Development and applications of single-particle tracking-FRET microscopy

by

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Abstract

Fluorescence microscopy techniques, including the fluorescence-based single-particle tracking (SPT) and fluorescence resonance energy transfer (FRET), have become powerful methods and have been extensively used to advance our knowledge in biological sciences. In this thesis, we introduce the development of an integrated fluorescence microscopy system for conventional single- and dual-color fluorescence imaging, single- and dual-color SPT and FRET measurement. Moreover, we discuss several biological applications using this microscopy system, including the investigations of the self-inactivation mechanism of stromal interaction molecule 1 (STIM1) as well as the stepping behavior and cellular functions of myosin X.

STIM1 is a calcium sensor protein located at the endoplasmic reticulum (ER) membrane. When calcium is depleted in the ER, STIM1 can activate calcium release activated calcium (CRAC) channels at the plasma membrane. Abnormal activation of STIM1 causes several diseases including Stormorken syndrome, York platelet syndrome and tubular aggregate myopathy. Thus, STIM1 should be tightly inactivated under the normal conditions. Recently, a conserved domain in STIM1, named inactivation domain of STIM (IDstim), has been discovered to play important roles in the self-inactivation of STIM1. Using live-cell intramolecular FRET, we have studied

the inactivation mechanism of IDstim. We found that the cytosolic region of STIM1, including coiled coil domain 1 (CC1), CRAC activation domain (CAD) and IDstim domain, formed compact conformation to maintain the self-activated status. We also found that such a compact conformation was abolished if the hydrophobic interaction between CC1 and CAD was destroyed.

As another application, we have studied the stepping mechanism of myosin X. Myosin X is a unique myosin motor that forms anti-parallel dimers. Recent studies have demonstrated that myosin X has flexible lever arm extensions and preferentially moves on bundled actin filaments. The unique structure of myosin X also leads to its special abilities at cellular level to move on and generate filopodia, which are membrane protrusions formed by bundled actin filaments. Using dual-color SPT, we have investigated the stepping mechanism of individual myosin X motors on bundled actin filaments. We found that single full-length myosin X took variable step sizes on bundled actin filaments. We also found that a mutant myosin X, whose anti-parallel coiled coil was destroyed, did not retain the capability to induce and promotes filopodia in cells whereas the full-length myosin X generated filopodia normally. In addition, we found that the ability of myosin X to induce and promote filopodia formation was inhibited by co-expressing the cargo binding domain (CBD) of myosin X.