

EGF-Induced Activation of the EGF Receptor Does Not Trigger Mobilization of Caveolae

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Caveolae-dependent endocytosis has been proposed in the uptake of Epidermal Growth Factor Receptor (EGFR) at high concentrations of ligand. However, we have previously shown that plasma membrane caveolae are stable membrane domains not involved in constitutive endocytosis [1]. We therefore investigated whether stimulation with high concentrations (100 ng/ml) of Epidermal Growth Factor (EGF) induced mobilization of plasma membrane caveolae, either as a bulk movement of cell surface caveolae towards the interior of the cell, or as an increased turnover of caveolae at the plasma membrane. Live-cell microscopy of cells expressing GFP-Caveolin-1 as a marker for caveolae revealed that no net movement of caveolae takes place in cells stimulated with high concentrations of EGF. In addition, Fluorescence Recovery after Photobleaching analysis of GFP-labeled plasma membrane caveolae showed that EGF stimulation does not increase the turnover of caveolae at the plasma membrane. Both in control cells and in EGF stimulated cells, the mobile fraction of caveolae was as low as 20-30%. In contrast, we found that endocytosis of EGFR was efficiently inhibited by knockdown of clathrin heavy chain, both at high and low concentrations of EGF. Our results show that caveolae are not involved in endocytosis of EGF-bound EGFR to any significant degree, and high concentrations of EGF do not mobilize caveolae. We further show that clathrin-mediated endocytosis is the major EGFR internalization mechanism irrespectively of EGF concentration [2].

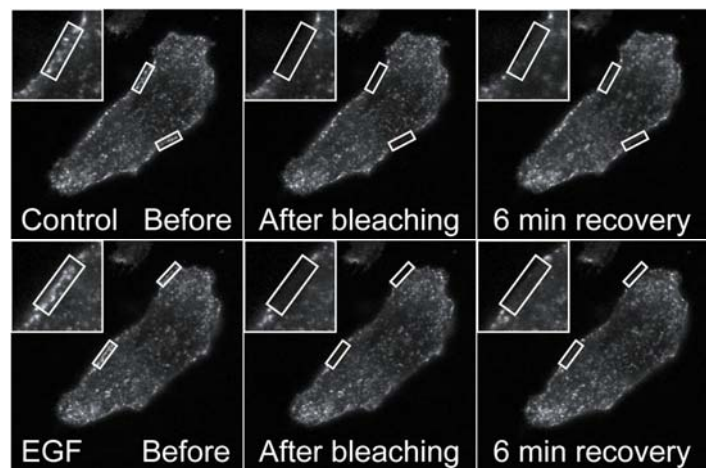


Figure 1 Peripheral caveolar regions of a live cell expressing GFP-caveolin-1 were bleached and the cell was followed by microscopy for 6 min. The same cell was subsequently stimulated with EGF, and after 1-2 min a similar bleaching experiment was performed. The upper panel shows the control experiment, and the lower panel shows the same cell after stimulation with EGF. Note the very limited fluorescence recovery in the bleached region. Figure modified from ref. [2].

[1] Thomsen, P., K.Roepstorff, M.Stahlhut, and B.van Deurs. Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking. *Mol. Biol. Cell* **13**, 238-250 (2002).

[2] Kazazic, M., K.Roepstorff, L.E.Johannessen, N.M.Pedersen, B.van Deurs, E.Stang, and I.H.Madshus. EGF-induced activation of the EGF receptor does not trigger mobilization of caveolae. *Traffic*. **7**, 1518-1527 (2006).