P-2.3

Single Molecule Analysis of the Interaction Between the Tight Junctional Protein zo-1 and actin

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Tight junctions (TJs) are fundamental cell-cell connections that act as selective barriers and as hubs for polarity, signalling, and mechanotransduction. TJ complexes are formed by transmembrane proteins (claudin, occluding, JAM), which connect adjacent cells and are intracellularly linked to adaptor proteins that, in turn, associate with the actin and microtubule cytoskeletons. ZO-1 is an adaptor protein essential for the formation and regulation of TJs, controlled by actomyosin-generated tension and cytoskeleton-associated proteins such as cingulin (CGN).

Here, we used ultrafast force-clamp optical tweezers to directly observe the load-dependent interaction of ZO-1 with actin. Unlike other adaptor proteins investigated so far, multiple ZO-1 molecules establish a weak and continuous interaction with actin that, under constant force, results in constant-velocity sliding. We named this interaction a "frictional bond" because it acts as a viscous drag on the actin filament. Actin sliding velocity increases linearly with force and asymmetrically with respect to actin filament orientation. A single ZO-1 molecule exhibits a viscoelastic response to force that can recapitulate the collective response of multiple molecules. Finally, CNG greatly enhances ZO-1's affinity for actin, supporting previous evidence that CNG is crucial for exposing ZO-1 actin-binding domain.