Genes controlling the development and function of plant vacuoles

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Summary

In this thesis the study of different aspects of plant vacuoles genesis, functions and dynamics is approached using different strategies based on the analysis of mutants, the study of transcriptomes, the comparison of different plant species showing specific adaptations.

In **Chapter** 2, to unravel the mechanisms responsible for copper hypertolerance in *Silene vulgaris*, a dominant species in metal-rich habitats, we analyzed homologs of the P_{1B}-ATPase copper transporter HMA5. We found that *Silene* has two such genes, *SvHMA5I* and *SvHMA5II*, originating from an ancient duplication. Both are up-regulated in a Cu-hypertolerant *Silene* population and confer Cu-tolerance by distinct mechanisms when expressed in *Arabidopsis*. When co-expressed, these two Cu transporters enhance growth both at toxic and nutritional copper concentrations. SvHMA5II and the *Arabidopsis* homolog AtHMA5 localize in the ER and upon exposure to Cu move to the plasma membrane from where they are internalized and degraded in the vacuole. This resembles trafficking of mammalian homologs and revealing an apparently very ancient mechanism of Cu detoxification. SvHMA5I, instead, resides always on the tonoplast, likely sequestering Cu to the vacuole. We present a model for the contribution of HMA5I and HMA5II in Cu tolerance and adaptation to polluted soils.

Petunia mutants with blue flowers uncovered a proton pump that controls vacuolar hyper-acidification in petal epidermal cells. This pump consists of two interacting P-ATPase, PH1 and PH5, residing in the tonoplast. PH5 is similar to plasma membrane proton P_{3A}-ATPase, whereas PH1 is the only known eukaryotic P_{3B}-ATPAse. As there are no indications that such a pump might operate in other species, we investigated the distribution of PH1 and PH5 homologs in the plant kingdom in **Chapter 3**. We show that homologs of PH1 and PH5 are common in the genomes of angiosperms and they appeared probably in gymnosperms. Phylogenetic analysis and swaps of protein domains suggests that PH5 originated from plasma membrane proton transporters by the acquisition of a new subcellular localization and a new regulation mechanism. PH1 shares its origins with bacterial magnesium transporters, and its appearance in plants could have involved horizontal transfer from bacteria to plants, although the acquisition in bacteria from plants is not excluded. PH1 is irregularly distributed within plant families, indicating it was possibly repeatedly lost. The wide conservation of PH1 and PH5, suggests that vacuolar hyper-acidification is required, not only for flower color determination, but possibly also for other processes. The challenge is now to uncover these processes are.

Since some 20 years, it is known that cells can contain multiple distinct vacuoles, however, the multivacuolar cell-type described so far and the mechanisms underlying vacuolar differentiation remained unknown. The lumen of vacuoles is typically mildly acidic, but can be lower in specialized tissues, like petals, where PH1 and PH5 are active. In **Chapter 4** we show that the sorting of this pumps and other vacuolar proteins to the CV involves transit (24 hours after transformation) through small vacuoles, vacuolinos, which are present in petal epidermal cells next to the central vacuole. 48 hours after transformation, vacuolar proteins reach the central vacuole.

Vacuolino formation is controlled by petal epidermis-specific transcription factors complex. This complex containing the factors AN1, PH4 and PH3, also regulates pigment synthesis and transcription of the PH1 and PH5 genes. Vacuolino fusion to the central vacuole and delivery of vacuolar proteins, requires vacuolar SNAREs interacting with the PH1-PH5 pump. This implies that structural tonoplast proteins can act as tethering factors between different vacuolar types, probably becoming a recognition factor for different compartments and defining in this way the interactions among their membranes. Vacuolinos and the collection of mutants affecting their genesis and fusion offer a model for the study of separate vacuoles and the traffic between these and the CV.

In order to identify candidate genes involved in the vacuolinos pathway, we analysis in **Chapter 5**the transcriptomes of petals from mutants for the transcription factors AN1, PH4 and PH3. We found large number of genes differentially expressed in these mutants as compared to wild type petals, indicating these transcription factors possibly control more process than we thought before. Among these differently expressed genes, we identify 31 genes significantly differentially expressed (> 5 fold change) in all three *an1*, *ph4* and *ph3* mutant petal as compared to wild type petals. These genes are potential vacuolinos genes. To date, we confirmed that 2 genes, *PAT1* (which encode patatin-like phospholipase) and *CAC12.3* (encoding a protein of unknown function), are indeed involved in the vacuolino pathway. In *pat1* knockdown petals, vacuolar proteins move to the central vacuole directly without transiting through vacuolinos, just as in mesophyll cells. In *cac12.3* knockdown petals, vacuolar protein moved to small dot-like compartments, where they remain stuck without ever reaching the central vacuole. These findings imply that *CAC12.3* and *pat1* are both required the formation of vacuolinos, however, at different points in their genesis pathway.

Besides the vacuolinos, the AN1-PH4-PH3 complex (WMBW) is shown to regulate the biosynthesis of anthocyanins and proanthocyanidins and to trigger vacuolar hyperacidification, whereas homolog WMBW complexes in *Arabidopsis* are known to regulate the biosynthesis of anthocyanins, proanthocyanidins and other processes such as mucilage production in seeds as well as trichome development on aerial tissues and non-hair cells in the root epidermis. Based on the comparison of the target genes of WMBW complexes in petunia and *Arabidopsis,* we found that their sets of target genes have only very partial overlap. From this observation it is possible to conclude that differences between *Arabidopsis* and petunia WMBW complexes with regard to their function and the subordinate suite of direct and indirect target genes are to a large extent due to differences in the promoters of the targets, rather than in changes affecting the regulatory proteins.