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EDITORIAL

Living interfaces watched with new tools

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Omne vivum ex ovo – "all life comes from an egg" concluded Francesco Redi (1668) after disproving, by a famous and elegant experiment, the Aristotelian concept of spontaneous generation. His statement made clear that life activity is bound to discrete units. But still, almost two centuries later, the botanist Schleiden, while correctly proposing the Cell Theory, together with his friend, the animal physiologist Schwann, assumed that the nucleus would crystallise from a broth of mucus and gum, and subsequently organise the surrounding cell (Schleiden 1838). However, since the second half of the nineteenth century, the idea of life activity as field or energy that can diffuse freely through space had been replaced by the concept of cells as "Atoms of Life", that cannot be generated de novo, but only derive from other cells. Thus, life needs borders that delineate inside and outside, but at the same time are permissive for the molecules needed to sustain life. While the textbook schemes imply the concept of borders that are solid and stable, the reality is much more dynamic, with continuous integration of material from exocytotic vesicles, and release of material for endocytotic vesicles. In a classical paper published in this journal (Phillips et al. 1988), the membrane of an auxin-stimulated epidermal cell of oat was found to be completely recycled once every 3 hours! How can a cell discriminate against this virtually boiling turnover of membranes, whether it is still intact, or whether its integrity has been irreversibly damaged, such that apoptotic death would be the appropriate response? This question is even accentuated by the fact that part of the "outside world" can be internalised as membrane-surrounded compartments into the cell. While for the case of animal lysosomes these "enclaves from the outside world" are relatively small, they can fill up almost the entire interior of expanding plant cells confining the cytoplasm to a very narrow rim of life activity bordered towards the outer world by a plasma membrane, while the tonoplast sequesters the acidic and potentially destructive content of the vacuole. We need new tools to follow, how these living borders are established, sustained, and eliminated. Two contributions to the current issue develop innovative approaches to provide insight into the dynamics of living borders.

The plasma membrane should not be seen as homogenous surface, but is regionally subdivided into domains with different function. A very impressive example of this surface patterning is the development of so called pH banding observed in certain chlorophytes, but also in aquatic angiosperms. The giant internode cells of the green alga *Chara* are particularly suited to study this phenomenon, and has been intensively studied with respect to electrophysiology and cell biology, providing a rich body of data on the dynamic self organisation of the plasma membrane. The banding pattern consisting of acidic and alkaline zones develops within minutes after illumination and is thought to be relevant for photosynthetic efficiency, because it allows for the concentrated uptake of carbonate. This phenomenon obviously requires rapid modulations in the activity of ion channels in the membrane, and it can be disturbed by ionic stress as it occurs by the impact of salinity. The contribution to the current issue by Absolonova et al. (2018) investigates the role of putative H^+/OH^- channels in the response to mild salt stress. While these channels are well known from animal models and have been genetically identified there, the presence and molecular identity of such channels has remained under debate in plant cells. The pH banding is rapidly perturbed in response to salinity, although there is no pH modulation that might explain activation of such channels requiring a mediating signal. As indicated by microirradiation in combination with a fluorescent sensor for reactive oxygen species (Eremin et al. 2013), this mediator might be reactive oxygen species (ROS) from the chloroplasts that open H^+/OH^- channels by oxidation of their sulphhydryl groups, a model, which is supported by experiments, where the banding can be quelled by ROS scavengers such as dithiothreitol or

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melatonin. Also the local activation of pH shifts in the medium in the response to local wounding (which is experimentally possible in these giant cells) supports a role of ROS as mediators (Foissner et al. 2015). Since zinc ions, known to inhibit the H^+/OH^- channels in animals, also eliminate the pH banding, and electrophysiological characteristics also have been detected, it is very likely that these channels exist in plants and that they represent a sensitive target to salt stress. However, since these channels have remained genetically elusive, the authors searched for a way to observe their activity and self-organisation more directly. They employed a fluorescent pH sensor (fluorescein isothiocyanate) coupled to a low-mobility tether (a 70 kDa dextran) to detect for local pH shifts and to follow the spatial patterns of their activation. Using confocal imaging, they were able to see the transporters in action. First, alkaline patches appeared and disappeared, while later some of these patches became stationary, which was explained by a self-amplification loop, where more active channels induce a more prominent alkalinisation in the cell wall, which in turn more efficiently activates additional channels in the neighbourhood. Thus, the regional partitioning of the membrane is generated by non-linear, dynamic activities, which can be inferred from the use of tools that are non-invasive and respond instantaneously to changes of the parameters they report.

The contribution by Nagy et al. (2018) moves to the opposite site, the border between cytoplasm and vacuole, the tonoplast. They have developed a novel, lipophilic group of fluorochromes based on a isocyano-naphthalene moiety and show that these dyes, ACAIN and CACAIN, can be used to label the tonoplast membrane in vivo. By the use respective loss-of-function mutants, as well as analysis of binding of the dye to specific bands in protein extracts, authors arrive at the conclusion that the tonoplast intrinsic proteins are the preferential target for these fluorochromes. In contrast to concurrent vital dyes, the new markers preferentially labels the tonoplast (unlike FM4–64 which also labels plasma membrane and endosomes) and does not impair the endomembrane system (unlike FM1-43 which, starting from a few minutes, causes swelling of the ER). To explore the application of their new tools, they employ the dyes to probe the mode of action of a cyanobacterial peptide toxin, microcystin-LR, which is a generic blocker of serine-threonine phosphatases. Using their tonoplast dye, they can show, how, in response to microcystin-LR, the tonoplast membrane is subdivided into autophagosomal vesicles, and can compare the efficiency to inhibitors (okadaic acid, tautomycin) that are targeted to subtypes of protein phosphatases.

Both contributions illustrate the point that we can only observe the observable. What is observable, depends on our tools. Therefore, new tools will lead to new concepts. In order to follow the dynamics of membranes, we need tools that can be used in living cells while perturbing the process to be observed to only a minimal degree. In the light of these dynamic tools, membranes transform from static borders into living interfaces, i.e. they appear as activities rather than as structures.

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