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SHORT COMMUNICATION

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Self-organizing actin patterns shape cytoskeletal cortex organization

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ABSTRACT
Living systems rely, for biological function, on the spatiotemporal organization of their structures. Cellular order naturally emerges by dissipation of energy. Consequently, energy-consuming processes operating far from thermodynamic equilibrium are a necessary condition to enable biological systems to respond to environmental cues that allow their transitions between different steady-states. Such self-organization was predicted for the actin cytoskeleton in theoretical considerations and has repeatedly been observed in cell-free systems. We now demonstrate in our recent work how self-organizing actin patterns such as vortices, stars, and asters may allow cells to adjust their membrane architecture without affecting their cell mechanical properties.

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One basic question in biology is how cellular structures are dynamically organized in space and time. While cell-biology has gained a good understanding of important processes including structural and functional knowledge of many molecules, mechanisms of organization underlying most dynamic features in living systems remain unclear. Two fundamentally different mechanisms exist to generate macromolecular structures in cells: self-assembly and self-organization. Self-assembly involves the physical association of molecules into an equilibrium structure with no energy dissipation and external intervention, purely driven by the tendency of systems to minimize their free energy in accordance with the second law of thermodynamics.[1,2] Self-organization requires the collective action of interacting molecules far from thermodynamic equilibrium driven by the constant input of energy into a steady-state structure.[3-5] Yet, in practice, cellular order results from both a combination of complex deterministic interactions (self-assembly) and of dynamical interactions between molecules that require energy dissipation (self-organization).[6,7] Notably, both organization mechanisms can lead to similar phenomenological patterns, but their pattern evolution may vastly differ on different length- and time-scales, as previously computed for membranous lipid-protein interactions *in situ*. For example, the self-assembly of such membrane domains led to periodic domains on the nanoscale, whereas the self-organization of those membrane domains resulted in a pattern wavelength comparable to a typical cell size.[8] Conclusively, one can say with certainty – self-organization is essential for living systems because in the absence of a continuous supply of energy cells die.

The cortical actin cytoskeleton full-fills all criteria of self-organization.[9] It is a complex system that comprises polydisperse filamentous actin (F-actin) of 2 different F-actin lengths undergoing continuous turnover with constant growth of the filaments at their barbed ends and shrinkage at their pointed ends.[10] These actin filaments have 10–20-fold differing kinetic binding rates of actin monomers and arise from distinct nucleation pathways[11]: (1) polymerization of long F-actin is driven by formin proteins, which associate with the fast-growing barbed end of actin; and (2) branching of short F-actin is driven by the Arp2/3 complex, which binds to pre-existing F-actin and nucleates new filaments. The latter population has been shown to account for 80% of the total F-actin in different cell types.[10,11] In addition, these filaments are crosslinked over finite periods of time and redistributed by the action of molecular motors, such as myosin-II.[12] The mechanical forces generated by these processes operate on multiple length- and time-scales.[13] Previously, these components of the cortical cytoskeleton including actin kinetics, dynamics, and mechanics could not sufficiently be studied, mainly due to technical limitations in the observation and probing technologies. Yet this is changing. Fortunately, with the development of novel optical fluorescence imaging modes,[10,14-17] and

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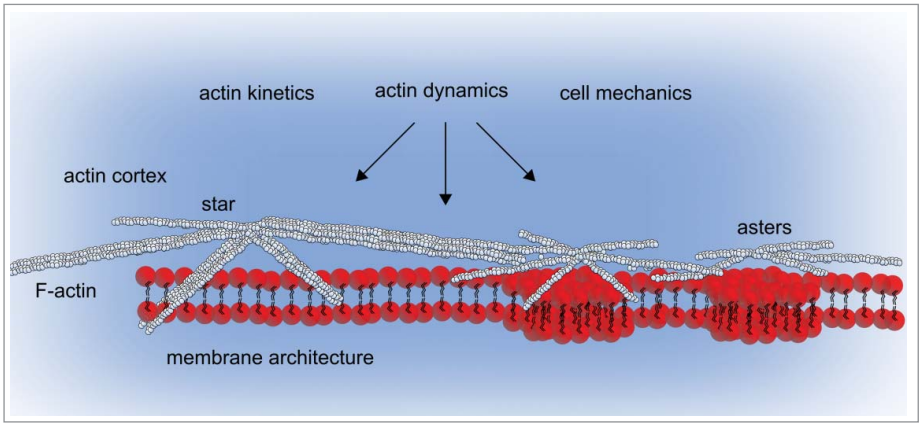

Figure 1. Actin kinetics, dynamics, and mechanics must be investigated to unravel the complexity and organization mechanisms of the cortical actin cytoskeleton and their effects on membrane architecture. To this end, actin kinetics refer to actin monomer association/dissociation into actin filaments, actin dynamics to the assembly of filaments into actin structures, and mechanics to the cytoskeleton that generates, senses, and transmits mechanical forces.

force probing technologies[18,19] we have now the ability to dissect actin architectures in enough detail to understand the organization mechanisms of the cortical actin cytoskeleton.

We recently demonstrated how the cortical actin cytoskeleton uses mechanisms of self-organization to dynamically generate different actin patterns in HeLa cells.[20] Employing state-of-the-art super-resolution microscopies allowed the monitoring of these transitions over time in living cells, which demonstrated that upon adherence of the cells an active multistage coarsening process naturally leads to the formation of actin vortices and subsequently into stars and asters. Unexpectedly, pattern dynamics were primarily driven by the nucleation of the Arp2/3 complex, but not by myosin motor proteins, which is in contrast to what has been theoretically predicted and observed *in vitro*. Myosin-II patches localized only to F-actin strands of both stars and asters but not to their cores, with an active mobility along actin filaments, suggesting that myosin-II was not actively participating to pattern nucleation and maintenance. Nevertheless, myosin-II was likely be involved in generating the intrinsic mechanical stress as it is required for the initiation of the transitions between different actin patterns at different steady-states. Effects of other crosslinking proteins must be investigated in more advanced experiments with the possibility to transiently activate and deactivate molecules involved in setting intra-cellular forces in the actin network. Measurements of cell mechanical properties and plasma membrane fluidity indicated that patterning alters cellular membrane architecture but occurs at constant cortical elasticity. Consequently, self-organizing actin patterns may allow cells to adjust their membrane architecture without affecting their macroscopic mechanical properties.

Future investigations should make use of the above described novel quantitative methodologies such as the computation actin filament lengths, complementary to super-resolution microscopy, to achieve deep mechanistic understanding of the physiological importance of self-organization compared with self-assembly. To unravel the active role and true complexity of the actin cytoskeleton from the bottom up in cellular function, the dynamic interplay of all 3 components including actin kinetics, dynamics, and mechanics must be evaluated. Especially, scenarios involving immune cell function, where a series of energy-consuming reorganizations of the cortical actin cytoskeleton is required on multiple length- and time-scales,[21,22] are likely to rely on self-organizing actin patterns to efficiently shape membrane architecture and cellular mechanics.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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