrote the paper: GS.

**Acknowledgments** This work was supported by Agencia (<http://www.agencia.mincyt.gob.ar>), grants PICT 2011–1325, PICT-2014-1397, PICT-2014-3659 and PICT-2014-3759.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Amin M, Elias SM, Hossain A, Ferdousi A, Rahman MS, Tuteja N, Seraj ZI (2011) Over-expression of a DEAD-box helicase, PDH45, confers both seedling and reproductive stage salinity tolerance to rice (*Oryza sativa* L.). *Mol Breed* 30:345–354
- Fox AR, Soto G, Mozzicafreddo M, Garcia AN, Cuccioloni M, Angeletti M, Salerno JC, Ayub ND (2014) Understanding the function of bacterial and eukaryotic thiolases II by integrating evolutionary and functional approaches. *Gene* 533:5–10
- Koroleva OA, Calder G, Pendle AF, Kim SH, Lewandowska D, Simpson CG, Jones IM, Brown JW, Shaw PJ (2009) Dynamic behavior of Arabidopsis eIF4A-III, putative core protein of exon junction complex: fast relocation to nucleolus and splicing speckles under hypoxia. *Plant Cell* 21:1592–1606
- Luo Y, Liu YB, Dong YX, Gao XQ, Zhang XS (2009) Expression of a putative alfalfa helicase increases tolerance to abiotic stress in Arabidopsis by enhancing the capacities for ROS scavenging and osmotic adjustment. *J Plant Physiol* 166:385–394
- Perez Di Giorgio J, Soto G, Alleva K, Jozefkowicz C, Amodeo G, Muschiatti JP, Ayub ND (2014) Prediction of aquaporin function by integrating evolutionary and functional analyses. *J Membr Biol* 247:107–125
- Pham XH, Reddy MK, Ehtesham NZ, Matta B, Tuteja N (2000) A DNA helicase from *Pisum sativum* is homologous to translation initiation factor and stimulates topoisomerase I activity. *Plant J* 24:219–229
- Phillips AJ (2006) Homology assessment and molecular sequence alignment. *J Biomed Inform* 39:18–33
- Sanan-Mishra N, Pham XH, Sopory SK, Tuteja N (2005) Pea DNA helicase 45 overexpression in tobacco confers high salinity tolerance without affecting yield. *Proc Natl Acad Sci USA* 102:509–514
- Soto G, Fox R, Ayub N, Alleva K, Guaimas F, Erijman EJ, Mazzella A, Amodeo G, Muschiatti J (2010) TIP5;1 is an aquaporin specifically targeted to pollen mitochondria and is probably involved in nitrogen remobilization in *Arabidopsis thaliana*. *Plant J* 64:1038–1047
- Soto G, Stritzler M, Lisi C, Alleva K, Pagano ME, Ardila F, Mozzicafreddo M, Cuccioloni M, Angeletti M, Ayub ND (2011) Acetoacetyl-CoA thiolase regulates the mevalonate pathway during abiotic stress adaptation. *J Exp Bot* 62:5699–5711
- Soto G, Alleva K, Amodeo G, Muschiatti J, Ayub ND (2012) New insight into the evolution of aquaporins from flowering plants and vertebrates: orthologous identification and functional transfer is possible. *Gene* 503:165–176
- Umate P, Tuteja R, Tuteja N (2010) Genome-wide analysis of helicase gene family from rice and Arabidopsis: a comparison with yeast and human. *Plant Mol Biol* 73:449–465
- Zhang X, Henriques R, Lin SS, Niu QW, Chua NH (2006) Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat Protoc* 1:641–646