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Conference Abstracts

P554 AtDOF6, an Arabidopsis DOF transcription factor putatively involved in the regulation of seed germination

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DOF proteins (DNA binding with One Finger) are transcription factors (TFs) that play diverse roles in gene regulation of plant-specific processes. In barley (*Hordeum vulgare*), 26 different DOFs have been described. Four of them (BPBF, SAD, HvDOF19 and HvDOF17) have been shown to play a role, not only in seed storage protein expression during seed maturation, but also in the regulation of hydrolase gene expression in the germinating aleurone cells.[1-6]

We have analyzed Dof gene expression by RTqPCR in Arabidopsis germinating seeds, and have identified *AtDof6* as an early and highly induced gene. Its expression profile precedes that of genes putatively involved in reserve mobilization (lipases, proteases) and in cell-wall modification (xyloglucan-endotransglycosylases, expansins). Since no KO mutants for this gene could be found in public data-bases, we have generated gain of function and amiRNA transgenic plants, in order to study the possible regulatory role of *AtDof6* within this physiological process.

Phylogenomic approaches have revealed the presence of conserved *cis* elements containing DOF-binding motives (5'T/A-AAAG 3') in hydrolase gene promoters in Arabidopsis and closely related Brassicaceae. We are performing transient expression assays to further characterize the function of AtDof6 in the transcriptional control through these motifs. To identify AtDof6 interacting partners, we are performing a yeast-2-hybrid screening with an Arabidopsis normalized TF library.

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4 Mena, M., *et al* (1998) Plant J 16, 53-62
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P555 The molecular mechanism of growth repression by BIG BROTHER

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The growth of an organ, i.e. biomass accumulation, is controlled by species-specific genetic mechanisms. The different pathways that promote or restrain cell and organ growth must be precisely coordinated. For organs to reach their specific size, growth must be terminated after a certain time.

One of the central negative regulators of organ growth is the *BIG BROTHER (BB)* gene. *BB* limits the growth of floral organs and the stem in a strictly dosage-dependent manner by restricting the period of proliferative growth. The *BB* gene encodes an E3 ubiquitin ligase, which contains a RING-finger domain. The E3 activity of BB protein suggests that BB limits organ size by marking crucial growth stimulators for proteasomal degradation. Since BB functions independently of the major phytohormones and known organ growth promoter, such as *JAG* and *ANT*, identification of its substrates may uncover essential novel activators of plant growth.

By employing the repressed transactivator system (RTA), a member of the homeobox gene family was identified that specifically interacts with BB. Our results suggest that BB restricts organ growth by marking key transcription factors for degradation.

P556 Active CLV3 is an arabinosylated glycopeptide

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Plants continuously produce organs from the self-renewing shoot apical meristem (SAM). A receptor kinase CLV1 and a secreted peptide CLV3 are key components of the regulatory network controlling stem cell renewal and differentiation in Arabidopsis. CLV3 belongs to the CLV3/ESR (CLE) family of peptides that contain a short conserved domain (CLE domain) at or near the C-terminus. Several lines of evidence suggest that the CLE domain, which is the only region with similarity among CLE family peptides, is the functional domain of CLV3. However, we have reservations about the proposed structure for the CLV3 functional form, mainly because exogenous application of the 12-amino-acid CLE domain peptide does not fully rescue *clv3* mutant phenotypes at physiologically relevant concentrations. In addition, although the CLE subfamily (such as genes *CLE1* to *CLE7*) can almost fully complement phenotypes of *clv3* mutant under the *CLV3* promoter, exogenous application of the corresponding 12-amino-acid CLE domain peptides does not rescue *clv3* phenotypes. We thus assume that mature functional form of CLV3 may have undergone as-yet undiscovered posttranslational modifications critical for their function. Here, we show, by nano-LC-MS/MS analysis of apoplastic peptides of Arabidopsis plants overexpressing CLV3, that active mature CLV3 is a 13-amino-acid arabinosylated glycopeptide. We treated *clv3-2* mutant seedlings with purified CLV3 glycopeptide and observed that the *clv3-2* SAM treated with CLV3 at 30 nM were substantially reduced in size comparable to wild-type levels. In contrast, synthetic peptide devoid of arabinose showed only weak activity, indicating that the arabinose chain of CLV3 is critical for full activity.

We also analyzed the binding affinity of CLV3 glycopeptide to CLV1 receptor kinase and confirmed that CLV3 glycopeptide interacted with the CLV1 ectodomain far more strongly than the non-arabinosylated forms. Collectively, we propose that active mature CLV3 is an arabinosylated glycopeptide.

Ohyama *et al* Nature Chem. Biol. (2009) in press.