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Single molecule imaging of actin-tropomyosin interaction

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Actin filaments with its binding partners play various roles in the cytoskeleton. They are involved in most cellular processes including determination of cell shape, cell migration, cell division, membrane functions, and intracellular transport. Tropomyosin with an unclear mechanism of sorting wrap around actin filament to create distinct actin populations. Sorting based on one hypothesis is partly due to differences in affinity of tropomyosin isoforms for different actin structures. The competition between tropomyosin isoforms and the differences instability as well as the number of Tms that leads to the formation of a nucleation spot on a single actin filament are not known. The main roadblock to addressing these questions is the lack of techniques and tools to elucidate underlying processes at the molecular level. Thus, we have successfully developed a combined microfluidic and single-molecule fluorescence imaging approach to visualize and quantify the assembly process by reconstituting actin filaments in the presence of labeled tropomyosin isoforms. We have also developed an image analysis tool that allows the investigation of the composition of tropomyosins on individual actin filaments below the point spread function. Therefore, cytoskeletal and skeletal tropomyosin isoforms were labeled using maleimide chemistry. Biochemical assays proved that labeled tropomyosins bind cooperatively to actin filaments. We then competitively bound different tropomyosin isoforms to actin filaments in a microfluidic flow channel and visualized early assembly intermediates utilizing TIRF microscopy. Our observations reveal nucleation of short stretches of tropomyosin polymers at multiple locations along the actin filaments. While on average there are twice as many skeletal than cytoskeletal tropomyosin dimers bound to individual filaments of both skeletal and cytoskeletal actin isoforms. We have also for the first time observed the cooperative binding of tropomyosins to some single actin filament in real time. Our results have implications for the assembly pathways of specialized actin filaments in cells.

Biography

Peyman Obeidy's fundamental research interest is the integration of cancer biology, immunology, and biophysics. During his postdoctoral work at Professor Wolfgang Weninger's laboratory, he manipulated the expression of Arp2/3, one of the critical actin nucleators, by the shRNA-mediated knockdown. Findings from this study expand our knowledge of the regulation of the actomyosin cortex in T cells. His PhD study was focused on dissecting the molecular mechanisms that underpin the functional specialization of the actin cytoskeleton. Using fluorescence imaging and single-molecule analysis, he developed microfluidics assays and succeed in detecting the binding of single tropomyosin's into polymers with actin *in vitro*. In the biomedical field, he worked on the development of applications and assays which pave the way for more accurate and accessible therapies.

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