Going in the Right Direction: Cellular Mechanisms Underlying Root Halotropism
Dhr. R.A. Korver

Summary

Worldwide salinization of the soils threatens our food supply. What makes salinization an even more urgent problem is that the areas that are affected most are in general regions with relatively lower food availability to start with. For these communities losing harvests to abiotic factors is devastating. Improvements on salt tolerant crops are being made, however, not in all cases the underlying mechanisms of the tolerance are known. Nevertheless, when the genes involved have no homologues or even orthologues in other crops, knowing the mechanism will help towards finding a solution in all crops. One major response to salinity in plants is the change of root system architecture, including root growth direction under influence of altered local concentrations of the plant hormone auxin, and auxin symmetry in the root. The current knowledge on salt-induced changes of root growth direction and the processes involved are summarized in **chapter 1**. Moreover, one essential cellular signalling pathway, phospholipid signalling, during salt stress is introduced in this chapter.

In **chapter 2,** the auxin-related changes during salt stress are summarized. From recent literature it is becoming clear that other auxin regulating processes besides polar auxin transport contribute more to the auxin-induced changes during salt stress than previously thought. Regulation of passive auxin transport and auxin storage is required to build, and even more importantly, maintain local auxin maxima. Also, local auxin biosynthesis and conjugation alters the abundance of free auxin available for processes such as auxin signalling. In a meta-analysis on recently published gene expression data it was revealed that IAA biosynthesis and conjugation related gene expression show distinct patterns in different root tissues during salt stress.

The complex interplay of factors regulating auxin flow in the root make it a system in which tweaking on small and large scale is possible. As a consequence of this complexity, it sometimes is difficult to comprehend and predict the changes that different stimuli cause to the system. In **chapter 3** the question if salt-induced changes in PIN2 polarity are sufficient for the alteration of the auxin symmetry in the root during halotropism is addressed. Due to the complexity of the auxin flow in the root it was expected that more changes would follow the PIN2 polarity change. Indeed, auxin feedback dependent changes of AUX1 were predicted and experimentally validated to be relevant. In addition, to regulate the timing of building auxin accumulations higher supply of auxin through a transient increase of PIN in the stele was found. These results show the importance of combining *in planta* experiments to determine the exact parameters to put into *in silico* analysis to accurately and rapidly predict what changes

occur in these complex processes. These predictions can then be verified by specific experiments. In this way, computational modelling will significantly speed up the discovery of factors involved in complex biological systems.

Local patches of signalling lipids in the membrane are signals for cytosolic proteins to bind the membrane and carry out their function. Phospholipase D ζ proteins hydrolyse the structural membrane lipid phosphatidyl choline (PC) into the lipid second messenger phosphatidic acid (PA). PLDζ derived PA has been suggested to recruit proteins to the membrane involved in endocytosis during salt stress. In **chapters 4 and 5** the roles of PLDζ1 and PLDζ2 during salt stress and in particular halotropism are studied. PLDζ1 was found to regulate PIN2 polarity in root epidermal cells. In addition, the PIN2 and AUX1 polarity shifts observed during salt stress are altered in a *pldζ1* KO mutant. No indication for involvement of PLDζ2 in PIN2 internalization or polarity have been found. PLDζ1 was also observed to be involved in root gravitropism, possibly through the PIN2 polarity in the root epidermal cells. This emphasizes the importance of proteins and processes regulating the polarity of auxin carriers. In addition, we report the discovery of excess membrane material which putatively affects auxin carrier endocytosis and cycling; osmotic stress-induced membrane structures (OSIMS). This excess membrane material appears shortly after exposure of the root to osmotic stress and is not found for longer then 30 minutes at the PM in wildtype cells. In the *pldζ1* KO mutant OSIMS are observed for longer than 60 minutes. How OSIMS influence auxin carrier internalization or polarity remains unknown.

How clathrin-mediated endocytosis (CME) is involved in the salt response of roots is unclear. Epsin-like clathrin adaptors (ECA's) are A/ENTH domain-containing proteins putatively involved in the assembly of clathrin coated vesicles (CCV's). Characterization of ECA's during salt stress was performed **(Chapter 6)** to help unravel the role of CME during the salt response in the root cells. ECA4 negatively regulates the halotropism response while ECA1 has a positive effect. This result suggests that CME is involved in a broad range of salt-induced processes. Next to that, ECA4 was found to negatively influence main root growth and lateral root development under control conditions, as well as growth during salt stress. However, no indication was found that ECA1 or ECA4 regulate PIN2 internalization or polarity. Therefore, it is proposed that CME is involved in many salt-induced processes, but not auxin carrier re-localization.

In **chapter 7** I discuss how these results fit into the current knowledge on halotropism and salt stress responses. I provide an outline for further studies on these subjects. It has become clear that the alteration of auxin flow through environmental stimuli is a complex process, which should be studied on both a cellular level and on a

whole root level using a combination of physiological and cell biological approaches, including computational models. The focus of research on the changes of the sub-cellular localization of auxin carriers should be more towards the manipulation of the re-cycling instead of the internalization. Furthermore, CME seems to play roles outside of auxin carrier endocytosis and its protein interactions point towards new cellular mechanisms involved in halotropism that have not been studied yet. Here lies a great opportunity to further increase our understanding of root tropic responses.