

# DEAD-Box RNA Helicases are among the Constituents of the Tobacco Pollen mRNA Storing Bodies

Said Hafidh<sup>1</sup>, David Potěšil<sup>2,3</sup>, Zbyněk Zdráhal<sup>2,3</sup> and David Honys<sup>1\*</sup>

<sup>1</sup>Laboratory of Pollen Biology, Institute of Experimental Botany ASCR, Rozvojova 263, 165 02 Praha 6, Czech Republic

<sup>2</sup>CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic

<sup>3</sup>National centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

**Keywords:** Translation; mRNA storage; RNA helicase; mRNP; Ribonucleoprotein; Pollen

## Introduction

Male gametogenesis in flowering plants is attributed by substantial changes in cellular reorganization, cell fate specification and changes in cellular metabolism including RNA metabolism. Sequestration from immediate translation of protein coding mRNAs has emerged as one aspect of mRNA post transcriptional regulation that is widespread during gametogenesis. Transcripts repressed from translation are confiscated into mRNA storing bodies and function later during development and at early stages of embryogenesis. In Arabidopsis, 88% and 12% of the zygotic transcriptome at 2-4 cells stage of development now known to originate from maternal and paternal gametes respectively [1]. Moreover, an activator of YODA (YDA) mitogen-activated protein kinase pathway in the zygote, the interleukin-1 receptor-associated kinase (IRAK)/Pelle-like kinase gene Short Suspensor (SSP) is specifically expressed in the Arabidopsis male gametophyte but only translated upon gamete fusion to promote suspensor development [2]. Previously we have reported the proteome of EPP complexes [3], the tobacco pollen large mRNPs granules containing translationally sequestered mRNAs, non-coding smRNAs and a repertoire of associated protein complexes including factors for initiation of translation [4]. Here, we have analysed our previous and new EPP proteome data set obtained from Orbitrap LC-MS/MS as well as tobacco pollen Agilent microarray data [3-5] and highlighted that RNA helicases, predominantly DEAD-box domain containing helicases are among the constituents of the EPP RNA granules. Their potential function tied to local translation is explored. In addition, we discuss the recent report of RNA helicases localization within peroxisomes bodies and speculate their likely role as an additional site for mRNA control in the male gametophyte alongside EPP mRNA storing granules. Thus, mRNA homeostasis in the male gametophyte might be subjected to multiple controls at diverse but likely interconnected cytoplasmic foci.

EPP complexes were isolated as described previously [3]. New EPP proteome dataset was obtained by Orbitrap LC-MS/MS. Agilent microarray data were from Hafidh et al. [4,5]. Phylogenetic tree construction was performed with MUSCLE 3.7 and ClustalW 2.0.3 multiple protein alignment programs, Gblocks 0.91b for alignment curation, PhyML 3.0 aLRT with bootstrap for reconstructing phylogenetic tree and TreeDyn 198.3 for tree visualization. Final tree was manually curated in Inkscape software ver. 0.48. Protein domains and families were analysed according to SMART and pfam annotation. Arabidopsis pollen grains expressing pLAT52::CFP::PTS1 [6] in a qrt/qrt background were visualized with NIKON TE2000-E fluorescence microscope (Nikon, Japan) under Hoffman modulation contrast. Images were captured with ProgRes C5 camera (JENOPTIK Laser optic system, Germany) using NIS-Elements AR3.0 software (Nikon instruments, Melville, USA) and edited in Adobe Photoshop CS6.

## Results and Discussion

Of the complex EPP proteome, we have identified three isoforms of the DEAD-box RNA helicase family eukaryotic translation initiation factor 4A (eIF4A)-8,9,13 as designated components of the EPP RNA granules. eIF4A is required for binding of capped mRNA to the 40S ribosomal subunit via eIF3, whilst its ATP-dependent helicase activity functions in unwinding the secondary structure of the 5' UTR to facilitate ribosomal binding and translation. In synapses, eIF4A is a target of smRNA (BC1) that blocks the eIF4A helicase activity and impose subsequent widespread translation repression [7]. We have extended our analysis to tobacco Agilent microarray data [4,5] to search for other helicases that could potentially be associated with EPP mRNA storing granules. Additional 49 helicases (including all three isoforms of eIF4A identified by Orbitrap LC-MS/MS) were identified and were expressed as late as 24 h of pollen tube growth (Table 1 and Figure 1A). For majority, their transcripts were also detected in EPP RNA granules [5]. Analysis of their expression profiles show differential abundance but no apparent specificity in the male gametophyte (Figure 1A). Structural analysis classified majority as a DEAD-box motif helicases of SF2 super family with two RecA-like domains and conserved C-terminal domain (Figure 1B and 1C). These helicases could potentially function alongside EPP-bound eIF4A-8,9,13. Among them, EMB2733/ESP3, a homologue of yeast PRP2 and related to the DEAH RNA helicases, is essential for embryogenesis and is extensively phosphorylated [8]. The role of these helicases in promoting gametogenesis and embryogenesis development is worth an investigation.

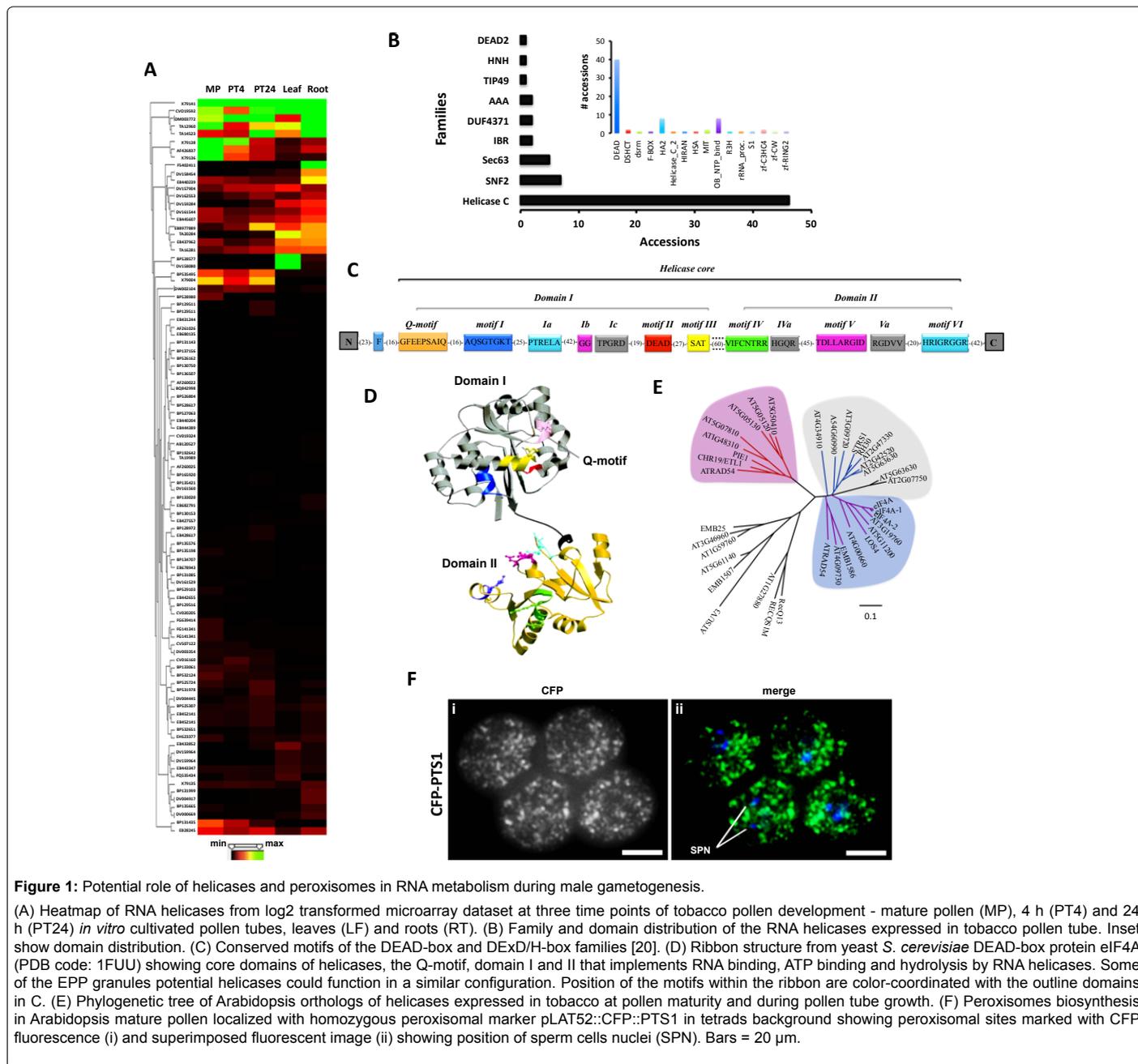
RNA helicases of the DEAD-box and the related DExD/H protein families are involved in almost all aspects of RNA metabolism and contain characteristic twelve conserved sequence motifs (Figure 1C). The identified tobacco eIF4A isoforms show 70% identity with *S. cerevisiae* eIF4A (data not shown). Solved structural features of *S. cerevisiae* eIF4A, domains of ATP binding, hydrolysis, RNA binding and their configurations, are proposed to convey conformational changes of bound transcripts as well as mRNP protein complexes including reshuffling of bound proteins to specify transcript fates (Figure 1C and 1D). These activities could be associated with mRNP localization once exported from the nucleus and most likely during EPP complex assembly. Within domain II, the three motifs, motif IV-

**\*Corresponding author:** David Honys, Laboratory of Pollen Biology, Institute of Experimental Botany ASCR, Rozvojova 263, 165 02 Praha 6, Czech Republic, Tel: +420-225-106-450; Fax: +420-225-106-456; E-mail: david@ueb.cas.cz

Received March 23, 2013; Accepted July 28, 2013; Published August 02, 2013

**Citation:** Hafidh S, Potěšil D, Zdráhal Z, Honys D (2013) DEAD-Box RNA Helicases are among the Constituents of the Tobacco Pollen mRNA Storing Bodies. J Plant Biochem Physiol 1: 114. doi:10.4172/2329-9029.1000114

**Copyright:** © 2013 Hafidh S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



VI, are known to facilitate cytoplasmic anchoring and contain putative phosphorylation sites that are likely to modulate helicase activities. In fact, phosphorylation of eIF4A-8 [9] and possibly eIF4A-13 [10] has been reported in tobacco pollen tubes. Interestingly the association of RNA helicases with repressed mRNAs during gametogenesis has been reported in a wide spectrum of organisms. Polar granules of *Drosophila* oocytes together with P-granules of *Caenorhabditis* germ cells, all contain diverse species of mRNAs that specify germ cell fates and are associated with Xp54, Me31B and CGH-1 RNA helicases respectively. In Arabidopsis, SLOWWALKER3, a DEAD-box RNA helicase plays a key role during female gametogenesis [8]. Phylogenetic analysis grouped all Arabidopsis pollen tube expressed helicases into four main branches, potentially implying their specified roles (Figure 1E).

To extend the link of RNA helicases likely role in gametogenesis

and embryogenesis, a recent report have localized two DEAD-boxes RNA helicases, RH11 and RH37, and four other RNA-binding proteins within leaf peroxisomes [11]. Both proteins are highly expressed and accumulated during pollen maturation. Moreover, at least two additional unknown DEAD/H helicases, AT2G42520 and AT3G58510, are also predicted to localize in peroxisome and show cumulative mRNA profile during Arabidopsis pollen maturation [6,12]. Peroxisomes are actively synthesised in the male gametophyte (Figure 1F) and recent studies have pointed at the unexpected role of peroxisomes in male and female gametophytes cross-talk during fertilization [13,14]. Plant peroxisomes are required to maintain cellular redox balance as well as production of signalling molecules such as Indole Acetic Acid (IAA), Reactive Oxygen Species (ROS) and Nitric Oxide (NO) [15]. In animals, ROS and NO are key signalling molecules for fertilization related processes

Tobacco accession	Arabidopsis best hit (<math>e^{-10}</math>)	Sequence description	Domain	Family	Tobacco accession	Arabidopsis best hit (<math>e^{-10}</math>)	Sequence description	Domain	Family
DW003772	AT3G19760	eukaryotic translation initiation factor 4A, putative/eIF-4A, putative/DEAD box RNA helicase, putative	DEAD	Helicase_C	DW003772	AT3G19760	Eukaryotic translation initiation factor 4A (eIF4A), putative DEAD box RNA helicase	DEAD	Helicase_C
EB428246	AT1G32490	EMB2733/ESP3 (EMBRYO DEFECTIVE 2733); ATP-dependent RNA helicase	DEAD/HA2	Helicase_C/OB_NTP_bind	EB428246	AT1G32490	EMB2733/ESP3 (EMBRYO DEFECTIVE 2733); ATP-dependent RNA helicase	DEAD/HA2	Helicase_C/OB_NTP_bind
DV161544	AT5G60990	DEAD/DEAH box helicase, putative (RH10)	DEAD	Helicase_C	EB440239	AT1G31970	STRS1 (STRESS RESPONSE SUPPRESSOR 1); ATP-dependent helicase	DEAD	Helicase_C
EB440239	AT1G31970	STRS1 (STRESS RESPONSE SUPPRESSOR 1); ATP-dependent helicase	DEAD	Helicase_C	BP532124	AT4G35740	RecQI3 (Recq-like 3); ATP binding / ATP-dependent helicase	DEAD	Helicase_C
BP532124	AT4G35740	RecQI3 (Recq-like 3); ATP binding / ATP-dependent helicase	DEAD	Helicase_C	BP130750	AT3G27730	RCK (ROCK-N-ROLLERS); ATP binding / ATP-dependent helicase	DEAD	Helicase_C
EB677889	AT3G58570	DEAD box RNA helicase, putative	DEAD	Helicase_C	EB444389	AT4G14790	ATSUV3 (embryo sac development arrest 15)	-	Helicase_C/SUV3
BP130750	AT3G27730	RCK (ROCK-N-ROLLERS); ATP binding / ATP-dependent helicase	DEAD	Helicase_C	BP532651	AT1G50410	SNF2 domain-containing protein / helicase domain-containing protein / RING finger domain-containing protein	zf-C3HC4_2	Helicase_C/SNF2_N
EB444389	AT4G14790	ATSUV3 (embryo sac development arrest 15)	-	Helicase_C/SUV3	DV159284	AT5G63120	ethylene-responsive DEAD box RNA helicase, putative (RH30)	DEAD	Helicase_C
BP532651	AT1G50410	SNF2 domain-containing protein / helicase domain-containing protein / RING finger domain-containing protein	zf-C3HC4_2	Helicase_C/SNF2_N	BP135576	AT3G12810	PIE1(PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1); ATP binding / DNA binding / helicase	HSA	Helicase_C/SNF2_N
DV159284	AT5G63120	ethylene-responsive DEAD box RNA helicase, putative (RH30)	DEAD	Helicase_C	BP529103	AT2G30800	HVT1 (HELICASE IN VASCULAR TISSUE AND TAPETUM); ATP binding / helicase/ nucleic acid binding	R3H/DEAD/HA2	Helicase_C
BP135576	AT3G12810	PIE1(PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1); ATP binding / DNA binding / helicase	HSA	Helicase_C/SNF2_N					
BP529103	AT2G30800	HVT1 (HELICASE IN VASCULAR TISSUE AND TAPETUM); ATP binding / helicase/ nucleic acid binding	R3H/DEAD/HA2	Helicase_C					
EB452141	AT3G19210	ATRAD54/CHR25 (ARABIDOPSIS HOMOLOG OF RAD54); ATP binding / DNA binding / helicase	-	SNF2_N/Helicase_c					
DW002104	AT4G00660	DEAD/DEAH box helicase, putative	DEAD	Helicase_C					

**Table 1:** Selected list of helicases identified in Agilent 44k Tobacco Array as expressed during tobacco male gametophyte development.

such as acrosome reaction, ovulation and fertilization [16-18]. Whereas in plants, the peroxisome-derived NO was shown to be essential for pollen tube reorientation [14] and to promote sperm cell discharge for gamete fusion [13]. Only recently, ROS was shown to play a crucial role during female gametogenesis and fertilization in plants [19]. Thus, it remains speculative whether localization of RNA helicases within peroxisomes might imply new role of peroxisomes in mRNA regulation during gametogenesis alongside EPP, P-bodies and stress granules and if the activities of these cytoplasmic foci's are linked.

The above-mentioned candidate helicases are likely to be integral for EPP mRNA granules assembly and precise development of both gametophytes as well events post-fertilization. Their ATP-mediated helicase activities and RNA binding ability are key elements known to regulate mRNA fates. The distribution of helicases within EPP complexes, actively translating polyribosomes and mRNPs is currently under investigation. A likely association of some of the helicases and other RNA-binding proteins to peroxisomes opens new avenues through which dynamic relationship between cellular compartments with respect to RNA fates can be addressed. Precise purification procedures combining biochemical and fluorescent assisted protein complex isolation techniques will be fundamental towards better understanding of the close interplay between subcellular compartments and the influence on developmental processes [20].

#### Acknowledgement

This work was supported by Czech Science Foundation grants P501/11/P321 and P501/11/1462, Ministry of Education, Youth and Sport CR project LD13049 and by the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

#### References

1. Autran D, Baroux C, Raissig MT, Lenormand T, Wittig M, et al. (2011) Maternal epigenetic pathways control parental contributions to Arabidopsis early embryogenesis. *Cell* 145: 707-719.
2. Bayer M, Nawy T, Giglione C, Galli M, Meinzel T, et al. (2009) Paternal control of embryonic patterning in Arabidopsis thaliana. *Science* 323: 1485-1488.
3. Honys D, Renak D, Fecikova J, Jedelsky PL, Nebesarova J, et al. (2009) Cytoskeleton-associated large RNP complexes in tobacco male gametophyte (EPPs) are associated with ribosomes and are involved in protein synthesis, processing, and localization. *J Proteome Res* 8: 2015-2031.
4. Hafidh S, Breznenova K, Ruzicka P, Fecikova J, Capkova V, et al. (2012) Comprehensive analysis of tobacco pollen transcriptome unveils common pathways in polar cell expansion and underlying heterochronic shift during spermatogenesis. *BMC Plant Biol* 12: 24.
5. Hafidh S, Breznenova K, Honys D (2012) De novo post-pollen mitosis II tobacco pollen tube transcriptome. *Plant Signal Behav* 7: 918-921.
6. Honys D, Twell D (2004) Transcriptome analysis of haploid male gametophyte development in Arabidopsis. *Genome Biol* 5: R85.
7. Lin D, Pestova TV, Hellen CU, Tiedge H (2008) Translational control by a small RNA: dendritic BC1 RNA targets the eukaryotic initiation factor 4A helicase mechanism. *Mol Cell Biol* 28: 3008-3019.
8. Liu M, Shi DQ, Yuan L, Liu J, Yang WC (2010) SLOW WALKER3, encoding a putative DEAD-box RNA helicase, is essential for female gametogenesis in Arabidopsis. *J Integr Plant Biol* 52: 817-828.
9. op den Camp RG, Kuhlemeier C (1998) Phosphorylation of tobacco eukaryotic translation initiation factor 4A upon pollen tube germination. *Nucleic Acids Res* 26: 2058-2062.
10. Fila J, Matros A, Radau S, Zahedi RP, Capkova V, et al. (2012) Revealing phosphoproteins playing role in tobacco pollen activated in vitro. *Proteomics* 12: 3229-3250.
11. Reumann S, Babujee L, Ma C, Wienkoop S, Siemsen T, et al. (2007) Proteome analysis of Arabidopsis leaf peroxisomes reveals novel targeting peptides, metabolic pathways, and defense mechanisms. *Plant Cell* 19: 3170-3193.
12. Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, et al. (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS One* 2: e718.
13. Boisson-Dernier A, Frietsch S, Kim TH, Dizon MB, et al. (2008) The peroxin loss-of-function mutation abstinence by mutual consent disrupts male-female gametophyte recognition. *Curr Biol* 18: 63-68.
14. Prado AM, Porterfield DM, Feijo JA (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. *Development* 131: 2707-2714.
15. Nyathi Y, Baker A (2006) Plant peroxisomes as a source of signalling molecules. *Biochim Biophys Acta* 1763: 1478-1495.
16. Herrero MB, de Lamirande E, Gagnon C (2003) Nitric oxide is a signaling molecule in spermatozoa. *Curr Pharm Des* 9: 419-425.
17. Kuo RC, Baxter GT, Thompson SH, Stricker SA, Patton C, et al. (2000) NO is necessary and sufficient for egg activation at fertilization. *Nature* 406: 633-636.
18. Thaler CD, Epel D (2003) Nitric oxide in oocyte maturation, ovulation, fertilization, cleavage and implantation: a little dab'll do ya. *Curr Pharm Des* 9: 399-409.
19. Martin MV, Fiol DF, Sundaresan V, Zabaleta EJ, Pagnussat GC (2013) oiwa, a Female Gametophytic Mutant Impaired in a Mitochondrial Manganese-Superoxide Dismutase, Reveals Crucial Roles for Reactive Oxygen Species during Embryo Sac Development and Fertilization in Arabidopsis. *Plant Cell* 25: 1573-1591.
20. Linder P, Jankowsky E (2011) From unwinding to clamping - the DEAD box RNA helicase family. *Nat Rev Mol Cell Biol* 12: 505-516.