Temporal resolution and Fluorescence Resonance Energy Transfer (FRET)-based subcellular visualization of receptor signaling

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Bacteria-triggered signaling events in infected host cells are key elements in shaping the host response to pathogens. We are interested in Carcinoemryonic antigen-related cell adhesion molecule 3 (CEACAM3)-mediated signaling. CEACAM3 is an immunoglobulin-related receptor expressed on human granulocytes and functions as a single chain phagocytic receptor for specific Gram-negative bacteria. The cytoplasmic domain of CEACAM3 contains an immunoreceptor tyrosine-based activation motif (ITAM)-like sequence that is phosphorylated upon receptor engagement. Biochemical assays have revealed several SH2-domain containing cellular proteins as binding partners of the phosphorylated CEACAM3 ITAM-like sequence and we have observed the colocalization of these proteins with CEACAM3 at sites of bacterial infection [1, 2]. However, it is unclear, if this spatial organization of different signaling molecules together with one particular receptor follows also a temporal hierarchy. To address this issue, we take advantage of the broad palette of fluorescence proteins to resolve the kinetics of SH2-domain-mediated recruitment to CEACAM3 by live cell microscopy. As the resolution of conventional light microscopy is limited to about 200 nm, we further employ Fluorescence Resonance Energy Transfer (FRET)-imaging to confirm the direct interaction of CEACAM3 and its partners (Fig. 1). Furthermore, the relevance of the interaction for phagocytosis of bacteria will be analysed. As this methodology is not restricted to CEACAM3-mediated signaling, it will be a valuable tool to dissect receptor induced protein-protein interactions in general.