

Live Cell Imaging Methods to Quantify Signaling Molecule Dynamics

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Examining protein distributions and dynamics in live cells using high-resolution bio-imaging methods and biophysical techniques can reveal information about the real-time activities of cell signaling networks. Modeling the temporal changes that can be observed with fluorescence microscopy and their relationship to protein function along complex signaling pathways can help to shed light on the underlying mechanisms that regulate cell signaling. Here, we describe the use of quantum dots (QDs) and Visible Fluorescent Protein (VFP) labels to track and model the dynamics of membrane receptors and cytoplasmic signaling proteins.

Keywords — IgE Receptor, FcεRI, quantum dots, single particle tracking, electron microscopy, ERK, translocation

I. BACKGROUND

The responses of a cell to its surrounding environment result largely from the transduction of signals from the outer cell surface to the cytoplasm and nucleus. Strict regulation of signal transduction is crucial for cell survival, differentiation and proliferation. Unregulated signaling is an important component in the pathogenesis of many diseases. However, many aspects of how the cell maintains spatio-temporal control of signaling pathways remain unclear.

Correlating protein activity with spatial distribution, dynamics and binding partners is essential for understanding cell function. Fluorescence imaging microscopy not only complements the existing biochemical, genetic and physiological techniques for studying cellular function, but also adds information on subcellular localization and temporal resolution. The development of genetically encoded VFP-tags and the use of QDs have significantly advanced the use of fluorescence microscopy in cell biology [1]. These advances have enabled the quantification of dynamic processes along signaling pathways in the context of the living cell.

II. MEMBRANE PROTEIN DYNAMICS

We have used QD-tags to characterize the motion and distribution of the high affinity IgE receptor, FcεRI. FcεRI is the principal multi-subunit immunoreceptor on the surface of human mast cells and basophils. Crosslinking of IgE-FcεRI complexes by multivalent allergen initiates complex

signaling pathways, leading to the release of mediators of allergic inflammation. To study FcεRI dynamics, we generated a monovalent QD-IgE and performed single QD tracking to measure diffusion of individual receptors under resting and activated conditions [2]. We employed wide field, confocal and TIRF microscopy for multi-color imaging and SPT. In the resting condition, we observed co-confinement of multiple FcεRI in the same region (up to 2 μm in size) and provide evidence that receptors have short residency in nanoscale microdomains. By combining single QD tracking and electron microscopy, we correlate FcεRI diffusional dynamics with topography and demonstrate that small, dynamic clusters reorganize into large, stable signaling domains within seconds of the addition of multivalent antigen.

III. CYTOPLASMIC-NUCLEAR SHUTTLING

One result of FcεRI activation is the initiation of the ERK signaling cascade. Upon activation, ERK1 and ERK2 translocate from the cytoplasm to the nucleus where they phosphorylate transcription factors. Thus, nuclear translocation is critical for successful relay of the signals and evocation of cell responses. However, the mechanism by which ERK1 and ERK2 pass through the nuclear membrane remains unclear.

Specific fluorescent tagging of ERK1 *in vivo* was achieved by fusion with GFP. We studied the real-time dynamics of GFP-ERK1 in living cells after transfection into ERK1-knock-out cells. We determined ERK1 translocation rates under different conditions by real-time imaging and through various FRAP protocols in which the cytoplasm, nucleus or parts of the nucleus were bleached. We also study the effect of ERK1 dimerization [3] on nuclear localization. Comparison with an ERK1 mutant that is dimerization impaired revealed the possibility of two mechanisms of nuclear entry. Mathematical modeling, based on the mechanism of Fujioka *et al.* [4], is being performed to determine the influence of active and passive nuclear entry.

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