Regulated intramembrane proteolysis in the plastid envelope

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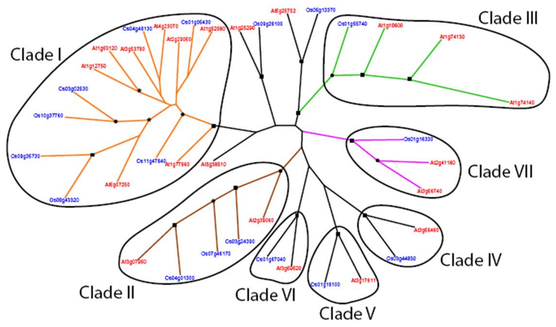
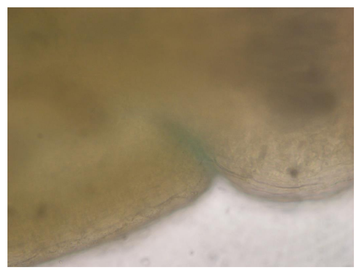
Rhomboid proteins were the most recently identified of the four families of intramembrane proteases. Found in almost all organisms, these serine proteases operate in a diverse range of pathways. They regulate *Drosophila* growth factor signalling (Urban et al., 2002), permit infection by apicomplexans (Dowse et al., 2005), play a key role in mitochondrial dynamics (Herlan et al., 2004), and allow bacterial quorum sensing via channel activation (Stevenson et al., 2007).

In plants, as in other eukaryotes, rhomboids are encoded by a multigene family. These are little researched to date but we documented previously that *A. thaliana* rhomboid RBL10 was situated in the plastid envelope and has roles in fertility and photosynthesis (Thompson et al., 2012).



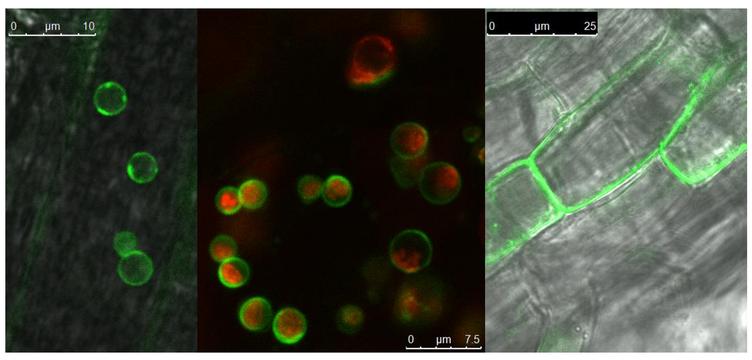
Arabidopsis and rice rhomboids (Tripathi & Sowdhamini, 2006).

GUS-promoter staining of RBL10 in apex of the stigma.



TISSUE LOCALISATION

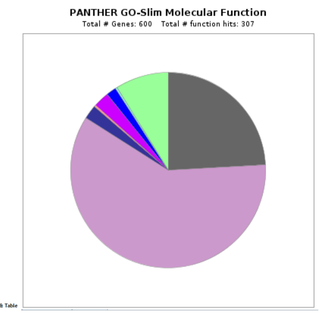
Transcript was amplified from seedlings (day 5 and day 6), mature rosette leaf, bolt stem, petiole, open flowers and immature siliques. The promoter of *rbl10* contains the core of the low-temperature responsive element of the *A. thaliana cor15a* gene but transcription was not environmentally regulated in our assays. Our GUS-promoter reporter showed only limited staining at the stigma apex.

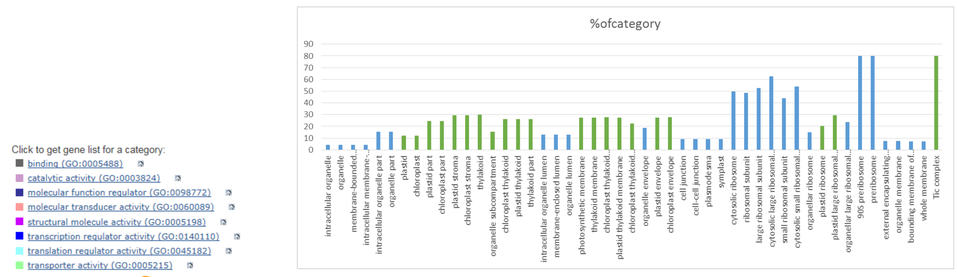


Day 4 chloroplast and root plastid GFP in outer membrane. Left, *RBL10*-GFP at day 20 in root plastids; Middle, d9 chloroplast envelope; Right, control extensin-GFP in root cell wall.

A CHLOROPLAST RHOMBOID

The predicted N-terminal transit peptide of RBL10 suggested a location in the chloroplast outer membrane, confirmed with our GFP-rhomboid transformed plants. Fluorescence was also observed in plastid-membrane links within the cells (Thompson et al., 2012).





RNASeq

Transcriptomes from floral tissues and rosette leaf of WT vs *RBL10* plants revealed numerous organellar and membrane-located genes among those that were significantly differently expressed (padj <0.05). Most GO hits for those transcripts' molecular function in flowers were 'binding', 'catalytic activity' and 'transport activity'. As might be expected, most downstream processes from the regulated transcripts were predicted (PANTHER) to involve signalling pathways.

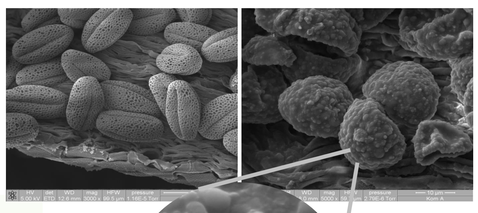
In the leaf, 133 'defence response' transcripts were identified among GO classifications: this is relevant to speculation that RBL10 effects on jasmonate pathways could link *RBL10* photosynthesis and fertility phenotypes (Knopf et al., 2012; Thompson et al., 2012). Suggestions that RBL10 has a role in phosphatidic acid metabolism (Lavell et al., 2019) could be supported by the 153 'lipid metabolic process' transcripts regulated in the mutant. 112 out of 410 'phospholipid metabolic process' genes. Specific regulated genes are under investigation.

Differentially transcribed genes in *RBL10* mutant leaf vs wild type: roles (left, above PANTHER and below REVIGO) and location of regulated transcripts (right)

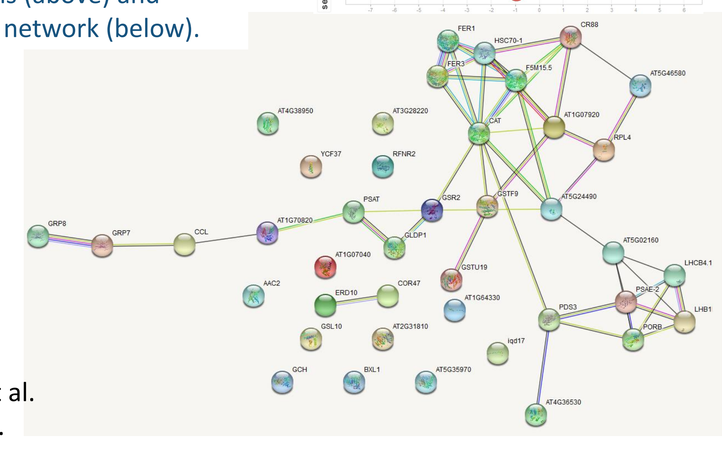
Chloroplast *RBL10* mutant pollen (inset, *KOM* mutant); seeds/silique; and floral abnormalities.

MUTANT PHENOTYPE

A proportion of *RBL10* pollen developed poorly: some of the heterogeneous pollen grains were half the length of WT pollen, or were collapsed or had flatter surface topology. The pollen phenotype, however, was not as severe as that seen in another *Arabidopsis* rhomboid mutant, *KOM*. *RBL10* showed other floral abnormalities, with the stigma commonly malformed. Few siliques developed successfully on the plant from early inflorescences and the number of seed per silique from primary inflorescences was significantly lower than in the WT. Mutant plants also showed increased anthocyanin levels and more lateral roots, relative to WT, and aberrant nonphotochemical quenching in FvFm assays.



For differentially regulated transcripts, most common biological role category for encoded proteins (above) and STRING association network (below).



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