Unraveling the auxin mechanism in 2,4-D induced somatic embryogenesis in Arabidopsis thaliana

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Summary

Plant clonal propagation can be achieved either through stem cuttings or shoot and subsequent root regeneration, in which cells go through a dedifferentiated state from which they can differentiate again into shoot or root forming cells. Alternatively, vegetative propagation can be established in a single step in which a shoot-root axis, a vascular system and functional meristems are produced by the induction of embryos without fertilization such as somatic embryogenesis (SE). In the model plant *Arabidopsis thaliana*, somatic embryos are efficiently induced by incubating immature zygotic embryos (IZEs) on SE induction medium (SEIM) containing high concentrations of the auxin analogue 2,4-dichlorophenoxyacetic acid (2,4-D). During SE somatic cells are reprogrammed to become totipotent cells, from which a new embryo cell fate is acquired in order to develop somatic embryos. The genetic and molecular mechanism by which 2,4-D induces SE initiation has not been elucidated yet and the aim of this PhD thesis was to identify which components of the auxin response pathway are involved in this developmental process.

Chapter 2 describes the establishment of a standardized system for SE initiation and outgrowth of somatic embryos using 12 days old *Arabidopsis* IZEs. In this system, *pWOX2::NLS-YFP* was used as a marker for embryonic cell fate and the first *WOX2-YFP*-reported embryonic cell clusters could be observed after 5 to 7 days of culture in SEIM. Interestingly, these clusters were located in regions of low auxin response. Cells neighboring these SE initiation regions with low auxin response expressed AUX1 influx carriers, which suggested the low auxin response regions might be generated by auxin influx into these neighboring cells. *aux1 lax* loss-of-function mutant IZEs showed enhanced shoot/root regeneration at the cost of SE, and studies using inhibitors of cellular auxin transport corroborated the important role for auxin influx proteins in SE initiation, whereas auxin efflux proteins only seemed to be important for establishment of the embryonic body pattern later during SE. .

Chapter 3 focuses on the role of auxin biosynthesis in SE. We used combinations of chemical biology, genetics and reporter studies to unravel the Trp-dependent auxin biosynthesis pathway during 2,4-D induced SE initiation. The best-characterized Trp-dependent pathway is the indole-3-pyruvic acid (IPyA) pathway, which includes two enzymatic steps that successively convert Trp into IPyA and IPyA to IAA. The first step comprises the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 / TRYPTOPHAN AMINOTRANSERASE RELATED1-4 (TAA1/TAR1-4) family of enzymes. The second step includes the YUCCA1-11 (YUC1-11) family enzymes. Histological analysis in combination with the use of YUC- or TAA1/TAR-specific inhibitors suggested that YUC activity is rather required for IZE survival during tissue culture and for later development of the initiated embryo, and that

TAA1/TARs are required to establish a dynamic auxin response that drives cell division during SE initiation.

In **Chapter 4** again a combination of chemical biology, mutants and promoter reporter lines was used to map the auxin response components involved in 2,4-D-induced SE on *Arabidopsis* IZEs. Blocking the formation of TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX (TIR1/AFB) co-receptor complex with the auxin antagonist auxinole showed that this co-receptor complex was required for SE initiation. Furthermore, we found that the activating AUXIN RESPONSE FACTOR (ARF) sister pairs ARF7/19 and ARF6/8 were both required for efficient SE initiation. Interestingly, SE was almost completely blocked in the semi-dominant *solitary root-1* (*slr-1*) mutant, indicating that 2,4-D-induced SE and lateral root initiation in *Arabidopsis* share the same auxin response module. The auxin response mutants that were blocked in SE also showed a reduced 2,4-D-induced root callus development, suggesting that this phenotype can be used to pre-select mutants affected in SE. Based on our results we propose a model in which *SLR/IAA14*–regulated dynamic auxin responses are required for both lateral root and embryonic founder cell specification and for the subsequent cell divisions starting respectively lateral root and somatic embryo development.

In conclusion, we propose that lateral root initiation is similar to SE initiation in that an auxin maximum followed by a minimum is required for both processes, and that the auxin responses in both systems are modulated by the same SLR/IAA14 module. Substantial work is still required to outline the details of downstream responses. They possibly include the regulation of auxin biosynthesis through TAA1/TAR and the coordinated expression of AUX1/LAX proteins to establish the dynamic auxin response leading to SE initiation.