

RETROGRADE FILOPODIAL TRANSPORT OF ACTIVATED EGF RECEPTORS: SINGLE MOLECULE SENSITIVITY WITH QUANTUM DOT LIGANDS

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The growth factor EGF bound to quantum dots (QDs) activates the cognate receptor tyrosine kinase (erbB1, EGFR) on cell surfaces. The QDs are readily internalized and traffic in the endosomal compartments [1]. Thanks to the great photostability of the QDs, these processes are readily visualized over extended periods of time by confocal laser scanning and wide-field microscopy. The QD ligands can be visualized down to the single nanoparticle level, leading to the unexpected finding of a mechanism for the systematic retrograde transport of QD-EGF-EGFR complexes along filopodial cellular extensions. This mechanism requires activation and interaction of at least two receptor molecules. The transport rates determined by particle tracking and MSD analysis are compatible with or exceed those characteristic of the treadmill activity of the actin bundles constituting the core of the filopodia [2]. Cytochalsin D, that disrupts polymerized actin cytoskeleton, and specific inhibitors of the EGFR kinase activity prevent transport but not diffusion of the receptor. Retrograde transport occurs prior to internalization of the ligand-receptor complex at the base of the filopodia and is presumed to be mediated by an actin-associated adapter and/or motor protein (Fig. 1). These results imply that filopodia serve as sensory organelles for the cell, probing for the presence and concentration of effector molecules far from the cell body, and thereby coupling remote sensing to cellular response via directed transport of activated receptors. QDs are excellent ligands for biophysical studies of cellular activities, particular in combination with the numerous expression probes of cell surfaces receptors, such as EGFR, available and under development.

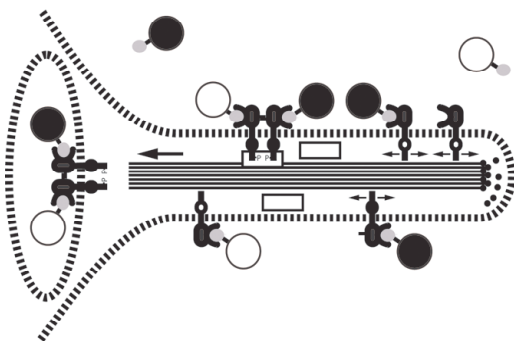


Figure 1: Scheme for retrograde filopodial transport of EGFR present in either monomeric or oligomerized (not shown) forms. Binding of EGF or EGF-QD (open and filled circles) leads to conformational rearrangements in ectodomain II (dimerization loop extended) and other domains (black cytoplasmic region) potentiating the stabilization of an “active dimer” competent for auto- and transphosphorylation. The phosphoprotein (-P) is recognized internally by adapters and/or motor proteins (rectangles) leading to a physical linkage of the receptor complex to F-actin filaments and a shift from random diffusional movement (pair of opposed arrows) to directed retrograde transport. Uptake into the cell body into endosomes occurs at the base of the filopodium, where clathrin coated pits and other components of the endocytic machinery are first available.

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