Plant Development

HOW TO FORM A SEX ORGAN: MOLECULAR AND GENETIC ANALYSIS OF *NOZZLE*, A GENE INVOLVED IN PATTERN FORMATION, GROWTH AND EARLY SPOROGENESIS IN *ARABIDOPSIS THALIANA*

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Sexual reproduction is a salient aspect of plants and elaborate structures, such as the flowers of angiosperms, have evolved that aid in this process. Within the flower the corresponding sex organs, the anther and the ovule, form the male and female sporangia, the pollen sac and the nucellus, respectively. However, despite their central role for sexual reproduction little is known about the mechanisms that control the establishment of these important structures. With the characterisation of the *NOZZLE* (*NZZ*) gene we aim at a better understanding of the molecular and genetic mechanism underlying pattern formation and growth control during floral organogenesis.

The *NZZ* locus excerts various functions during sex organ development. In several *nzz* mutants the nucellus and the pollen sac fail to form. It indicates that *NZZ* plays an early and central role in the development of both types of sporangia and that the mechanisms controlling these processes share a crucial factor. In addition, *NZZ* may have an early function during male and female sporogenesis as well. In the ovule *NZZ* plays a central role in the formation of the nucellus at least in part through antagonizing the activities of two genes, *AINTEGUMENTA* (*ANT*) and *INNER NO OUTER* (*INO*), in the prospective nucellar region of the primordium. The two genes encode putative transcription factors of the AP2 and YABBI class, respectively. We provide evidence that *NZZ* and *BELL* (*BEL1*) share overlapping functions in establishing the central region, the prospective chalaza, of the ovule. *BEL1* encodes a homeodomain protein and thus yet another putative transcription factor. *NZZ* also plays a role in controlling the length of the funiculus again through antagonizing *ANT* and possibly *INO* function. In addition, *NZZ* is required for the development of the integuments. We show that during the initial phase of this process *NZZ* is transcriptionally regulated by *ANT* and *INO*. *NZZ* thus represents an early direct or indirect target of these two genes in the integument development pathway. Finally, our results indicate that *NZZ* plays an important role in the earliest steps in the formation of the sporogeneous lineage during early anther development.

In addition we present the molecular characterization of *NZZ* (Schiefthaler et al, 1999). *NZZ* encodes a putative protein of unknown function. However, based on sequence analysis we speculate that NZZ is a nuclear protein and possibly a transcription factor. The expression pattern of *NZZ* during ovule and anther development correlates with the observed phenotype in *nzz* mutants. However it is expressed in other tissues, such as seedlings, stems, petals or nectaries as well. Loss of *NZZ* function in these tissues does not lead to a detectably altered phenotype.

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CONTROL OF LEAF FORM AND GROWTH: OF SUGARS AND CELLS

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Leaf formation is a fundamental aspect of plant biology which contributes to the generation of the wide variety of plant form observed. Despite the spectrum of leaf form apparent in different plants, it is likely that the basic elements by which leaf formation occurs is conserved between plant species. However, although regulatory elements involved in leaf formation have been identified, the downstream target processes remain unknown. For example, although on purely theoretical grounds it is clear that cell expansion and division must be involved, the relative importance of these cellular processes in determining/limiting leaf morphogenesis and growth has been a matter of some dispute. To address this problem, we have adapted the tetracycline-inducible promoter system to allow us to locally manipulate cell expansion and division processes both within the apical meristem and young leaf primordia. Our results show that local tissue expansion (but not cell division) is sufficient to induce leaf formation, but that local manipulation of cell division is sufficient to alter leaf morphology. Thus, the role of cell division in plant morphogenesis appears to be context-dependent.

To identify molecular processes occurring during the earliest stages of leaf formation, we took a differential display approach. A number of cDNAs encoding key enzymes of carbohydrate metabolism were identified which showed altered transcript patterns during leaf initiation (ADPglucose pyrophosphorylase; SNF1 kinase; sucrose synthase). Furthermore, we could show that the pattern of sucrose synthase mRNA accumulation was sugar-responsive, whereas that of ADPglucose pyrophosphylase was not. These data indicate an unexpectedly complex spatial pattern of carbohydrate metabolism within the meristem which is itself responsive to the sugars supplied to the tissue. The function of local alterations in carbohydrate metabolism in meristem function is being further investigated.

Finally, at the whole organ level, we have investigated the function of the cell wall protein expansin in the regulation of grass leaf extension growth. Our data indicate (surprisingly) that the previously well characterised alpha-expansin proteins are not essential for growth in grass leaves. Indeed, in leaf tissue the expression of alpha expansins is restricted to xylem parenchyma cells destined to form supportive fibres within the vasculature. We postulate that, due to the special molecular architecture of their cell walls, a novel family of beta expansins has evolved to regulate leaf expansion in grasses. Genes encoding these proteins are now being characterised to investigate their potential role in the regulation of leaf growth.

Key Literature:

Cell division, expansion and plant form

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TREHALOSE METABOLISM AFFECTS DEVELOPMENT OF ARABIDOPSIS BY REGULATING THE SUGAR SENSING MECHANISM

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A major question in biology is how external stimuli affect the genes that regulate development of organisms. Plants are ideally suited to address this question since organogenesis, e.g. development of the root system, occurs mainly postembryonically and is strongly dependent on external factors (nutrient status, light, presence of pathogens or symbionts). These external factors are sensed by the plant and transduced into growth and developmental processes, such as e.g. flowering. Our group is interested in identifying the mechanisms how external stimuli are perceived and transduced in the plant in order to understand their impact on developmental programs.

Trehalose is a signal in carbohydrate allocation

The non-reducing disaccharide trehalose is composed of two glucose units. It is in lower organisms such as bacteria, fungi and invertebrates, where it plays a role as carbon source and stress protectant. Although plants generally do not produce detectable levels of trehalose, they are potentially exposed to trehalose by pathogenic or symbiotic micro-organisms. In soybean, key enzymes involved in source-sink relationships, such as sucrose synthase and invertase, are altered in roots of plants that were grown in a high trehalose concentration (Müller et al., 1998). In barley, exogenously applied trehalose is able to induce the activity of 6-SFT from barley, a key enzyme in fructan biosynthesis (Müller et al., 2000). Trehalose, thus appears to be a signal in carbohydrate allocation. Plant-associated micro-organisms might release trehalose in order to influence the carbohydrate flow of the plant to their favor. Trehalase, the enzyme that degrades trehalose is however constitutively expressed and may function as a safeguard that allows the plant to keep control over its sugar sensing system. (Aeschbacher et al., 1999; review: Müller et al., 1999).

Trehalose affects development of Arabidopsis by inducing starch biosynthesis.

Using the Arabidopsis root as a model system, we found that root expansion was strongly inhibited in a concentration dependent manner by exogenously fed trehalose. This effect was drastically pronounced when the trehalase activity was inhibited by the specific inhibitor validamycin A (Fig 1) (Aeschbacher et al., submitted). We observed a strong accumulation of starch in the cotyledons in young seedlings grown on trehalose. In vitro activity of ADP-glucose pyrophosphorylase (AGP), a key enzyme in starch biosynthesis, increased by about 60% in these seedlings (see abstract by Wingler et al.). Interestingly, trehalose specifically induces expression the ApL3 gene, one of the ADP-glucose pyrophosphorylase genes known to encode the large subunit of AGP. Trehalose, thus affects carbohydrate metabolism by changing the expression of an important enzyme in starch biosynthesis.

Trehalose metabolism may be an endogenous metabolite in Arabidopsis.

So far, it was generally believed that higher plants do not produce endogenous trehalose. However, we, and others, recently identified and cloned A. thaliana genes for the two steps of trehalose formation from UDP-glucose and glucose-6-phosphate (Blazquez et al., (1988) Vogel et al., (1998); review: Müller et al., 1999). In order to obtain gene probes also for trehalase, we purified the enzyme from soybean nodules and cloned its cDNA using degenerate primers derived from micro-

sequenced peptides in RT-PCR reactions (Aeschbacher et al., 1999). We also identified and characterized a trehalase homolog from Arabidopsis by functional complementation of a yeast trehalase mutant (Aeschbacher et al,, submitted). The expression of these trehalases is generally low, but constitutive in many tissues.

The identification and cloning of all genes involved in the synthesis and degradation of trehalose from common precursors in higher plants indicates that plants potentially metabolize trehalose endogenously (mini-review by Goddjin and Smeekens, 1998, review: Müller et al., 1999).

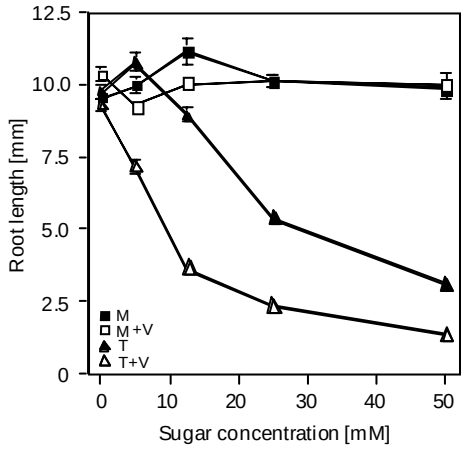
Arabidopsis Mutants resistant to trehalose.

In order to isolate potential trehalose signaling mutants, we performed a mutant screen in Arabidopsis looking for seedlings resistant to the growth inhibitory action of trehalose. Two mutants were identified that had a much longer root on trehalose compared to wild type when grown in the presence of trehalose. Fig. 2 shows such a trehalose resistant T-DNA mutant after 14 days of growth on a trehalose containing medium, compared to a sensitive wild type seedling.

Prospects

We are currently expressing trehalose metabolism genes of Arabidopsis under the control of constitutive and tissue-specific promoters in transgenic Arabidopsis and Medicago plants in order to specifically modulate the endogenous trehalose content. This will allow to analyze the role of trehalose as a developmental signal and the analysis of the interactions of the trehalose signal with other plant signaling systems. Our research may lead to novel insights in plant signaling mechanisms. Ultimately, it may open the way to overproduce trehalose in transgenic crops, which may render them more resistant to stress and especially to drought (review: Müller et al., 1999).

Fig. 1: Root length of Arabidopsis seedlings grown on different concentrations of trehalose (T), or mannitol (M) in the absence, or presence of 10uM of the trehalase inhibitor validamycin A (+ V). Arabidopsis seedlings were axenically grown in tissue culture and root length was measured after 14 days of growth. Mean values and S.E.M. are indicated.



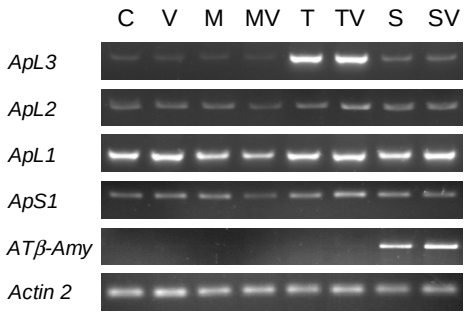


Fig 2: Arabidopsis Wild type (A) and trehalose resistant mutant (B). Both seedlings were axenically grown in tissue culture on media containing 50mM trehalose for 14 days.

Fig 3: RT-PCR of cotyledons of 12 day old Arabidopsis seedlings grown in the light. The following sugars were added at 25mM to the growth medium: C: no sugar (control); V: no sugar plus 10 uM Validamycin A (Val A), M: Mannitol, MV: Mannitol plus Val A, T: Trehalose, TV: Trehalose plus Val A, S: Sucrose, SV: Sucrose plus Val A. The probes were: ADP-glucose pyrophosphorylase genes S1, L1, L2 and L3 (ApS1, ApL1, ApL2, Apl3); beta-amylase (Atß-amy) and actin2 (Actin2: constitutive control).

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NEW TOOLS TO INVESTIGATE PLANT CELL POLARITY PROCESSES

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Cell polarity is the ultimate reflection of complex mechanisms that establish and maintain functionally specialised domain in living cells. These determined domains facilitate processes as diverse as cell growth and differentiation, cytokinesis and pattern formation or vectorial transport of molecules across cell layers. Based on studies in budding yeast and mammalian cells, a common pathway for establishment of cell polarity has been proposed (Drubin and Nelson, 1996). This hierarchical pathway can be described in 3 steps: in response to spatial cues, the first step marks and interprets the cue through receptors and signal transduction networks (Rho GTPase), the second reinforce the cue through a reorganisation of the actin cytoskeleton and establishment of targeting patches and the third step propagates the cue through reorientation of microtubules and secretory pathways. The maintenance of cell polarity is achieved by feedback regulation at each stage.

Cell polarity in plants is poorly understood at the genetic level. So far only 3 mutations have been identified that interfere with cell division orientation; the ton (Traas et al., 1995) and the fass (Torres-Ruiz and Jürgens, 1994) mutants of Arabidopsis and the tan-1 mutant in maize (Cleary and Smith, 1998; Smith et al., 1996). Both the ton and the tan-1 genes have been cloned but sequences are not yet available to the scientific community. Interestingly, phenotypic analysis of these mutants revealed that the mutation is associated with alteration of the cytoskeleton but also indicates that improper orientation of cell division does not impair morphogenesis.

We have chosen to develop a model genetic system to study plant cell polarity in the moss Physcomitrella patens, since (1) this moss is the only plant so far where targeted disruption and allele replacement of endogenous genes is efficient, (2) cell polarisation can be initially investigated at the single cell level during polarotropic division of protoplasts, and its role in further development can be studied during unidimensional apical growth in the protonema and 3-dimensional caulinary growth in the leafy shoot, (3) microtubule preprophase bands that mark the future position of the phragmoplast are absent in the protonema but present in the leafy shoot: this may facilitate studies of the respective role of microfilaments and microtubules in the orientation of cell division. Thus we focus our studies on the genetic control of the behaviour of the cytoskeleton during cytokinesis with special emphasis on actin dynamic.

Furthermore we have decided to base our search for moss genes involved in polarised cell division on the genetic knowledge available in fission yeast (Gould and Simanis, 1997). This strategy is based on the following observations: (1) in fission yeast and mosses, the position of the future division plate is determined by the position of the nucleus, indicating that the initial cue for cell polarisation in cytokinesis is probably of nuclear origin, and (2) conditional expression of Arabidopsis cDNAs in fission yeast has identified plant cytoskeletal, cell cycle-related and polarity-related plant genes (Xia et al., 1996).

Two different approaches were used to identify moss genes involved in cellular polarity. The first one can be considered as a global genomic strategy. The 20 genes of fission yeast described in the review from Gould and Simanis (Gould and Simanis, 1997) were compared with the plant sequences available in Genbank. Significant homologies with plant genes were found for the 9 genes listed in Table 1, and genes involved in actin dynamic were chosen to screen a moss cDNA library using the corresponding plant sequences amplified by PCR as heterologous probes.

Results and prospective of this part of the work are described on the poster from P.-F. Perroud et al. (D5). The second approach, which is similar to that performed by Xia et al. (1996), is based on a functional dominant interacting screen of moss cDNAs conditionally expressed in fission yeast. The overall strategy and preliminary results are described on the poster of P.-F Perroud et al. (D5).
To validate this approach we have cloned the Arabidopsis ton-1b cDNA in the thiamine repressible vector pREP 3 and transformed fission yeast strain leu 1-32 (in collaboration with D. Bouchez, INRA Versailles and V. Simanis, ISREC, Lausanne). Overexpression of At ton-1b in yeast induces a dramatic phenotype characterised by asymmetric division, errors in nuclear placement, and considerable loss of cell polarity. This phenotype is not reminiscent of previously observed phenotype in fission yeast mutants and strongly validate such type of approach. Preliminary attempts to isolate the corresponding moss homologue using the Arabidopsis cDNA as heterologous probe or by PCR using degenerate primers designed on conserved sequences of TON homologues were not successful. Recently, and antibody against the Arabidopsis protein was obtained in Versailles and used to probe moss protein extracts. The antibody detects a single band in moss extracts and we hope to be able to isolate the corresponding moss gene by screening a moss expression library with this antibody.
The comparison of fission yeast cell polarity genes with the plant sequence database has allowed the identification of several known and new plant genes possibly involved in cell polarity. Among those, genes encoding proteins of the Arp2/3 complex were identified and the first plant cDNA sequence encoding arp-3 has been isolated. The functional dominant interacting screen performed following fission yeast transformation with a moss cDNA library has identified several moss genes interfering with fission yeast polarity processes among which one of the first rab-GAP plant cDNA sequence. Such approach was further validated by the observation that overexpression of one of the 2 plant cell polarity genes isolated to date, the ton-1b gene of Arabidopsis, induces a dramatic loss of cell polarity in fission yeast. Thus the use of the genetic knowledge available in yeast to identify plant genes involved in cell polarity processes provides an extremely efficient new tool.

Table 1

Plant genes showing significant homology with fission yeast genes involved in septum formation (NCBI blast).

Yeast gene	Access. #	Gene product	Blast score	Plant gene	Access. #
act-1	Y00447	Actin	688	A.thal. actin	U37281
cdc-3	Z30648	Profilin	1127	A.thal. profilin	S82691
cdc-4	L42454	EF hand protein	290	Petunia cam53	M80831
myo-2	U75357	Myosin II	540	A.thal. gDNA	AC002334
arp-3	M81068	Actin related protein 3	238	A.thal. gDNA	AC007537
sop-2	Y08998	Arp2/3 complex	224	A.thal. gDNA	AC006593
nuc-2	X07693	APC component	273	A.thal. gDNA	AC006081
ppb-1	D28955	Calcineurin Pase	211	A.thal. Ser-Threo Pase	P48485
tea-1	Y12709	TEA1p	395	Zea mays cDNA	AI668113

Acknowledgement

This work is supported by the Swiss National Science Foundation grant # 31-51853.97

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BIOCHEMICAL CHARACTERIZATION OF TOBAMOVIRUS MOVEMENT PROTEIN-MICROTUBULE INTERACTIONS

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The movement protein (MP) of tobacco mosaic virus (TMV) is essential for intercellular spread of the viral genome during plant infection. Consistent with this function, MP has been demonstrated to target and increase the size exclusion limit (SEL) of plasmodesmata (Pd) in MP-transgenic and TMV-infected plants. In addition, MP binds single-stranded nucleic acids *in vitro* and forms unfolded, elongated ribonucleoprotein (RNP) complexes with viral RNA (vRNA), which are presumably compatible in size and shape with the modified Pd. It is therefore postulated that viral movement is facilitated by MP-dependent modification of Pd, followed by trans-plasmodesmal trafficking of the viral-RNP (vRNP).

Transfection of *Nicotiana benthamiana* leaf epidermal cells or BY-2 protoplasts with virus containing MP fused to green fluorescent protein (GFP) has shown that, during early infection, MP accumulates in ER membrane-associated "inclusion bodies" which are putative sites of virus replication. However, later during infection fluorescent MP:GFP appears to be predominately co-aligned with microtubules (MTs) (figure 1). This redistribution of MP during virus infection has led to the hypothesis that MP-MT interactions may play an important functional role in the intracellular trafficking of vRNPs from their site of synthesis to Pd.

In support of this hypothesis, we have a number of MP-mutagenized viral constructs that correlate a deficiency in viral movement to an absence of MP accumulation on MTs. In addition, we have temperature-sensitive mutants that exhibit similar MP-subcellular distribution and viral-movement characteristics to wild type virus at 22 ^{o}C. However, when shifted to 32 ^{o}C, these mutants are dramatically inhibited in their ability to move from cell-to-cell, which also appears to directly correlate with an absence of MT-associated MP.

MP-MT complexes from TMV (MP:GFP)-infected BY-2 cells can be easily co-isolated and are found to be stabilized against disruption by cold/Ca^{2+}, and high salt treatments. Furthermore, in MP-transfected animal cells, it was established that MTs were highly resistant against cold temperature and MT depolymerizing agents such as nocodazole and vinblastine, suggesting that MP may act in a manner distinct from the 'classical' MAPs such as neuronal MAP2 and Tau.

By biochemical analysis of MP-MT complexes formed both *in vivo* and *in vitro*, we are investigating the molecular mechanisms governing intracellular vRNP trafficking. We are particularly interested in determining if host components other than tubulin contribute to the MP-MT interaction, and whether vRNA itself acts as a regulatory-determinant of MP-MT complex formation. The possibility that MP may mimic certain domains of tubulin, as predicted by multiple alignment data, is also under investigation.

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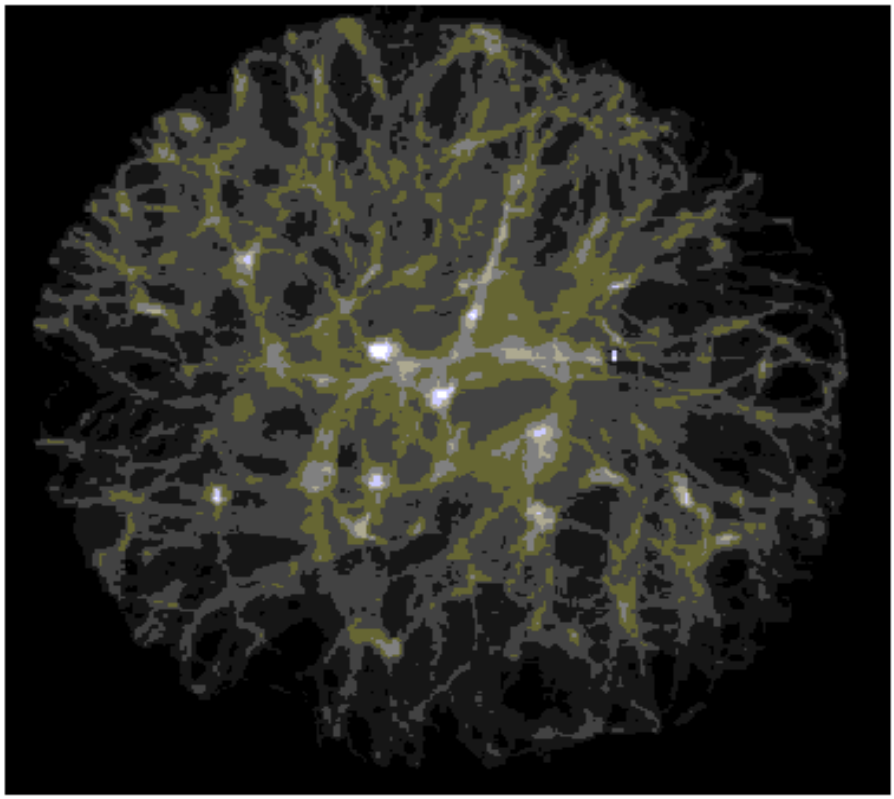

Figure 1. Tobacco BY-2 protoplast infected with TMV (MP:GFP). Filamentous structures are MP in co-alignment with microtubules.

CHARACTERIZATION OF TRANSGENIC *ARABIDOPSIS* EXPRESSING A FUNGAL CUTINASE

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A major structural component of the cuticle of plants is cutin. The functional analysis of cutin *in vivo* has been limited because no mutants with specific defects in cutin have been characterized. Therefore, transgenic *Arabidopsis* that express and secrete a cutinase from *Fusarium solani* f. sp. *pisi* were generated (Figure 1). Arabidopsis expressing the cutinase in the extracellular space show an altered

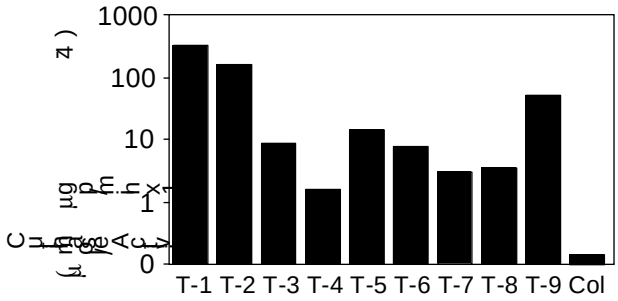

Figure 1. Cutinase activity of transgenic *Arabidopsis* expressing a fungal cutinase (lines 1-9).

ultrastructure of the cuticle and an enhanced permeability of the cuticle to solutes (Figure 2). In addition, pollen germinate on fully differentiated leaves of cutinase-expressing plants, whereas they do not on control leaves. These differences coincide with strong postgenital organ fusions (Figure 3). In leaves, the cell walls of the fused epidermal cell layers come often into direct contact and only remnents of the cuticle

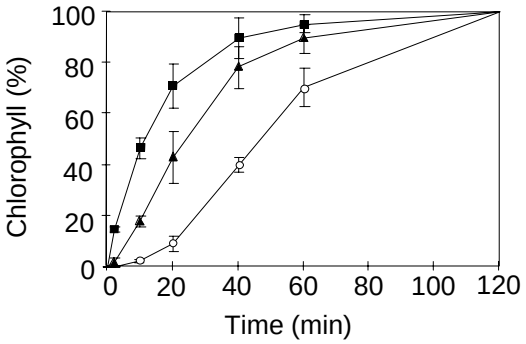

Figure 2. Chlorophyll release from submerged leaves of cutinase-expressing transgenic *Arabidopsis* (squares, triangles: lines T-2 and T-5, respectively, circles: control plants).

Figure 3. Organ fusion between a sepal (s) and a cauline (c) leaf of cutinase-expressing *Arabidopsis*.

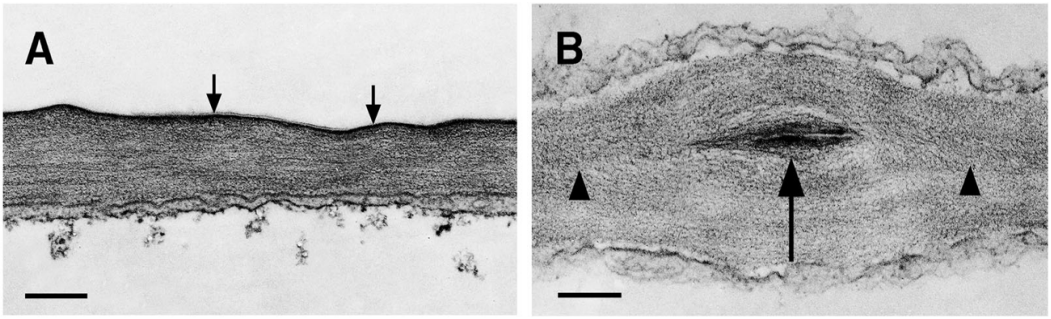

Figure 4. Structure of the extracellular matrix of the epidermis of leaves.

(A) In wild type plants, the cell wall of leaf epidermal cells is covered with a cuticle (arrows).

(B) In cutinase-expressing plants, the epidermal cell layers fuse via direct contacts in the cell wall (arrow heads) and the cuticle is only present as remnants (arrow). are found (Figure 4).The cell walls of fusion zones are rich in pectic polysaccharides. As fused organs grow apart from each other, organ deformations and protrusions composed of epidermal cells develop at positions with high mechanical stress. These results demonstrate that an intact cutin layer is not only important for plant/environment interactions, but also prevents fusions between different plant organs and is therefore necessary for normal epidermal differentiation and organ formation.

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Poster D1

THE EFFECT OF TREHALOSE ON STARCH SYNTHESIS IN *ARABIDOPSIS* - IMPLICATIONS FOR THE RESPONSE OF PLANTS TO TREHALOSE-PRODUCING MICRO-ORGANISMS

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Introduction

The disaccharide trehalose (α-D-glucopyranosyl-[1,1]-α-D-glucopyranoside) has been shown to affect plant carbohydrate metabolism, e.g. by inducing enzymes of fructan biosynthesis in barley (Wagner et al., 1986; Müller et al., 2000) or sucrose synthase in soybean (Müller et al., 1998). Since trehalose is a common sugar in micro-organisms, including many plant pathogens and symbionts, plants are probably regularly exposed to trehalose of microbial origin. In addition, genes encoding enzymes of trehalose synthesis have been found in *Arabidopsis* (Vogel et al., 1998; Blázquez et al., 1998), suggesting that plants might also produce trehalose. Here, we tested how *Arabidopsis* reacts to exogenously supplied trehalose and to a trehalose-producing pathogen.

Results and Discussion

The presence of 25 mM trehalose inhibited the elongation of *Arabidopsis* roots by about two-thirds, compared to seedlings grown without external sugar or on 25 mM mannitol, and caused the accumulation of starch in the shoots (Fig. 1). Since addition of 25 mM sucrose or glucose restored root growth in the presence of trehalose, the inhibitory effect of trehalose was probably due to a starvation of the roots. Starch also accumulated when the seedlings were grown in the presence of trehalose plus sucrose, showing that this effect was not caused by a retardation of growth. To study if trehalose directly enhanced starch formation, we measured the activity of ADP-glucose pyrophosphorylase (AGPase), the first enzyme of starch synthesis. AGPase activity was significantly increased after growth in the presence of 25 mM trehalose (Fig. 2). This increase was accompanied by a specific induction of the expression of the AGPase gene, *ApL3* (see abstract by R. Aeschbacher et al.).

We became interested to find out if some of the known effects of plant-associated micro-organisms on plant development and metabolism are due to the release of microbial trehalose into the plant. To study this, we chose clubroot, a disease of crucifers caused by the pathogen *Plasmodiophora brassicae*. One of the symptoms of clubroot is an accumulation of starch in the hypocotyls (Williams et al., 1968). We infected *Arabidopsis* plants with *P. brassicae*, and found that trehalose accumulated in the roots (2.4 ± 0.8 mg g^{-1} DW), whereas it was hardly detectable in the leaves. We do not know yet whether or not trehalose is released by the pathogen. However, we found that the trehalose-cleaving enzyme trehalase was strongly induced in infected roots (Fig. 3). This might be a response of the plant to the release of trehalose by the pathogen, as a safeguard to prevent excess accumulation of

the sugar and its transport from the roots to the leaves. Alternatively, the trehalase in the infected roots might be produced by the pathogen. We will use expression analysis by RT-PCR to assess the origin of trehalase in the infected roots. We will also study whether the accumulation of starch in the hypocotyl of *P. brassicae* is due to an induction of *ApL3* expression.

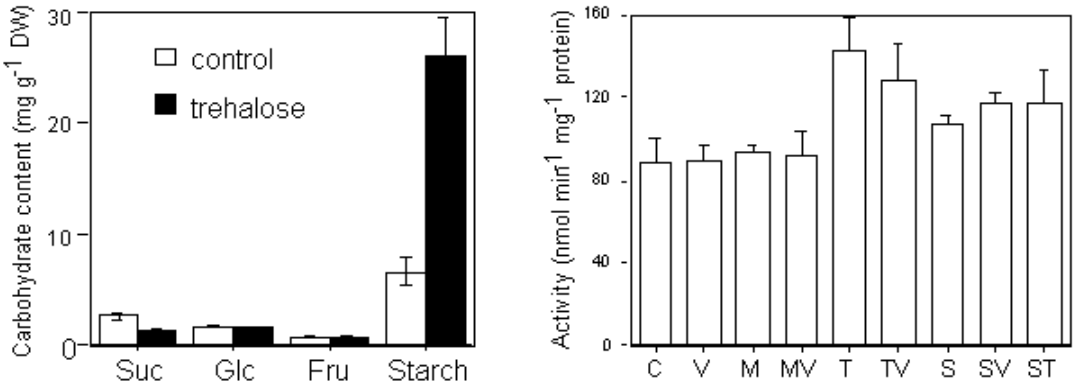

Fig. 1. Contents of sucrose (Suc), glucose (Glc), fructose (Fru) and starch in total shoots of *Arabidopsis* seedlings grown for10 d on half-strength MS-medium without (control) or with addition of 25 mM trehalose. Data are means ± SE of seedlings harvested from three different agar plates.

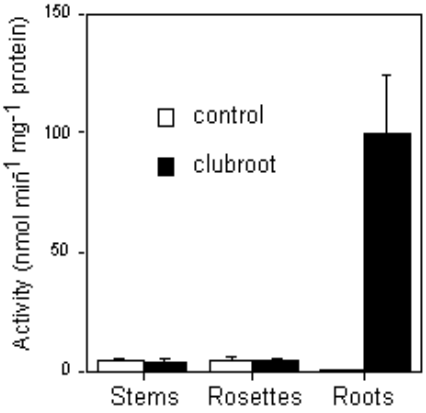

Fig. 2. Activity of ADP-glucose pyrophosphorylase in total shoots of *Arabidopsis* seedlings grown for 10 d on half-strength MS-medium (C) supplemented with 10 µM of the trehalase inhibitor validamycin A (V), 25 mM mannitol (M), 25 mM trehalose (T), 25 mM sucrose (S) or combinations thereof. Data are means ± SE of seedlings harvested from 3-4 different agar plates.

Fig. 3. Activity of trehalase in healthy *Arabidopsis* plants (control) and plants infected with *P. brassicae* (clubroot) 23 d after inoculation. Data are means ± SE of 3-4 plants.

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Poster D2

THE GLYCINE-RICH STRUCTURAL PROTEIN GRP1.8 OF BEAN SHOWS HYDROPHOBIC INTERACTIONS IN THE CELL WALL

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Glycine-rich proteins (GRPs) represent a major class of structural proteins and are localized in the plant cell wall. GRP1.8, a glycine-rich protein of bean (*Phaseolus vulgaris*), consists mainly of the repeat $(G\text{-}X)_n$, where X is often a glycine. GRP1.8 has been shown to be expressed in the vascular tissue and is an abundant protein in the modified primary cell wall of protoxylem elements. Protoxylem elements are the first xylem elements developed by a plant and are functional during the elongation growth of the plant. In this study, we have investigated the interaction of GRP1.8 in the cell wall. Different domains of GRP1.8 were fused to a reporter protein and the fusion proteins were expressed in the vascular tissue of transformed tobacco (Fig. 1). A chitinase of cucumber, which shows little interaction in the cell wall matrix, was used as the reporter protein. Extraction experiments indicate that the chitinase/GRP1.8 fusion proteins show stronger interaction in the extracellular matrix than the chitinase alone. A low-salt buffer (50 mM Na-citrate, pH5.5) was sufficient for complete extraction of the chitinase. In contrast, fusion protein could be extracted with Na-citrate, 1% SDS even after washing with Na-citrate (Fig. 2). Further experiments showed that the fusion proteins show hydrophobic interactions in the extracellular matrix. A detergent (Na-citrate, Triton X-100) but not a strong salt ($CaCl_2$) was able to extract additional fusion protein (Fig. 3). Similar experiments were done to analyze the interaction of endogenous GRP1.8 in the extracellular matrix of bean hypocotyl. The results confirmed the hydrophobic property of GRP1.8 found with the reporter protein system in transgenic tobacco (data not shown).

These results reveal a so far unknown characteristic of GRP1.8 and possibly of other glycine-rich structural proteins. Structural proteins were so far thought to form ionic interactions in the cell wall. The hydrophobic property of GRP1.8 and its abundance in the modified primary cell wall of protoxylem suggest a function of the protein in a repair mechanism of the protoxylem to strengthen the vessel cell wall that are weakened through the passive stretching during elongation growth of the plant.

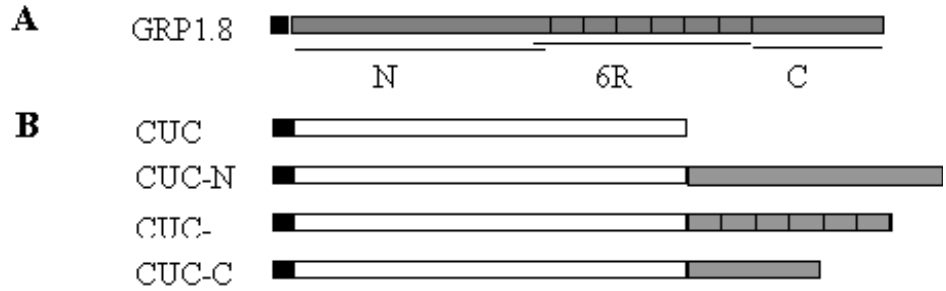

Fig. 1 Different constructs used for tobacco transformation

(A) Scheme of GRP1.8 and the three domains used for the chitinase/GRP1.8 fusion proteins. N-terminus: N; repetitive middle domain: 6R; C-terminus: C.

(B) Chitinase and chitinase/GRP1.8 fusion protein constructs used for *N. tabaccum* transformation. Chitinase: CUC; : signal peptide

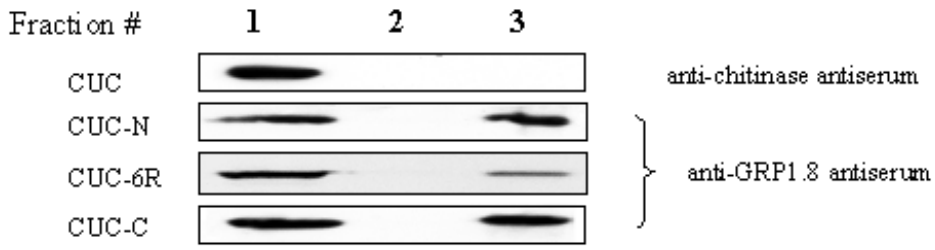

Fig. 2 The different proteins expressed in tobacco show increased interaction in the cell wall.

Ground stem material of the different transgenic lines was extracted with Na-citrate (Fraction # 1), the cell wall fraction was washed with Na-citirate and an aliquot of the last washing kept to check complete removal of proteins soluble in Na-citrate (Fraction # 2). The last extraction was done with Na-citrate, 1% SDS (Fraction # 3).

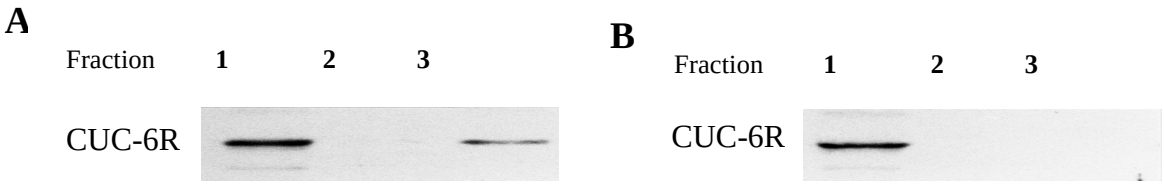

Fig. 3 The different proteins expressed in tobacco show hydrophobic interaction in the cell wall.

Ground stem material of the lines CUC-6R was extracted with Na-citrate (Fraction # 1), washed with Na-citirate and an aliquot of the last washing was kept to check complete removal of proteins soluble in Na-citrate (Fraction # 2). The last extraction was done with (**A**) Na-citrate, Triton X-100 (Fraction # 3) or (**B**) 500 mM $CaCl_2$.

The different fusion proteins gave the same results; only CUC-6R is shown.

Poster D3

CONTROL OF PHYLLOTAXIS

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Leaf primordia develop in regular patterns from the shoot apical meristem. In the majority of flowering plants the leaves are arranged in spirals with the divergence angle between successive leaves approaching the Fibonacci angle of 137.5°, the golden ratio. Although a number of early studies indicate that auxin can influence development of the shoot apex, its specific role in organ initiation and positioning has not been determined. Auxin is thought to be produced in young leaves and transported downwards to the maturing stem and to the roots by a polar transport system, that can be blocked with specific inhibitors. To assess the role of auxin in the regulation of phyllotaxis we micromanipulated auxin levels by interfering with auxin transport, and by local application of auxin to the meristem. Our results show that auxin controls the initiation and radial positioning of leaf primordia in tomato. A comparable role for auxin in the positioning of floral primordia is inferred from experiments with the auxin transport mutant *pin-formed1-1* of *Arabidopsis*. We propose that polar transport of auxin to the meristem determines the radial position and the size of lateral organs, but not the apical-basal position nor the identity of the induced structures.

Poster D4

INTERCELLULAR COMMUNICATION THROUGH PLASMODESMATA

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Coordination and regulation of growth and development in higher multicellular organisms is dependent, in part, on the controlled short- and long-distance transport of signaling molecules. Specifically, in plants, cell fate decisions rely on positional information rather than on cell lineage. The mechanisms and the nature of the signals exchanged beween cells and tissues are largely unknown. However, several studies indicate that one means of cell-to-cell communication is provided by trans-wall co-axial membranous tunnels termed plasmodesmata (Pd). By connecting the cytoplasm of neighboring cells, Pd integrate cells symplastically and provide a direct pathway for diffusion or transport of informational molecules.

Until recently, Pd were considered to be non-selective pores allowing the passive movement of molecules less that 1 kDa. However, the discovery that plant viruses as well as endogenous proteins, mRNAs, and silencing signals proposed to be nucleic acids utilize Pd for cell-to-cell movement provides compelling evidence that Pd channels are highly dynamic structures modulating their pore size to support cell-to-cell transport of these macromolecules. In fact, the Pd system is believed to form a regulated communication network throughout the plant, providing supracellular control to orchestrate plant development and defense responses against pathogens. The goal of our research is to use viruses as cellular probes to identify and to functionally characterize cellular components and factors involved in the targeting of informational macromolecules to Pd.

The cell-to-cell movement of viruses through Pd requires specific virus-encoded movement proteins (MP) and the MP of tobacco mosaic virus (TMV) has been most throughly analysed. In infected as well as in transgenic tobacco plants, the MP accumulates in Pd and alters their size exclusion limit. It also binds single-stranded nucleic acids in vitro, resulting in unfolded and elongated protein-nucleic acid complexes. This observation led to the hypothesis that the virus moves from cell-to-cell in the form of a ribonucleoprotein complex (vRNP) compatible in size and structure with the modified Pd.

Since the MP mediates the interaction between the virus and the host cell for intercellular spread, this protein can be used as a probe to identify host cell components involved in Pd targeting and intercellular communication. A TMV derivative modified to encode the MP as a fusion with green fluorescent protein (GFP) and in planta fluorescence techniques led to the identification of host structures in which the MP accumulates during infection and which may be involved in virus replication, Pd targeting and intercellular spread. These structures include the endoplasmic reticulum (ER) and microtubules (MT). Our main goal is to determine the functional significance of the MP:MT interaction and to identify other host factors that are involved in this complex.

To determine the functional significance of intracellular localization sites of MP in intra-and intercellular trafficking of vRNA, we have introduced deletion and amino acid exchange mutations into the MP fused to GFP. We found several mutations in the MP affecting both the virus transport function of the protein as well as the ability of the protein to associate with MT. Some of the mutations have a temperature-sensitive phenotype, i.e. they allow MT interaction of the MP:GFP and virus spread in leaves at permissive (22°C) but not at non-permissive temperatures (32°C). Whereas the MP targets Pd without interacting with microtubules, intercellular trafficking of vRNA requires the association of MP with microtubules as well as with Pd. We also identified domains in the MP that are functionally required for Pd targeting and MT association. Evidence from our laboratory that MP contains sequences similar to tubulin:tubulin assembly surfaces suggests that the protein may co-assemble with tubulin. In fact, MP:MT complexes formed in plant cells or upon transfection in animal cells are very stable and resistant against salt, cold temperature and microtubule-disrupting agents. We also have modified virus RNA for visualization *in planta*. Although we are able to detect replication sites so far we are unable to detect association of vRNA with microtubules.

We are using several approaches to identify genes encoding proteins interacting with MP. One approach is affinity isolation of host factors interacting with MP. Our aim is to isolate MP:MT complexes from in fected cells and to analyse their composition by mass spectroscopy. We are also using the yeast two-hybrid system and a first screen of an *Arabidopsis* cDNA library led to the identification of several possible candidate proteins. Finally, we have initiated the screening of *Arabidopsis* EMS mutants to detect mutants that are affected in genes required for cell-to-cell movement of viral RNA.

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Poster D5

USING FISSION YEAST TO IDENTIFY PLANT CELL POLARITY GENES

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Cell polarity organization during cell cycle is capital for growth regulation in living organisms. Different works on budding yeast (for review see Pruyne and Bretscher a and b) and fission yeast (for review see Gould and Simanis 1997) have permitted to define specific actors implicated in the coordination and the regulation of cytoskeleton structural elements like actin during this process. In plants, in spite of the description of mutants specifically impaired in cell polarity during mitosis (e.g. Trass et al. 1995), the genetic knowledge remains very poor.

The aim of this work is to identify genes implicated in cell polarity organization in *Physcomitrella patens* using molecular knowledge and functional opportunities that offers to us *Schizosaccharomyces pombe*, the fission yeast.

The first approach used is heterologous screening of moss phage library (Fig 1). 20 genes involved in the control of septation in fission yeast (Gould and Simanis 1997) have been compared to plant gene and ESTs databases. *Myo*2 (myosin 2), *arp*3 (actin related protein), *sop*2 (a component of the Arp2/3 complex) and *pro* (profilin) have been selected for high sequence homology between yeast and plant. Arabidopsis sequences of this genes were then use as heterologous probes to screen a moss cDNA library. Until now, this strategy permitted us to isolate a complete clone of *pro* (450 bp) and, for the first time at the level of cDNA in plant, a partial clone of *arp*3 (600 bp).

The second approach used to isolate genes implicated in cell polarity processes is based on a functional screen of fission yeast overexpressing moss cDNA (Xia et al. 1996) (Figure 1). After transformation of yeast strains with a moss cDNA library, phenotypic screening is possible based on characters as differential phloxin accumulation, a marker of cell viability, morphological changes during division as well as specific staining of cellular elements (DAPI for nucleus, calcofluor for parietal compounds).

In a first time, we have transformed fission yeast strain leu 1-32 with a moss conditional expression cDNA library. From 100 000 transformed colonies, screening on characters described above permitted to identify 4 clones with a phenotype in relation with alteration of cell polarity. The corresponding moss cDNA responsible for these alterations were isolated and characterized (homologues to a GST (partial), to a cDNAs of unknown function(partial), to a rab-GAP (full length) and to a gene induced upon nodulation in Medicago (full length).

To obtain more specifically genes altering cell polarity, we have transformed two thermo-sensible mutants affected in cell polarity, cdc4-8 and myo2. 30000 transformants of cdc4-8 and 10000 of myo2 have been screened at permissive temperature. Clones which presented modified polarity phenotypes have been isolated and preliminary results will be presented.

Acknowledgement

This work is supported by the Swiss National Science Foundation grant # 31-51853.97

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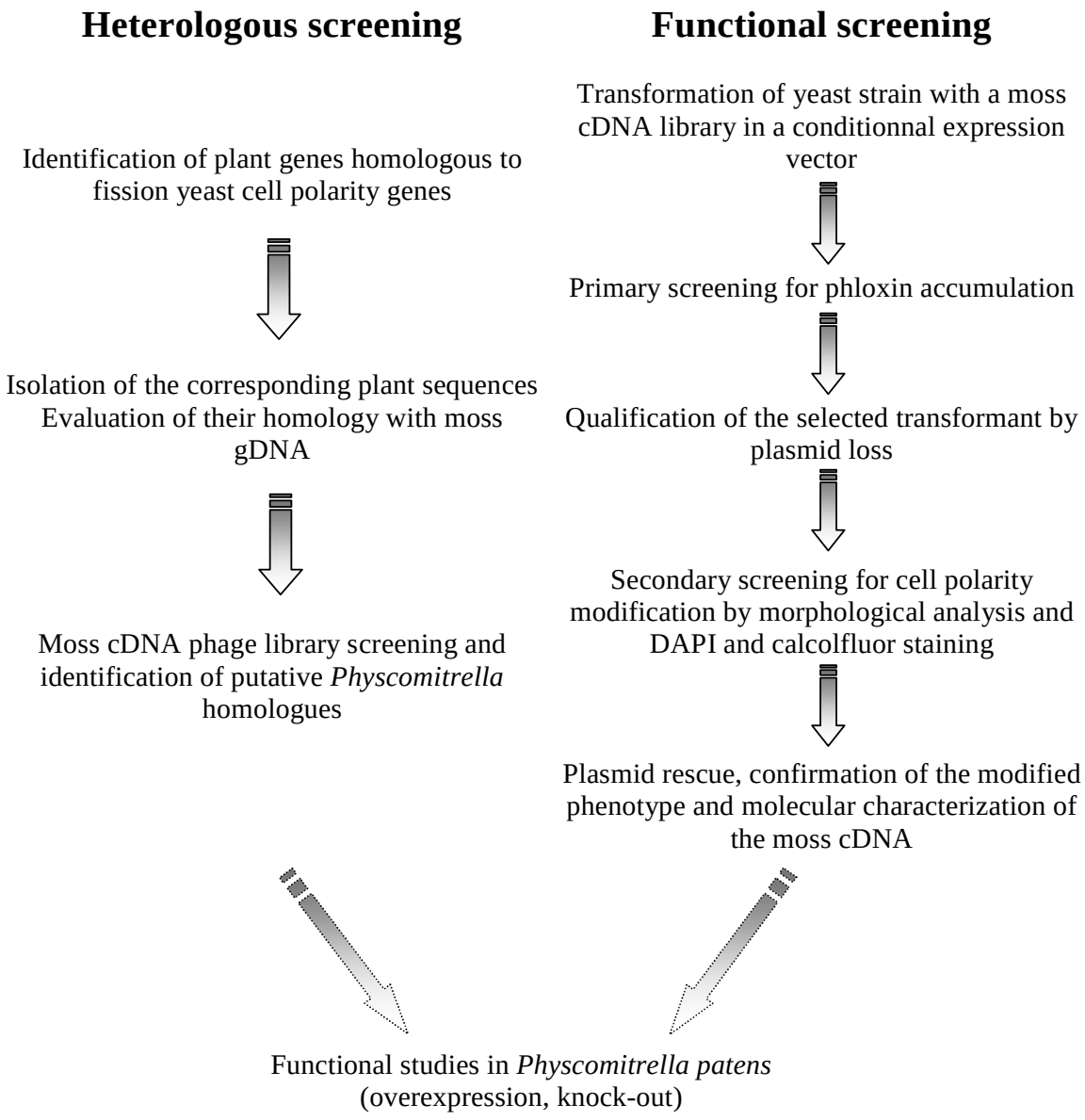

Figure 1: General pathway to isolate genes implicated in cell polarity in *Physcomitrella patens* using the fission *Schizosaccharomyces pombe*

Poster D6

IN VIVO OBSERVATION OF THE ACTIN NETWORK IN THE MOSS *PHYSCOMITRELLA PATENS*

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According to the general model proposed by Drubin and Nelson (Drubin and Nelson, 1996), the actin network plays a central role in the establishment and maintenance of cellular polarity in eukaryotes. It reinforces the spatial cue perceived by receptors and interpreted by Rho GTPase and promotes its further propagation to microtubules and secretory pathways. Therefore, we have decided to focus our studies on actin dynamic in our project to develop a genetic model system in *Physcomitrella patens* to investigate plant cell polarity processes.

In yeast, the actin cytoskeleton is organised in at least 4 distinct structures: cortical patches that are discrete cytoskeletal bodies, cortical cables formed by long bundles of F-actin filaments, the cap that consists of a polarised accumulation of cytoskeletal and regulatory protein at the site of growth and the cytokinetic ring (Pruyne and Bretscher, 2000). In plant cells, the following actin structures have been described: a net of F-actin around the nucleus, an extensive network of cortical cables, a cap-like structure associated with tip growth in root hairs and pollen tubes and single F-actin filaments associated with microtubules (de Ruijter and Emons, 1999). At the functional level, actin is involved in several polarity-related processes such as tip growth, mitosis and cytokinesis.

The actin network in moss protonema has been studied by rhodamin phalloidin staining (Doonan and Duckett, 1988). Cortical F-actin bundles and accumulation of actin at the growing tip of filamentous cells was detected in *Physcomitrella patens* and the involvement of F-actin in tropic responses of caulonema cells of *Ceratodon purpureus* was reported (Meske and Hartmann, 1995; Meske *et al*., 1996). Yet a complete description of the actin network in moss cells is still missing and this is probably due to the difficulties to maintain actin structures intact during cell fixation before staining. The recent development of a GFP fused to the F-actin binding domain of the mouse talin protein provides a reliable marker for *in vivo* studies of the actin microfilaments (Kost *et al*., 1998). We have transformed *Physcomitrella* with a 35S-GFP-talin cassette and report here a preliminary *in vivo* characterisation of the moss actin network.

In protonemal cells observed in confocal microscopy, GFP brightly labels a cortical branched network of actin bundles. These cables are aligned parallel to the axis of the cells and preliminary observation suggests that this network may be more dense in chloronema than in caulonema. Accumulation of GFP was also detected on both sides of the cell wall separating 2 adjacent filamentous cells as well as at the tip of actively growing apical cells, confirming thus previous observation suggesting that actin is actively involved in tip growth. A weaker fluorescence was also detected that surrounds the nucleus of each cell, indicating that F-actin forms a net around the nucleus.

Finally, brightly labelled cortical star-like structures connected with actin cables were observed in many cells. To our knowledge, such structures have not yet been described and we hypothesised that they could correspond to microfilaments organising centres.

If this is true, these centres could correspond to the site where actin polymerisation and bundle formation occur. The fact that such structures were not previously observed could indicate that they are very labile and are destroyed upon fixation prior to rhodamin phalloidin staining. The same features were observed in protoplasts and in leaf cells but surprisingly not in buds where GFP labelling is very weak and seems to colocalise with chloroplasts. The absence of GFP labelled F-actin in young buds may indicate that either a moss talin-like protein is highly expressed at this developmental stage, thus preventing GFP-talin to bind to F-actin, or that classical F-actin structures are almost absent from buds: this needs to be further investigated.

The use of GFP-talin provides an outstanding tool to visualise F-actin *in vivo* in moss cells with an accuracy that is not possible to reach with standard fixation and coloration techniques. The data presented here are extremely preliminary and deserve further studies. Time lapse video microscopy will be performed to describe the dynamic of F-actin structures during cell growth and cell division. Experiments with specific inhibitors of microfilaments and microtubules will also be conducted to analyse the effect of these drugs on the observed structures. We will also investigate the behaviour of F-actin during polarotropic division of protoplasts to try to establish the role of F-actin in the establishment of cell polarity. Finally we intend to use these strains to perform gene disruption experiments of the putative polarity genes isolated in the other approaches conducted in this project (see abstract D5) to be able to directly monitor the effect of loss of function of these genes on the F-actin cytoskeleton.

Nota bene

The front page of the abstract book represents moss protonema cells labelled with GFP-talin and observed by confocal microscopy.

Acknowledgement

This work is supported by the Swiss National Science Foundation grant # 31-51853.97

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Poster D7

ETHYLENE PRODUCTION IN *SOLANACEAE*: POTATO ONE JUMP AHEAD OF TOMATO!

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Terrestrial plants respond to flooding with enhanced ethylene production. The ethylene produced by flooded plants causes epinasty and is interpreted as an early warning of deteriorating soil conditions, inducing changes above ground to increase stress tolerance (1).

The roots of flooded plants produce 1-aminocyclopropane-1-carboxylic acid (ACC) via the key enzyme of ethylene synthesis, ACC synthase. ACC is transported from the root to the shoot where it is converted to ethylene by ACC oxidase (Fig. 1). The system is a good example for the spatial separation of a biosynthetic pathway and the best example of root to shoot communication involving a plant hormone (1). Flooding-induced ethylene is of agricultural importance, for instance in cotton where it causes losses due to premature abscission of cotton bowls after waterlogging.In the roots, ACC is synthesized by ACC synthase (ACS) which is encoded by a gene family of nine members in tomato. Six ACS genes were mapped to diverse locations on the tomato genome. Three of them, *Le-ACS*2, *Le-ACS*3 and *Le-ACS*7 are involved in the flooding response, with *Le-ACS*7 expressed early on (Fig. 2). The ACS genes involved in flooding appear to form a gene expression cascade. An ACS gene expression cascade is observed in the fruit where ACC produced by Le-ACS6 (3) leads to a low level ethylene production that kickstarts the ethylene-inducible ACS genes *Le-ACS*2 and *Le-ACS*4 and triggers subsequent autocatalytic ethylene production during the climacterium (2, 4).

Figure 1: Response of terrestrial plants to flooding.

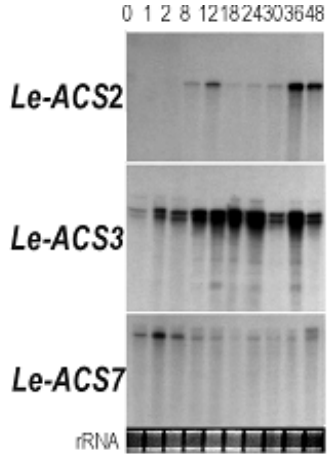
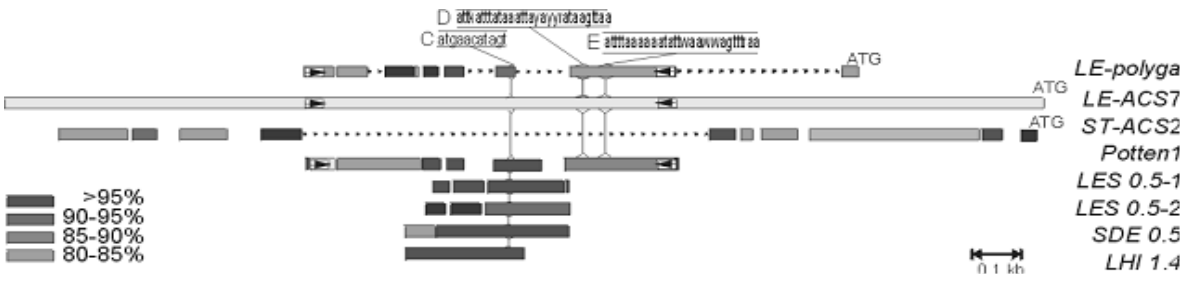


Figure 2: Time course analysis of ACS transcripts expressed in flooded tomato roots. roots.

In the tomato, *Lycopersicon esculentum*, the promoter of the early flooding-induced ACS gene, *Le-ACS*7, is tagged by a nonautonomous transposable element (Fig.3) (5). This transposon belongs to



the Sol3 class (6), a class restricted to the *Solanaceae* with highly conserved terminal inverted repeats (TIR) and approximately 50-100 copies in potato and tomato. A *Sol3* transposon is also present in the potato solanidine glucosyltransferase promoter (Belknap, pers. comm.) and the tomato polygalacturonase promoter to which it conferred regulatory elements (6, 7). Thus, *Sol3* transposons may affect the regulation of *LE-ACS7*, and ultimately, root to shoot communication of flooded tomato plants. The promoter of a member of the ACS gene family in the potato, *Solanum tuberosum*, *St-ACS2*, exhibits high sequence identity to *Le-ACS*7 (Fig. 3). However, the *St-ACS*2 promo-ter lacks the Sol3 transposon. Since the identity of these ACS promoters is unusual and since the genomes of tomato and potato (1n= 12) are syn-tenic the hypothesis was tested that *St-ACS*2 and *Le-ACS*7 are orthologs. RFLP analysis of *St-ACS*2 revealed that this gene resides on the top of potato chromosome 2, at the location corresponding to *Le-ACS*7 in the to-mato (Fig 4). This demonstrated that *St-ACS*2 and *Le-ACS*7 are orthologous genes. PCR analysis with an amplimer distal to the inverted repeats and complementary to the promoters of both, *Le-ACS*7 and *St-ACS*2 was used to test promoters of ACC synthase genes for the presence of the transposon (Fig. 5). The extensive know-ledge of the phylogeny of the *Solanaceae* al-lows one investigate if the Sol3 element was present in the promoter of the ancestral ACS genes and excised from *St-ACS2*, or if it tagged *Le-ACS*7 after the potato and tomato separated into distinct and uncrossable genera. Results on the phylogeny of this transposition event will be presented that suggest that the potato is one jump ahead of the tomato.

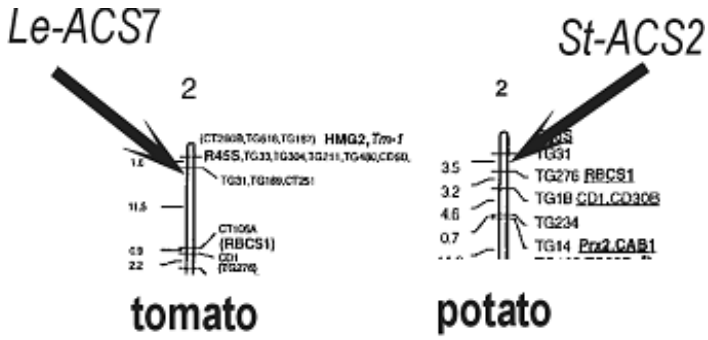


Figure 4: Chromosomal map locations of the ACC synthase genes *Le-ACS7* and *St-ACS2* in tomato and potato, repectively.

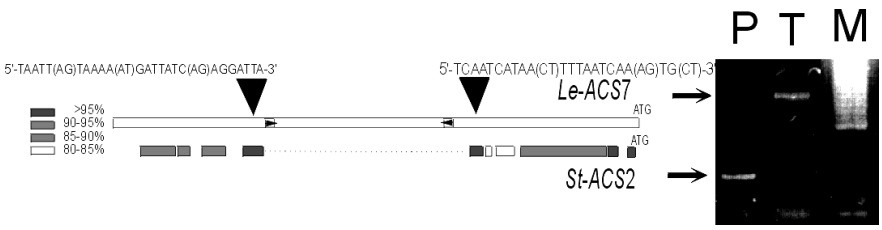


Figure 5: Amplification of genomic DNA with primers will yield different size fragments, depending on presence or absence of the transposon.

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