

INVITED LECTURE H7

Mechanical property of mechano-responsive proteins : possible mechanical roles for non-globular domains

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Molecular mechanisms of how cells sense mechanical forces are poorly understood. While we found that p130Cas acts as an ion channel-independent cytoskeletal mechano-sensor through its extension-induced enhancement of susceptibility to phosphorylation, i.e. substrate priming (Sawada et al. *Cell* 2006), it remained unclear whether p130Cas was extended by forces directly exerted on it.

To determine the force required to extend the p130Cas substrate domain, we conducted a single molecule study using an atomic force microscope (AFM). We found that p130Cas could be extended by the force below the detection limit of AFM (5 – 10 pN). Thus, p130Cas (substrate domain) appeared not to be mechanically stable and hence may not be a “force sensor.” Alternatively, we suggest that p130Cas is a “strain sensor” that responds to the changes in the dimension of the force-bearing complex to which p130Cas is anchored.

We sought the identities of the proteins that were tyrosine phosphorylated by cell stretching, because they likely act as strain sensors through substrate priming (Tamada, Sheetz, and Sawada, *Dev Cell* 2004). We found stretch-dependent phosphorylation of Gab1 and Shc in HEK293 cells and N-WASP in mouse fibroblastic L-929 cells, while there was no detectable stretch-dependent increase of tyrosine phosphorylation in IRS-1 and FRS2 in either type of cells.

While we are conducting proteomic analysis of mechanical force-responsive phosphorylation, we performed a computational analysis of amino-acid sequence. We found that stretch-responsive positive proteins so far (Gab1, Shc, N-WASP, and p130Cas) have all contained notable proline-serine rich non-globular (PSRNG) domains, suggesting the mechanical roles for those domains.