Abstract

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Thesis title: A study of cytoplasmic macromolecular crowding, reactive oxygen species, and microrheology from the perspective of mammalian cell volume regulation

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Synopsis

Cells can regulate their volume to acclimatize to the changing physicochemical external environment, like osmolarity of the extracellular fluid, as well as a response to various intracellular biochemical signalings, like cell cycle, apoptosis, necrosis, and transepithelial migration. In the aspect of cell volume regulation, the identity of a cell volume sensor remains unclear. The cell is effectively a closed system of densely packed macromolecules, any change in the intracellular macromolecule density can impact the thermodynamic activity of numerous biochemical processes. Hence, it is often proposed that the macromolecular crowding (MMC) in the protoplasmic fluid acts as a cell volume sensor. To establish intracellular MMC as a cell volume sensor, a reliable, quantitative probe is essential and currently lacking. We established that fluorescence anisotropy of EGFP (r_{EGFP}) can serve as a fast, high throughput probe for MMC, both in vitro and in vivo. We showed that r_{EGFP} scales linearly with increments in macromolecule density and is more sensitive to protein density than smaller molecules like polysucrose, amino acids, and salts. Further, r_{EGFP} is independent of solution pH, viscosity, and EGFP concentration. We found that the average intracellular MMC is distinguishably different among diverse cell lines. Intracellular r_{EGFP} measurements showed that the cytoplasmic MMC is spatially heterogeneous despite free diffusion of macromolecules, and the organization of the actin cytoskeleton separates the regions of varying cytoplasmic MMC. We then used r_{EGFP} to study the intracellular MMC homeostasis and tested the role of MMC as a cell volume sensor. We found that cells maintain MMC homeostasis during volume recovery from osmotic shocks, but perturbing the intracellular MMC isosmotically does not trigger any cell volume change. Furthermore, cell spreading and microtubule depolymerization enlarge cell volume, ignoring the changes in intracellular MMC. Hence, we hypothesized that MMC is not a cell volume sensor, but alternately proposed sensors like the plasma membrane may be more relevant. We found that pharmacologically inhibiting TNFR1 (Tumor Necrosis Factor Receptor 1) activity increases plasma membrane tension and arrests cell volume regulation during anisotonic challenges, microtubule disruption, and cell spreading. Hence, we propose that the plasma membrane is a more relevant cell volume sensor. We then investigated the biochemical influences of cell volume and cell morphology on the physiology of adherent cells. We found that detaching cells from their adhesion points leads to oxidative stress, while the reintroduction of adhesion-promoting substrates rescues the cells from oxidative stress. However, not allowing cells to spread after attachment makes the oxidative stress persist, and the persistence of intracellular oxidative stress increases the mechanical viscoelasticity of the cytoplasm.

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