

The plant cytoskeleton – new jobs for a versatile network

The cytoskeleton has been perceived over many years as a structural, rigid lattice that basically functions as a supporting scaffold or even barrier. This view was probably biased due to our methods of visualization – by electron microscopy or immunofluorescence the true dynamic nature of the cytoskeleton could be detected only upon a specific experimental design. It was clear, though, that both microtubules and actin filaments are very dynamic when they were analyzed *in vitro*. However, our perception of the cytoskeleton as a “skeleton” remained amazingly unaffected by these biochemical results. This perception became increasingly challenged during the last decade of the last century, when *in vivo* imaging of microtubules and actin filaments revealed that they are endowed with complex and highly nonlinear dynamics. Thus, in addition to their traditional structural functions, the cytoskeleton must play a role in more volatile events that have to be organized in space and time. It has become clear that both microtubules and microfilaments are subject to numerous signaling chains and that this is especially important in plants, where morphogenesis is under tight control of a broad panel of environmental cues. Traditionally, the field has been separated into an actin and a microtubule community. It becomes increasingly clear, however, that, especially in the context of these signaling functions, the cytoskeleton has to be seen as a functional and sometimes even structural entity. The scope of this special issue is therefore to highlight a few newly emerging functions in a more or less integrative approach.

Novel tools produce novel concepts

As pointed out above, our concepts on the cytoskeleton were basically shaped by the techniques that were available. It was the combination of fluorescent-protein technology with confocal imaging that scattered the traditional, “structuralist” view of the cytoskeleton. In a method-oriented review, A. Yoneda et al. summarize the state of the art in vital labelling of plant cells. To visualize microtubules, the binding domains or full-length constructs of microtubule-binding domains have been used successfully along with fluorescently tagged tubulin isoforms. In addition, it became pos-

sible to visualize microtubule polarity using plus-end markers. Conversely, actin filaments have been visualized by the actin binding domain of mouse talin and plant fimbrin. To exploit the potential of these powerful markers, imaging has to be complemented by three-dimensional reconstruction methods. As pointed out by Yoneda et al., the maximum intensity projection method is suitable for cytoskeletal structures, while contour-based surface modelling possesses many advantages for vacuolar membranes. A rapidly expanding field of *in vivo* imaging is the visualization of intracellular membrane flow that has become accessible to observation using new markers such as intrinsic tonoplast proteins or syntaxin-related proteins. In this context, the vacuole, a compartment unique and central for higher plants, has shifted into the center of interest. M. Sheahan et al. demonstrate how vacuolar architecture can be visualized in protoplasts by fusions of the green fluorescent protein to a δ -tonoplast-intrinsic protein (δ TIP). They are able to document how vacuoles are reorganized during protoplast regeneration and how numerous transvacuolar strands develop until the cell restores its competence for division, in a process that is driven mainly, if not exclusively, by actomyosin.

Cytoskeleton and plastids

Plastids represent a unique and conceptually exciting achievement of plants, because they originate from prokaryotic ancestors that had been domesticated during a long evolution. As part of this domestication process, the cytoskeletal systems of host and endosymbiont had to cooperate, for instance, during the division of plastids. Although it is clear that there must be functional and physical links between the plasto- and the cytoskeleton, surprisingly little is known about these links. Two publications in this issue show that both, microtubules and actin filaments participate in these interactions and that this process is regulated in a specific (and complex) manner. S. Zaffryar et al. investigate the interaction between microtubules and amyloplasts during dormancy and development in bulbs of *Narcissus tazetta*, one of the major geophyte crops worldwide. They show that the microtubules surround the amyloplasts at a

specific developmental time and they confirm this interaction by *in vitro* experiments with fluorescent brain microtubules and amyloplasts isolated at different developmental stages. The binding is not removed by protease treatment, suggesting that the interaction of microtubules to the amyloplasts must involve factors that are intrinsic to the outer amyloplast membrane. H. Paves and E. Truve analyze the role of the cytoskeleton in light-triggered chloroplast movement. By using different inhibitors directed against myosins, they can discriminate between light avoidance (independent of myosins) and light accumulation (dependent on myosins) movements of chloroplasts. The works of the two groups illustrate that plastids are linked to both elements of the cytoskeleton and that this interaction is specifically regulated and tuned depending on both exo- and endogenous signals.

Signaling to actomyosin in tip growth

Plant cells are endowed with polarity, i.e., an innate “direction”, and this polarity can respond to environmental cues. This phenomenon is most manifest in tip-growing cells and has attracted the interest of many researchers over the years. An impressive body of evidence has shown the impact of the actomyosin system for the induction, maintenance, and reorganization of cell polarity in tip-growing cells. Since tip-growing cells respond readily to a panel of exogenous, mostly chemical, signals, the signaling towards the cytoskeleton has been intensively investigated in those cells. H. Ren and Y. Xiang review our knowledge on the role of actin-binding proteins as linkers between signaling and dynamic changes of actin filaments that eventually culminate in changes of cellular architecture. Within this conceptual framework, H. Zi et al. focus on kinase-dependent signaling. By inhibiting tyrosine phosphatases and kinases, respectively, they can induce malformations of pollen tubes and even inhibit pollen germination. In the next step, they dissect the effects and show that tyrosine phosphatase function is required generally for pollen germination and pollen tube extension and that an inhibition of phosphatase function can be at least partially rescued by stabilization of actin filaments. This indicates that tyrosine phosphatases act to keep actin filaments in a dynamic state necessary for these functions. In contrast, inhibition of tyrosine kinase function by genistein more specifically affects reorientations of pollen tube growth. The work by E.-L. Ojangu et al. is aimed on the binding partner of actin, the motor protein myosin. Although it has long been discussed that myosins are important for tip growth, it has remained unclear whether this task can be assigned to spe-

cific myosins. Myosins form a large superfamily with plant-specific classes that are organized in small- to medium-sized gene families. For instance, there exist 13 potential myosin class XI genes in *Arabidopsis thaliana*. As a result of a broad-scale search for functional null-mutants of these different myosins, Ojangu et al. can define specific roles for the myosin XIK. They show evidence for a role of this myosin subtype in tip growth of root hairs, but in addition for the elongation of stalks and branches in trichomes. The finding that specific myosin types fulfill specific cellular functions indicates that the gene families common for most cytoskeletal proteins in higher plants (including tubulins, actins, but also motor proteins) have evolved to allow for defined interactions with specified signaling chains.

Signaling at the plasma membrane

If the diversity of cytoskeletal proteins is related to signal processing, the so-called cortical subpopulations of both actin filaments and microtubules are of special interest. Three contributions to this issue therefore deal with signal responses of the cortical cytoskeleton. In the review by D. Konopka-Postupolska, the role of the evolutionary conserved annexin family is analyzed in more detail. Annexins bind in a calcium-dependent manner to membrane phospholipids and not only can regulate dynamic changes in membrane architecture but also participate in the interaction between secretory vesicles and the plasma membrane. Interestingly, some members of the annexin family were also identified as actin-binding proteins providing a direct link between signaling in and at the plasma membrane and the organization of the actin skeleton. The close association of the cortical cytoskeleton with the plasma membrane has led in the past to the concept of a cell wall–membrane–cytoskeleton continuum as a plant-specific functional entity relevant not only for the regulation of expansion growth but also for signaling from the apoplast into the cytoplasm. However, experimental evidence for this continuum is still relatively scarce and some of the molecular components proposed from analogy with mammalian cells, such as the integrins, have remained elusive so far. To identify the cell wall components involved in this continuum, P. Wojtaszek et al. have chosen the approach to induce plasmolysis in root apex cells of maize in combination with enzymatic digestion of specific polymers of the cell wall. They can show that pectins at the cross wall are especially important for the proper organization of actin filaments, whereas the side walls are dominated by cellulose-hemicellulose networks. J. Müller et al.

investigated the response of the cortical cytoskeleton to a physical signal, heat stress. They observe disruption and subsequent recovery of microtubules and actin microfilaments in response to a transient heat shock and discuss this cytoskeletal response in the context of intracellular signaling leading to increased adaptation to abiotic stress.

During the past decades, the plant cytoskeleton has been identified as an important target for a manifold of signal chains that are triggered either by environmental cues such as light, temperature, gravity, or touch or by internal signals including hormones or developmental inputs. However, it has remained a bit more implicit that the cytoskeleton is more than a mere target of signaling. It participates

very actively in signal transduction itself. Due to their innate nonlinear dynamics both microtubules and actin filaments could serve as amplifiers of even minute and noisy input at the plasma membrane. In addition, they are virtually plastered by numerous associated proteins including elements of signaling cascades. They therefore could serve as platforms where different signaling components can interact and where this interaction could be regulated by the dynamic assembly or disassembly of the cytoskeleton. The role of the plant cytoskeleton for signal integration and processing has just begun to emerge.

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