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EDITORIAL

Phase in space

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When the young Robert Hooke took over a royal commission from his more established colleague, Sir Christopher Wren, to test a newly invented instrument called microscope, he first improved lighting, angles and distance, before applying the new device to all sorts of objects including a louse sucking blood from his hand, as well as his famous piece of cork. Thus, the new concept that living beings are composed of "cells" (Hooke 1665) was actually an outcome of advances in experimental technology. A second advance in technology, electron microscopy, allowed us to see the inner structure of these "cells" with unprecedented detail. However, the need to prepare the specimens for the use in extreme vacuum, requiring chemical fixation, dehydration, embedding, and ultra-sectioning, promoted a concept of "cells" as entities with complex internal architecture that is delineated into organelles, compartments and tethered by a filamentous "cytoskeleton". When, at the end of the last century, the new GFP technology, in combination with advances in fluorescence microscopy, allowed to follow the inner dynamics of cells with a new level of detail, this changed our concept of "cells" for a third time: The fine structure revealed by the electron microscope now turned out to be a snapshot in a very dynamic movie, where structures and membranes continuously merge and separate, leading to the question, how order can be generated and maintained on the background of an ever changing flow.

When we see "cells" as self-perpetuating activities rather than as static entities with fixed borders, we have to ask ourselves, how the observed form can be reinforced and sustained. The underlying mechanisms seem to be clearly non-linear, and, as worked out by Alan Turing (Turing 1952), they must include processes that are self-amplifying, while they suppress competing processes in their neighbourhood. The initial steps of such spatial phase transitions are, by their very nature, delicate and subtle. Two contributions to the current issue allow to catch a glimpse on such primordial moments of cellular self organisation.

To be able to talk about cellular metabolism, we have to use visualisations, where the sequence of events is sketched down as pathways. Nevertheless, we do not expect to see, while scrutinising a chloroplast, the Calvin-Benson cycle as circular structure cycling in the stroma. On the other hand, the times, where cells were conceived as "bag of enzymes", are definitely over. The argument put forward by Paul Srere (Srere 1967), that enzyme concentrations *in vivo* can locally be several orders of magnitude higher than those seen *in vitro* (after the cell had been homogenised), is still valid. However, there are few examples, where metabolic phase transitions have been made visible. One of these examples is the contribution by Yamano et al. (2018) in the current issue. This work deals with a phenomenon commonly found in aquatic plants, where the diffusion of carbon dioxide can become limiting for photosynthesis, such that carbon concentration mechanisms, for instance bicarbonate transporters, provide a strong selective advantage. Based on a mutant approach in the green alga *Chlamydomonas*, authors had identified a calcium-binding protein, CAS, as regulator of this carbon concentrating mechanism. Since *Chlamydomonas* harbours only one chloroplast, which is quite large, they used this novel regulator as tool to visualise metabolic phase transitions within the chloroplast in response to reduced carbon dioxide levels. For this purpose, they generated transgenic lines, where CAS was fused through a steric linker to the fluorescent reporter Clover, a GFP variant that had been tailored for the use in Green Algae. When carbon dioxide was abundant, this reporter was organised in a mesh-like pattern. Upon transfer to low carbon, the spatial distribution changed drastically: Within two hours, the CAS-Clover construct was shifted to the pyrenoid, a plastidic substructure found in many algae, and organised into a wheel-like structure, where fluorescent spokes were emanating from one center. This phenomenon is nothing else than the structural manifestation of a metabolic switch, where carbon concentration is repartitioned along the thylakoid to specific sites, where it can then proceed even under unfavourable concentrations of

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substrate. The mechanism leading to this phase transition is still unclear. Authors describe two plausible models – either the thylakoids themselves could undergo remodelling, or the binding of CAS to the thylakoid might be modulated depending on its phosphorylation status. The self amplifying mechanism needed for this phase transition could be brought about by formation of complexes of CAS with other components of the carbon concentration mechanism that depend on the local abundance of carbon dioxide.

Also the second contribution to be highlighted in the context of spatial phase transitions stays with Green Algae, but moves one level up in organisation. This group of algae provides numerous instructive examples illustrating different paths towards multicellularity. While already the agglomeration of several cells *per se* provides a selective advantage, because a larger organism is less readily devoured than a single cell, the full potential of this innovation is only released, when the cells differentiate. Using a very simple multicellular model, the foliose alga *Prasiola japonica*, the contribution by Mine et al. (2018) addresses the question, how the cell walls delineating groups of cells change during formation of a multicellular thallus. Multicellularity in this group of algae originates from incomplete separation of daughter cells that remain joined together, because the cell wall of the mother cell continues to ensheath the resulting pair of daughters. With time, this ancestral cell wall becomes thinner, but still constrains the different pairs of daughters and granddaughters. Using the stilbenic dye Calcofluor White, which is binding to cellulose microfibrils (Herth and Schnepf 1980), the authors show that the label decreases with progressive maturation of the cell wall over these three levels from a unicellular towards a multicellular boundary. They then use a panel of lectins to search for markers that compensate for the loss of anisotropic cell wall components. In fact, they succeed to identify three lectins, soybean agglutinin, jacalin, and *Vicia villosa* lectin that meet this criterion and specifically stain wedge-shaped regions in the mature cell boundaries that are not marked by Calcofluor White. This leads authors to the question, whether it is the gradual replacement of cellulose microfibrils by a more amorphous matrix, which is required to achieve true multicellularity. These lectin-binding components (probably N-acetyl-galactosamin like molecules) would thus be the functional analogue of pectins in terrestrial plants. Note: When single cells are surrounded by highly anisotropic cellulose, while the multicellular thallus is embedded in a more isotropic matrix, mechanic strain is expected to be repartitioned, which again should feed back to the axiality of cell expansion and would then, by unknown mechanisms, shift metabolism from cellulose synthesis towards N-acetyl-galactosamins. Since the structure of the ensuing cell wall will modulate the pattern of mechanic strain, again, a self-amplifying circuit would be generated.

Both contributions, although regarding different levels of organisation, share a common theme: The intra-plastidic pattern of the carbon-concentrating complexes in *Chlamydomonas* can be explained by a phase transition that depends on the metabolic activity, the super-cellular pattern of cell divisions in *Prasiola* can be explained as phase transition that presumably depends on the mechanic properties of the cell wall that constrains and guides this pattern. In both cases, shape is the solidified trace of activity.

Compliance with ethical standards

Conflict of Interest The author declares that there is no conflict of interest.

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