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Commentary

Signal transmission forces at the cell membrane under debate

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The advancement of techniques to measure nano- to piconewton forces has found a natural arena in Life Sciences in recent years. Countless biological processes have been examined, from DNA replication to protein–protein interaction, and many others (Clausen-Schaumann et al., 2000; Vinckier et al., 1998; Weisel et al., 2003). Commonly used techniques employed in these measurements are atomic force microscopy (AFM) and optical tweezers. More recently, biological researchers have tailored the application of these techniques to address questions of folding and refolding forces of single molecules and to measure the rupture force of molecular adhesion (Grubmueller et al., 1996; Rief et al., 1997). Forces for single molecules to unfold using AFM, like individual domains of titin, ranged from 150–300 piconewton depending on the pulling speed (Rief et al., 1997), whilst the rupture force, e.g. of a fibronectin–integrin complex, using AFM and laser tweezers was on average between 80–120 piconewton (Li et al., 2003; Litvinov et al., 2002). Recent structural analyses of $\alpha v\beta 3$-integrin have shed more light on how it interacts with fibronectin (Xiong et al., 2001), and how internal, as well as external, forces act on cells (Xiong et al., 2003). The research interest of many laboratories has focused on how the mechanical link between integrins, membrane-associated proteins like talin, and the actin cytoskeleton mediates signal and force transduction across the plasma membrane (Garcia-Alvarez et al., 2003; Von Wichert et al., 2003; Zhang et al., 2002).

Past experimental data have shown that talin serves as a key protein linking the cytoskeleton to the extracellular matrix (Isenberg and Goldmann, 1992). The binding of talin to actin has independently been reported by two groups (Kaufmann et al., 1991; Muguruma et al., 1990), and stopped flow measurements indicated 'on' and 'off' rates of 7×10^6 M^{-1} s^{-1} and 2–3 s^{-1}, respectively (Goldmann and Isenberg, 1991), which are similar to integrin binding to talin and fibronectin (Goldmann, 2000). From the biochemical point of view, these kinetic data reflect average strong and stable binding, which cannot be broken by an externally applied force of only 1–2 piconewton, as recently reported by Jiang et al. (2003), when the rupture of the fibronectin–integrin complex requires much higher forces.

To explain the findings of Jiang et al. (2003), one has to consider that talin not only binds to actin as a dumbbell-shaped, 51 nm long, flexible homodimer (Goldmann et al., 1994) whose subunits are arranged in an antiparallel fashion, but also inserts into the hydrophobic region of lipid membranes, allowing the promotion of actin filament assembly proximal to the membrane (Kaufmann et al., 1992). A competent sequence domain for membrane-anchoring, amino acids 385–406 just preceding the calpain cleavage site, has been identified, and it has been demonstrated that this peptide folds into a five-loop α-helix in the presence of lipid vesicles prior to binding to lipid bilayers (Seelig et al., 2000). The strongly amphipathic α-helix (H17) has been resolved by crystal structure of the talin head portion (Garcia-Alvarez et al., 2003), and competitive binding of β_3-integrin cytodomain and PIP_2-kinase to the same segment has recently been demonstrated (Barsukov et al., 2003). The binding of H17 to lipid bilayers most likely occurs in an oblique orientation (Isenberg et al., 2002), with one site facing the membrane, leaving the other, more charged half for integrin and PIP_2-kinase binding. Binding of the helical peptide alone to lipid bilayers was measured, rendering a partition coefficient of between 6.2×10^3 and 1.2×10^4 M^{-1}, depending on concentration (Seelig et al., 2000). This

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relatively weak affinity, competent for transient interactions with phospholipid bilayers, may well be disturbed by 1–2 piconewton forces, which seems more plausible to us. Or are the forces simply the result of thermal noise in the system?

In conclusion, more detailed experimental work needs to be carried out in order to understand the complex nature of integrin-membrane-associated proteins like talin, vinculin, filamin (ABP-280), alpha-actinin and the actin cytoskeleton with regard to bi-directional force transmission and biochemical signaling.

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