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## Spotlight

## When Separation Strengthens Ties

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**Phase separation underlies functional compartmentalization in living systems. Two recent studies (Beutel *et al.* and Schwayer *et al.*) show that zonula occludens (ZO) proteins of tight junctions (TJs) condense into compartments within the cytoplasm that display liquid properties. This ability to condense predicts normal TJ assembly and epithelial barrier function which are essential for vertebrate embryogenesis.**

All forms of life as we know it are likely composed of intermingled liquid phases whose separation results in compartmentalization of biological functions. At the cellular scale, lipid membranes are liquid crystal films that form selectively permeable boundaries between aqueous fluids, thereby organizing the intracellular ecosystem of organelles in eukaryotes. By contrast, proteins typically exist as solutes that may assemble into solids whose shapes, ordered structures, and mechanical properties, such as those of cytoskeletal filaments, are essential to cell physiology. Such protein assemblies may nonetheless be dynamic because of catabolism, and may act as compartments by accumulating specific interacting partners.

Observations on marine animal eggs more than a century ago led to the hypothesis that the cytoplasm also consists of a liquid–liquid emulsion [1]. Moreover, liquid–liquid phase separation (LLPS) was long ago proposed as a primordial step in the origin of life [2]. The past decade has seen a flurry of studies investigating how protein condensates can

form such emulsions, and how liquid behavior could make them special [3]. Today, hundreds of proteins have been identified as undergoing LLPS [4].

Two recent studies published in *Cell* now add the ZO proteins of TJs to the growing list of proteins that are capable of LLPS [5,6]. TJs are intercellular adhesion complexes that form a selective diffusion barrier between epithelial or endothelial cells, thereby regulating tissue permeability, a crucial function for embryogenesis and compartmentalization at the organ scale. In addition to transmembrane proteins that interact between adjacent cells, TJs require the assembly of scaffolding proteins on their cytoplasmic sides, among which are the ZO proteins (Figure 1).

By means of experiments *in vitro* and in cultured epithelial cells, Beutel *et al.* [5] have provided evidence that ZO proteins can form condensates that display liquid-like properties: their roundish shapes fluctuate, they coalesce, and their internal contents are highly mobile. In epithelial cells these condensates are not distinguishable from TJs at physiological concentrations, but can be revealed by cytoskeleton depolymerization or intercellular adhesion disruption. Both treatments disrupt the continuous TJ belt, and this breaks up into disconnected condensates reminiscent of a fluid thread as a result of Rayleigh–Plateau instability. In gastrulating zebrafish embryos, Schwayer *et al.* [6] showed that, in the yolk syncytium, ZO proteins form non-junctional condensates that subsequently fuse with TJs at the boundary between the yolk syncytial layer and the enveloping cell layer. The shapes and sizes of these non-junctional condensates are externally constrained by the surrounding actin cytoskeleton meshwork, reminiscent of how the size and shape of a liquid are limited by its container.

Beutel *et al.* used a collection of partial deletion mutants to demonstrate that

multivalent interactions in ZO1 underlie its phase separation (as in many other proteins [4]), and that these essentially involve its PSG (PDZ3-SH3-GuK) supradomain. These interactions are antagonized by the nearby U6 (unique-6) domain, and this autoinhibition is itself opposed by the long and mostly disordered C terminus. Dephosphorylation also promotes phase separation, which thereby could be involved in density-dependent enhancement of epithelial cell barrier function [7]. In addition, Beutel *et al.* show that the C terminus provides fluidity to the condensates. The C terminus also contains an actin-binding region (ABR) that does not strongly influence phase separation but substantially contributes to C terminus-dependent fluidity. Within TJs, however, ZO proteins are less mobile than in ectopic or non-junctional condensates.

Functionally, ZO proteins are able to selectively accumulate diffusible interactants in *in vitro* condensates, ectopic condensates in TJ-less cells [5], and non-junctional condensates in zebrafish [6]. Therefore, ZO condensates act as compartments. At the tissue scale, Beutel *et al.* reveal that the ability of ZO1 protein to phase-separate scales with its abilities to accumulate at TJs and build an impermeable epithelial barrier in cysts, in a manner that is mostly independent of the ABR when grown in Matrigel [8]. In zebrafish, however, a function for phase separation is unclear, but Schwayer *et al.* show that the ABR is required for efficient incorporation of non-junctional clusters into TJs – which scales with actomyosin tension and retrograde flow – as well as for normal gastrulation. Because the ABR provides both actin binding and fluidity to ZO condensates, the respective contributions of each feature remain an open question in both contexts.

Addressing whether these liquid condensates result in functions that cannot be achieved by solid counterparts, and

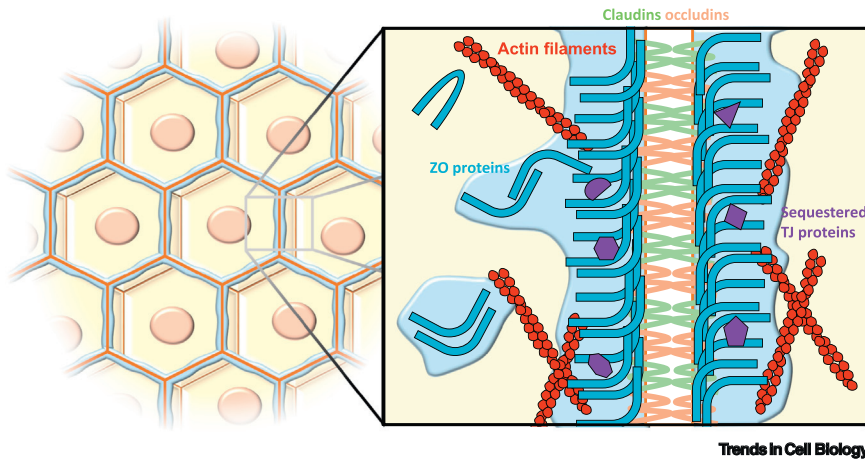


Figure 1. At Epithelial Cell Intercellular Contacts, Zonula Occludens (ZO) Proteins Condense into a Liquid-Like Phase To Assemble the Tight Junction (TJ) Belt.

identifying physiological or pathological cues that may cause this transition, are some of the next exciting steps. Indeed, a distinguishing feature of liquids is that their size and shape are instructed from outside; therefore, any functional benefit must outcompete the energy cost. This question is all the more valid for TJs where mechanosensitive stretching organizes ZO proteins into a 2D film that is very different from the shapeless droplets typical of liquid condensates [9]. ZO proteins also function in the nucleus [10], and the findings of

Beutel, Schwayer, and colleagues are likely to have implications beyond cell–cell adhesion. In any case, they advance our understanding of TJ biogenesis and assembly, and provide many exciting questions to be addressed in the future.

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