

## Implications of an Evolutionary Analysis on the TMBIM Superfamily of Proteins for the Field of Plant Research: Expansion and Function of the LFG Family in Plants

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### Commentary

The TMBIM (Transmembrane BAX Inhibitor Motif containing) superfamily comprises a group of cytoprotective (in most cases) transmembrane proteins conserved in prokaryotes and eukaryotes. In eukaryotes this superfamily is divided in the BI (BAX-Inhibitor) and LFG (Lifeguard) families [1]. In our recently published article we made an overview of the phylogenetic relationships of the TMBIM superfamily of proteins across Archaea, Bacteria, and Eukarya domains [2]. Furthermore, we made a close up to the evolution of the BI and LFG families in plants through the analysis of their orthology and synteny relationships [2]. In the present commentary, we summarize current studies about the LFG homologues in plants and expand our discussion about the implications of our previous findings about their phylogeny [2] for the field of plant research.

The BI-1 homologues of plant species have been extensively studied, especially in *Arabidopsis* (*Arabidopsis thaliana*). On the contrary, the plant LFG homologues have received less attention. *Arabidopsis* and barley (*Hordeum vulgare*) possess five LFG homologues each: AtLFG1-5, and HvLFGa-e, respectively [3]. AtLFG5 is placed within the plant LFG I clade, and AtLFG1-4 are placed within the LFG II (A-B) clade (Table 1) [2]. The phylogenetic placement and orthology relationships of HvLFGa-e from barley remain unclear as they were not included in our previous analysis [2]. However, from previous work of other authors [3] and BLAST similarity searches (against the protein dataset employed for the reconstruction of the plant LFG phylogeny in [2]) we can infer that HvLFGe (the closest homologue to AtLFG5 [3]) is placed within the LFG I clade probably sharing the "OG 4" with most land plants, and that HvLFGa-d Author Agreement are within the LFG II (A) clade (Table 1). HvLFGb-c probably share the "OG 8" with other Poaceae species (Table 1).

To our knowledge only four research articles have been published specifically addressing the biological functions of the LFG genes in plants [3-6]. In such publications, three genes from *Arabidopsis*, i.e. AtLFG1 (GAAP1/BIL4-H3), AtLFG2 (GAAP2/BIL4), and AtLFG3 (GAAP3/BIL4-H2), and one from barley, i.e., HvLFGa, have been characterized by means of over-expressing or silencing constructs, and/or *Arabidopsis* mutants (Table 1). All of these functionally characterized homologues are included within the LFG II (A-B) clade (Table 1). Moreover, AtLFG1, AtLFG2 and AtLFG3 proteins belong to "OG 12", "OG 7", and "OG 6", respectively (Table 1), which are orthologous groups specific for the Brassicaceae family [2].

Interestingly, AtLFG1 and AtLFG3 are preferentially expressed in reproductive tissues [2,5], and they are also related to ER-stress defense [5]. AtLFG2 is expressed in several plant tissues [2,6] and acts as a positive regulator of the Brassinosteroid signaling pathway [6],

indicating a possible role in development. Furthermore, HvLFGa, AtLFG1, and AtLFG2 support PMPI (powdery mildew pathogen infection) [3] (Table 1). These data suggest that a plant-pathogen interaction function might be conserved across the LFG II (A-B) clade in both monocots and eudicots. However, in Brassicaceae a sub-functionalization might have occurred within specific OGs leading to differences in gene expression and additional functions (ER-stress and Brassinosteroid signaling) of these proteins (Table 1). Further research on the function of these proteins is necessary to clarify this issue. In addition, the TaGAAP gene from wheat, which is placed within the LFG IIA clade and shares orthology with the "OG 5" and most seed plants [2], is activated upon PEG (polyethylene glycol) and heat treatments, and it is directly activated by the TaHsfA6f transcription factor from the Heat Shock Factor family [7]. Overexpression of TaHsfA6f confers thermo-tolerance [7]. If overexpression of TaGAAP or other LFG proteins from the LFG II (A-B) clade also contribute to thermo-tolerance remains open for further research.

Moreover, it is worth noting that all of the reported plant LFG proteins within the LFG II (A-B) clade (monocots and eudicots) are located in the plasma membrane, tonoplast, and endoplasmic reticulum (Table 1). None of them has been reported to be located in the Golgi apparatus, even when this is one of the characteristic that gave the name (GAAP) to these proteins [8].

What about the LFG I clade? To our knowledge, no study addressing the function of any protein within the LFG I clade in plants has been performed so far. However, the AtLFG5 (LFG I clade) is highly expressed in most tissues [2], probably fulfilling a key function in the maintenance of the whole organism. It is possible that the proteins within this clade are actually located in the Golgi apparatus. It is also worth noting that the LFG I clade in plants, remained with few duplicates [2]. This low number of duplicate genes in the LFG I clade and the expansion of the LFG II (A-B) clade in plants, mirrors the conservation of the LFG4 clade and expansion of the LFG1-like clade in animals [1,2]. It might be possible that the homologues of the LFG I in plants exist in few copies and located in the Golgi apparatus (and possibly other organelles), while the proteins of the LFG II (A-B) were expanded to regulate cytoprotective functions in different organelle membranes, somehow similar to what has been found in animal models [9,10]. Would the expansion of the LFG I clade in plants and LFG1-like clade in animals be related with the complexity in these eukaryotes? Do the different plant LFG duplicates play a role in different programmed cell death pathways as seen in animals [9]? These questions remain open to be answered by future research.

In this brief commentary we attempted to draw attention for the study of more LFG genes/proteins in plants under different contexts,

and we hope that our previous evolutionary analysis on the TMBIM superfamily [2] would be useful for interpretation of future research in this field.

Clade	Orthologous Group (OG)	ID	Symbol Names	Short functional description*	Refs.
LFG I	4 (Land Plants)	AT4G15470	AtLFG5 GAAP5 BIL4-H4	NA	
?	4?	TA32154_4513 AK249851	HvLFGe	NA	
	5 (Seed Plants)	AT1G03070	AtLFG4 GAAP4 BIL4-H1	NA	
	5?	TA49291_4513	HvLFGa	Supports susceptibility to PMPI. Locates in the PM. Relocates to endosomal compartments and tonoplast upon successful PMPI.	[3]
LFG IIA	6 (Brassicaceae)	AT4G02690	AtLFG3 GAAP3 BIL4-H2	Regulates the expression of some elements of the UPR signaling pathway. Increase tolerance to ER stress, and inhibits ER-stress induced PCD. Locates in PM and ER. Interacts with IRE1. Preferentially expressed in reproductive organs. Expression enhanced upon TM treatment or NaCl stress.	[5]
	7 (Brassicaceae)	AT3G63310	AtLFG2 GAAP2 BIL4	Positive regulator of BR signaling. Supports susceptibility to PMPI. Locates in tonoplast and endosomes. Interacts with BRI1 in endosomes, and alters its cellular trafficking and degradation in vacuoles. Expressed in most plant tissues.	[3,4,6]
?	8? (Poaceae)	TA42670_4513	HvLFGb	NA	
?	8?	TA42670_4513	HvLFGc	NA	
?	?	TA39011_4513 AK251691	HvLFGd	NA	
LFG IIB	12 (Brassicaceae)	AT4G14730	AtLFG1 GAAP1 BIL4-H3	Supports susceptibility to PMPI. Regulates the expression of some elements of the UPR signaling pathway. Increase tolerance to ER stress, and inhibits ER-stress induced PCD. Locates in PM and ER. Interacts with IRE1. Preferentially expressed in reproductive organs. Expression enhanced upon TM treatment or NaCl stress.	[3,5]
				Expression enhanced upon TM treatment or NaCl stress.	

**Table 1:** Summary of the LFG genes from Arabidopsis and barley.

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