**Structured illumination microscopy reveals novel insights into actomyosin arc formation at the T cell immune synapse.**

S. Murugesan, J. Hong, J. Yi, D. Li, L. Shao, X. Wu, E. Betzig, J.A. Hammer

Cell Biology and Physiology Center, NHLBI, NIH

ABSTRACT

The T cell’s actin cytoskeleton undergoes striking rearrangements (IS) upon contact with an antigen presenting cell to form the segregated domains that comprise the immunological synapse- the distal, peripheral and central supramolecular activation clusters (dSMAC, pSMAC and cSMAC respectively). Here we addressed the mechanism of formation and function of the concentric actomyosin II arcs that populate the pSMAC. Using total internal reflection fluorescence structured illumination microscopy (TIRF-SIM), as well as 3D-SIM to visualize the architecture of F-actin networks, we show that actomyosin arcs are bonafide structures in both Jurkat cells and primary mouse CD4/8+ T cells. A popular model suggests that the branched actin network comprising the dSMAC, which is created by Arp2/3-dependent nucleation, is converted into the concentric arcs by debranching and crosslinking. In contrast to this idea, we observed linear actin filaments that arise at the plasma membrane and project inward such that they are perpendicular to the plasma membrane and embedded in the branched actin network of the dSMAC. Morevover, as these filaments exit the inner aspect of the dSMAC, they splay out in the pSMAC and reorient into concentric arcs with an inherent antiparallel organization that is optimal for supporting contraction by bipolar myosin II filaments. Importantly, the perpendicular actin filaments in the dSMAC are greatly accentuated by inhibiting Arp2/3-dependent actin polymerization, which collapses the branched actin array and provides additional monomer for the other actin nucleators like formins. Consistently, several formins including Inf2 and mDia1, are concentrated at the tips of these perpendicular structures, and addition of a formin inhibitor disrupts arc architecture and prevents further arc formation in a reversible manner. Importantly, inhibition of myosin II using para-nitro-blebbistatin results in loose, disorganized filaments within the pSMAC that fail to reorient into concentric arcs. Together, these observations argue that arc assembly occurs through a formin-dependent mechanism (i.e. independent of Arp2/3-mediated nucleation), and that myosin II contractility is required for reorienting the perpendicular filaments emanating from the dSMAC into the concentric arcs in the pSMAC.