Varieties of mechanotransduction: the cytoskeletal stress fibre as a force transmitter and a mechanosensor

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It has been established that bacterial mechanosensitive (MS) channels are activated directly by stress in the membrane. However, whether eukaryote MS channels need some other accessory proteins, typically cytoskeletons, for their activation has been a pending problem. Theoretically the cytoskeleton would be a more efficient force transmitter than the membrane owing to its larger elastic modulus. It is likely that higher organisms have utilized such a device to increase the sensitivity of their MS channels. However, no direct evidence has been provided to show the role of cytoskeleton in MS channel activation.

We have developed two methods by which we can stretch the actin-based cytoskeleton (stress fibres) while monitoring MS channel activities either by Ca^{2+} influx or whole cell currents measurements in cultured endothelial cells (HUVECs). In one method a fibronectin coated glass bead, which is attached on the apical cell surface and connected to the basal focal adhesions *via* stress fibres, was mechanically moved to stretch the attached stress fibres. In the other method, phalloidin coated beads microinjected into the cell where they attached to stess fibres. One of these was pulled with laser tweezers to stretch the attached stress fibre. In either way, we could consistently record stretch activated currents and Ca^{2+} transients that originated from the activation of cation selective MS channels carried by HUVECs. The force required for a single MS channel activation was estimated as low as 1-2 pN. Ultra fast near field Ca^{2+} imaging resolved the Ca^{2+} influx spots across individual MS channels near basal focal adhesions. Simultaneous imaging of integrin molecules indicated that MS channels are located near integrin molecules as close as a few hundred nm. Forces originally created by membrane deformation and transmitted through a stress fibre/integrin complex seem to activate MS channels.

MS channels serve as a typical fast mechanosensor, however, they are not the only mechanosensor in the cell. Turning to slowly going mechanotransduction, we can see another world of mechanosensors. Endothelial cells *in situ* exhibit a spindle-like shape, aligning their long axis running along the vessel. They lose this characteristic shape when cultured in dish. However, they recover their original shape and alignment when subjected to uniaxial cyclic stretch (20% at 1Hz) that mimics circumferential cyclic stretch in the vessel. In other words, cells can detect the direction of applied forces and convert this information into their morphology. We found that Ca^{2+} influx *via* MS channels was indispensable for this mechanically induced cell shape change, but that the Ca^{2+} signal by itself could not indicate the direction of the force to the cell. As the stress fibres in the stretch axis were preferentially disrupted by cyclic stretch in a few minutes and then reorganized perpendicular to the stretch axis, we suspected that the stress fibre, particularly its major component actin fibre, might be a force direction sensor. To test this hypothesis in a more direct way, we examined the dynamics of single actin fibres in response to mechanical stretch. Surprisingly, relaxing but not stretching an actin fibre caused its rapid depolymerization with an aid of cofilin, a soluble actin-depolymerizing factor. In living cells disruption of stress fibres caused by actin fibre depolymerization activates several downstream signal molecules around the focal adhesion, which eventually leads to a cell shape change. In this sense, the stress fibre (actin fibre) is eligible as a mechanosensor.

In conclusion, the stress fibre serves as a force transmitter in fast mechnotransduction, and acts as a mechanosensor with direction sensitivity in slow mechanotransduction.