PLANT signaling & behavior

Plant Signaling & Behavior

ISSN: (Print) 1559-2324 (Online) Journal homepage: http://www.tandfonline.com/loi/kpsb20

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To cite this article: R. Glen Uhrig & Greg Moorhead (2017) AtSLP2 is an intronless protein phosphatase that co-expresses with intronless mitochondrial pentatricopeptide repeat (PPR) and tetratricopeptide (TPR) protein encoding genes, Plant Signaling & Behavior, 12:4, e1307493, DOI: 10.1080/15592324.2017.1307493

To link to this article: https://doi.org/10.1080/15592324.2017.1307493

PLANT SIGNALING & BEHAVIOR
2017, VOL. 12, NO. 4, e1307493 (4 pages)
http://dx.doi.org/10.1080/15592324.2017.1307493

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SHORT COMMUNICATION

AtSLP2 is an intronless protein phosphatase that co-expresses with intronless mitochondrial pentatricopeptide repeat (PPR) and tetratricopeptide (TPR) protein encoding genes

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ABSTRACT
Shewanella-like PPP family phosphatases (SLPs) are a unique lineage of eukaryote PPP-family phosphatases of bacterial origin which are not found in metazoans.[1,2] Their absence in metazoans is marked by their ancient bacterial origins and presence in plants.[1] Recently, we found that the SLP2 phosphatase ortholog of Arabidopsis thaliana localized to the mitochondrial intermembrane space (IMS) where it was determined to be activated by mitochondrial intermembrane space protein 40 (MIA40) to regulate seed germination.[3] Through examination of *atslp2* knockout (accelerated germination) and 35S::AtSLP2 over-expressing (delayed germination) plants it was found that AtSLP2 influences *Arabidopsis thaliana* germination rates via gibberellic acid (GA) biosynthesis.[3] However, the exact mechanism by which this occurs remains unresolved. To identify potential partners of AtSLP2 in regulating germination through GA, we undertook a gene co-expression network analysis using RNA-sequencing data available through Genevestigator (https://genevestigator.com/gv/).

ARTICLE HISTORY
Received 24 February 2017
Revised 11 March 2017
Accepted 13 March 2017

KEYWORDS
Arabidopsis thaliana; co-expression network; intronless genes; mitochondria; protein phosphatase

Gene Co-expression analysis reveals potential connections between the AtSLP2-AtMIA40 protein complex and accelerated germination

Using the normalized RNA-sequencing data available through Genevestigator, we compiled a list of co-expressed genes common between *AtSLP2* and *AtMIA40* which exhibit a positive correlation (Fig. 1). Through this we identified 96 co-expressed genes common between *AtSLP2* and *AtMIA40* that exhibited a positive correlation (Pearson Correlation Coefficient (PCC) > 0.95; Table S1). Gene Ontology analysis of co-expressed genes common between *AtSLP2* and *AtMIA40* performed using the cytoscape plugin BinGo[4]; http://apps.cytoscape.org/apps/bingo) found that these 96 genes are involved in cellular metabolism, translation, gene expression and RNA processing (Fig. 2A). Further SUBA3 ([5]; http://suba3.plantenergy.uwa.edu.au/) *in silico* subcellular localization analysis found that these 96 gene products are primarily targeted to the nucleus, cytosol and mitochondria (Fig. 2B).

With a total of 35.4% of all co-expressed genes common between *AtSLP2* and *AtMIA40* localized to the mitochondria and the AtSLP2-AtMIA40 protein complex targeted to the mitochondrial membrane space (IMS), it is possible that the primary function of the AtSLP2-AtMIA40 protein complex is to regulate the import and/or processing of mitochondrial targeting peptide (mTP) containing proteins destine for the mitochondrial matrix (Figs. 1 and 2). Interestingly, a large portion of the mitochondrial targeted proteins found in the 96 *AtSLP2-AtMIA40* co-expressed genes are involved in gene expression, RNA processing and translation. In particular, pentatricopeptide repeat proteins (PPR), tetratricopeptide proteins (TPR) and ribosomal subunits, loss of which, can lead to impaired mitochondrial function.[6–8] TPR and PPR proteins are especially interesting as they have been proposed to be involved in mitochondrial RNA editing and possess multiple short repeat regions that facilitate protein-protein interactions (Table S2).[8] Given this, the co-expression results suggest that the germination phenotypes observed in *atslp2* / 35S::AtSLP2 plants may be related to the regulation of mitochondrial protein import of nuclear encoded proteins involved in the transcriptional (TPR/PPR) and translational (ribosome) regulation of mitochondrial encoded genes/proteins. Alternatively, the predicted cytosolic and nuclear proteins of the identified co-expressed genes may represent direct or indirect targets of AtMIA40 activated AtSLP2. As mitochondrial IMS proteins do not maintain a conventional mitochondrial targeting peptide, predicting whether or not there is a direct and indirect connection between positively correlated, co-expressed *AtSLP2-AtMIA40* genes is more difficult. However, similar to the mitochondrial targeted proteins, many of the nuclear and cytosolic proteins are involved in transcription and translation regulation (Table S1).

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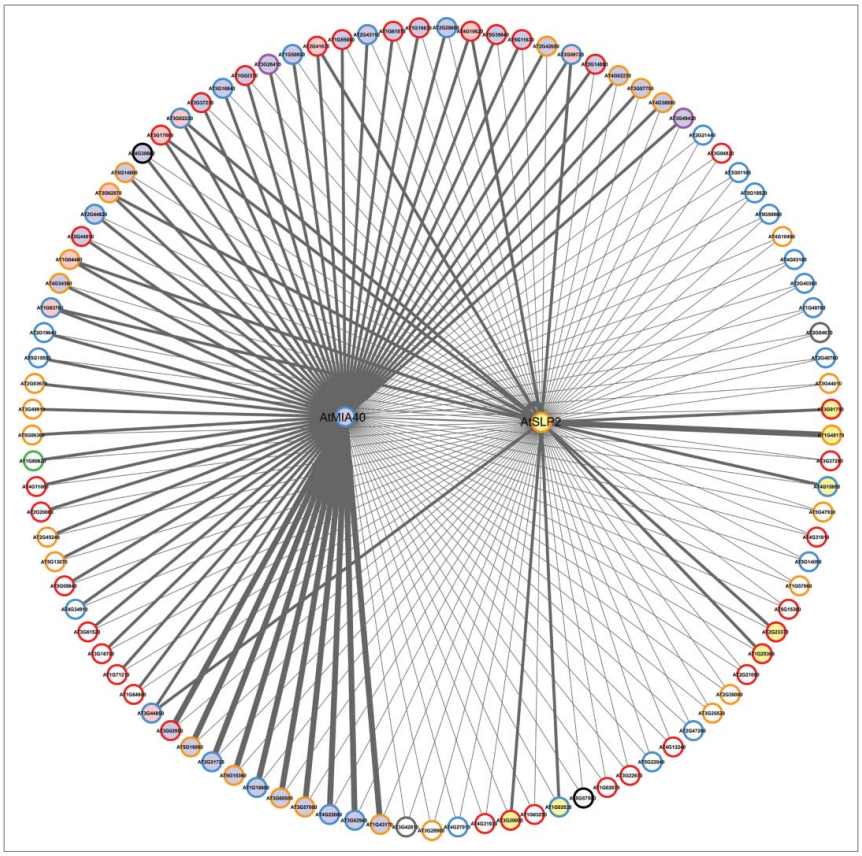

Figure 1. *AtSLP2* and *AtMIA40* co-expression network. The top positively co-expressing genes (Pearson correlation coefficient (PCC) > 0.95 shared between AtSLP2 (At1g18480) and AtMIA40 (At5g23395). Gene co-expression was determined using available normalized RNA-sequencing data (Genevestigator; https://genevestigator.com/gv/). Edge thicknesses represent the PCC values 0.950 - 0.984 (thin), 0.985 - 0.994 (medium) and 0.995 - 1.0 (thick). Yellow and blue filled circles represent genes (> 0.985) with a prefered positive correleation with either AtSLP2 or AtMIA40; respectively. Red filled circles represent genes (> 0.985) with shared co-expression between *AtSLP2* and *AtMIA40*. The outline of each circle represents the predicted subcellular localization of the protein product from each corresponding AGI (SUBA3; http://suba3.plantenergy.uwa.edu.au/). Cytosol (orange), extracellular (black), mitochondria (red), nucleus (blue), plasma membrane (purple) and plastin (green) are shown.

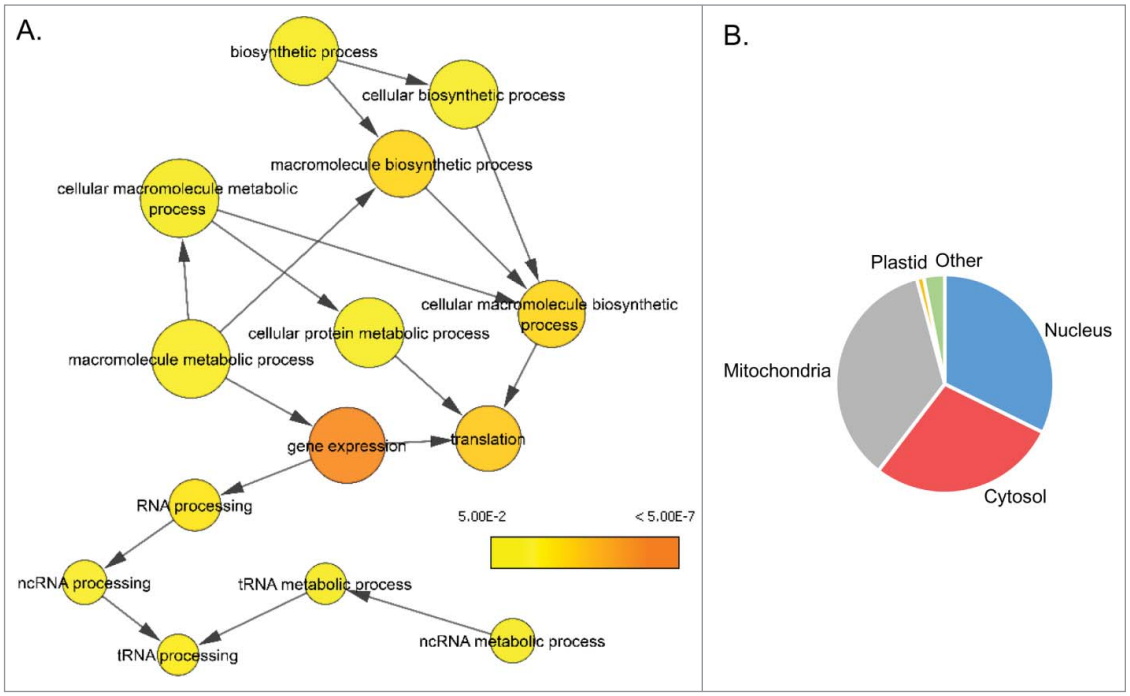

Figure 2. Biological term gene ontology analysis and subcellular localization distribution of co-expressed genes common between *AtSLP2* and *AtMIA40*. (A) Using the cytoscape plugin BinGo the biological terms enriched within the *AtSLP2/AtMIA40* co-expression network are shown. Coloring of the nodes represents the term enrichment (FDR pval ≤ 0.05). (B) In silico predicted subcellular localization of *AtSLP2 / AtMIA40* co-expressed genes. Colors represent nuclear (light blue), cytosolic (red), mitochondrial (grey), plastidial (yellow) and other (green) subcellular localization.

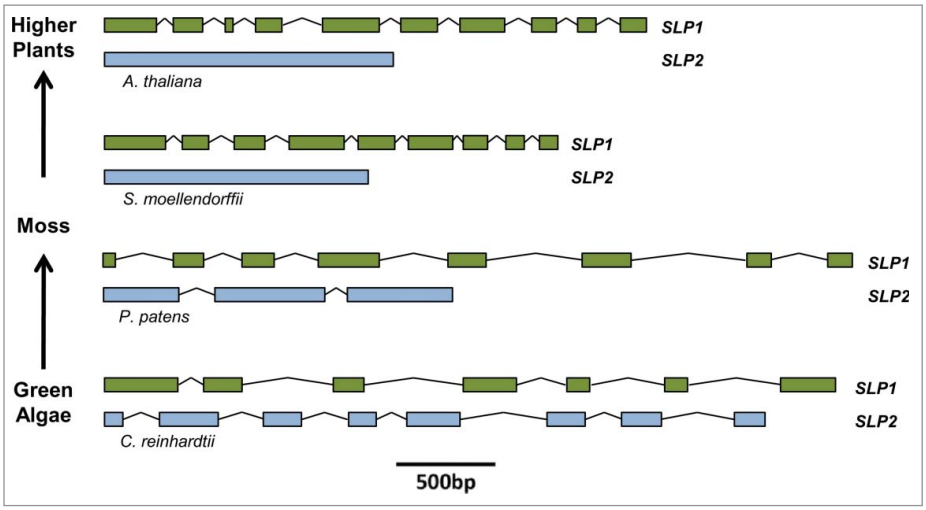

Figure 3. Gene structure of eukaryotic *SLP* phosphatases. *SLP1* and *2* phosphatases maintain similar gene structures in lower Eukaryotes. Exon-to-intron ratio increases in *SLP1* phosphatases versus complete intron loss in *SLP 2* phosphatases over the course of photosynthetic eukaryote evolution. Representative *SLP1* and *2* phosphatases are shown in green and blue, respectively.

AtSLP2 is an evolved intronless gene that co-expresses with several mitochondrial targeted intronless genes

Upon further targeted examination of the *SLP* gene structure, we found that the closely related *SLP1* and *SLP2* orthologs notably diverge over the course of photosynthetic eukaryote evolution.[1,2] In particular, *SLP1* orthologs maintain introns, while *SLP2* orthologs are intronless (Fig. 3; Table 1). Intronless genes can be found throughout eukaryotes, and have been suggested to be derived from either prokaryotic lateral gene transfer (LGT) events or retrogene insertions from cDNA.[9,10] Interestingly, despite their close relationship to bacterial phosphatases,[1] *SLP2* orthologs initially maintain introns in early photosynthetic eukaryotes (e.g. green algae) and progressively become intronless over the course of photosynthetic eukaryote evolution (Fig. 3; Table 1).

Table 1. Intron quantity for photosynthetic Eukaryote SLP phosphatases. Highlighted in bold are green algae (*Chlamydomonas reinhardtii*), lycophyte (*Selaginella moellendorffii*), moss (*Physcomitrella patens*) and *A. thaliana SLP1* and *SLP2* phosphatases. 1 Additional information includes their corresponding '*SLP1*' and '*SLP2*' tree identifiers[1], as well as organismal and gene identifier information. Gene models were obtained from Phytozome v7.0 (www.phytozome.net).

	SLP1	Organism	Gene Identifier	Intron #	SLP2	Organism	Gene Identifier	Intron #
Dicots	***AtSLP1***	***Arabidopsis thaliana***	**At1g07010**	**9**	***AtSLP2***	***Arabidopsis thaliana***	**At1g18480**	**0**
	AlSLPa	*Arabidopsis lyrata*	470724|PACid:16040444	9	*AlSLPb*	*Arabidopsis lyrata*	472067|PACid:16060211	0
	MeSLPb	*Manihot esculenta*	cassava4.1_009955m	7	*MeSLPa*	*Manihot esculenta*	cassava4.1_008887m	0
	BrSLPb	*Brassica rapa*	Bra015544|PACid:22693638	7	*BrSLPa*	*Brassica rapa*	Bra025903|PACid:22696925	0
	CsSLPb	*Citrus sinensis*	orange1.1g016381m|PACid:18111291	9	*CsSLPa*	*Citrus sinensis*	orange1.1g041373m|PACid:18124504	0
	CsatSLPa	*Cucumis sativus*	Cucsa.151840.1|PACid:16964486	9	*CsatSLPc*	*Cucumis sativus*	Cucsa.075800.1|PACid:16955776	0
	GrSLPb	*Gossypium raimondii*	Gorai.013G271300.1|PACid:26789935	9	*GrSLPa*	*Gossypium raimondii*	Gorai.006G232000.1|PACid:26833651	0
	GmSLPb	*Glycine max*	Glyma14g37780.1|PACid:26287143	9	*GmSLPa*	*Glycine max*	Glyma19g13655.1|PACid:26327286	1
	MgSLPb	*Mimulus guttatus*	mgv1a007297m|PACid:17681917	9	*MgSLPc*	*Mimulus guttatus*	mgv1a007897m|PACid:17678561	0
	MtSLPb	*Medicago truncatula*	Medtr5g081100.1|PACid:17467021	8	*MtSLPa*	*Medicago truncatula*	Medtr7g005770.1|PACid:17472861	1
	PvulSLPb	*Phaseolus vulgaris*	Phvul.008G256500.1|PACid:27155250	9	*PvulSLPa*	*Phaseolus vulgaris*	Phvul.005G085100.1|PACid:27150157	1
	PtSLPa	*Populus trichocarpa*	Potri.009G077900.1|PACid:26987030	9	*PtSLPb*	*Populus trichocarpa*	Potri.013G127500.1|PACid:26995499	0
	VvSLPb	*Vitis vinifera*	GSVIVT01024637001|PACid:17832792	9	*VvSLPa*	*Vitis vinifera*	GSVIVT01035133001|PACid:17840382	2
	AcSLPa	*Aquilegia coerulea*	Aquca_030_00216.1|PACid:22035664	9	*AcSLPb*	*Aquilegia coerulea*	Aquca_042_00158.1|PACid:22033007	1
Monocots	*OsSLPa*	*Oryza sativa*	LOC_Os10g25430.1|PACid:21883573	6	*OsSLPb*	*Oryza sativa*	LOC_Os11g15570.1|PACid:21945373	0
	SbSLPa	*Sorghum bicolor*	Sb01g023200.1|PACid:1952078	10	*SbSLPb*	*Sorghum bicolor*	Sb05g006560.1|PACid:1969658	1
	SiSLPa	*Setaria italica*	Si036062m|PACid:19679889	10	*SiSLPb*	*Setaria italica*	Si026407m|PACid:19708378	0
	BdSLPa	*Brachypodium distachyon*	Bradi3g25370.2|PACid:21831821	10	*BdSLPb*	*Brachypodium distachyon*	Bradi4g20750.1|PACid:21811426	0
	SmSLPa	***Selaginella moellendorffii***	**123773|PACid:15403392**	**8**	***SmSLPb***	***Selaginella moellendorffii***	**65599|PACid:15406428**	**0**
	PpaSLPc	***Physcomitrella patens***	**Pp1s412_38V6.1|PACid:18061202**	**8**	***PpaSLPb***	***Physcomitrella patens***	**Pp1s98_64V6.1|PACid:18070451**	**2**
Green Algae	*VcSLPb*	*Volvox carteri*	Vocar20007918m|PACid:23138954	5	*VcSLPa*	*Volvox carteri*	Vocar20010810m|PACid:23129267	7
	CrSLPa	***Chlamydomonas reinhardtii***	**Cre03.g185200. t1.2|PACid:26901432**	**8**	***CrSLPb***	***Chlamydomonas reinhardtii***	**Cre17.g718800. t1.2|PACid:26897048**	**7**
	CocSLPa	*Coccomyxa sp.*	jgi|Coc_C169_1|30480|	7	*CocSLPb*	*Coccomyxa sp.*	jgi|Coc_C169_1|35854|	5

In genome-scale analyses of arabidopsis and rice, hundreds to thousands of intronless genes have been found.[10] The protein products of these genes were found to be involved in specific cellular processes such as protein translation, energy metabolism as well as amino acid biosynthesis, and consist mainly of ribosomal, F-box, auxin-responsive, pentatricopeptide repeat (PRR), leucine-rich repeat, transcription factors, and cytochrome p450 proteins.[10] Interestingly, many of these genes are also co-expressed with both *AtSLP2 / AtMIA40* (Fig. 1; Table S1). In fact, we found that 18% of all co-expressed genes common between *AtSLP2 / AtMIA40* were intronless[10]; Table S1), while 20% of all *Arabidopsis thaliana* genes have been shown to be intronless.[10] The majority of these intronless *AtSLP2 / AtMIA40* co-expressed genes are transcription factor, PRR / TPR and ribosomal proteins (Table S1). Furthermore, analysis of intronless gene expression have found that they are more rapidly expressed in response to stress[11] and development[12] than intron containing genes. This suggests that AtSLP2 may be primarily regulated at the level of transcription and part of a complex intronless gene regulation network located in the mitochondria.

Conclusion

Given the pervasive nature of intronless genes in plants, their involvement in key aspects of plant cell function, and their potential for rapid transcriptional induction, it is possible that the positively correlating *AtSLP2* and *AtMIA40* co-expressed genes identified here form a regulatory network ranging from indirect transcriptional- to direct post-translational protein-regulation of mitochondrial protein import and metabolic processes. Further targeted experimentation is required to validate direct (protein-protein) and/or indirect (genetic) interactions between AtSLP2 and the identified, positively correlated co-expressed *AtSLP2 / AtMIA40* genes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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