Cell-derived plasma membrane vesicles as minimal cells for analysing transmembrane signalling

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Abstract

Cellular signalling is classically investigated by measuring optical or electrical properties of single or populations of living cells. Here we show how cell-derived vesicles can be used for analysing transmembrane signalling. The vesicles are derived from live mammalian cells by using either chemicals, or by optical tweezers and they comprise parts of the plasma membrane and cytosol of the mother cell. We measured in vesicles derived from individual cells with single molecule sensitivity the different steps of G protein-coupled receptor mediated signalling like ligand binding to receptors, subsequent G protein activation and finally receptor deactivation by protein-coupled receptor mediated signalling like ligand binding to receptors. Observing cellular signalling the smallest autonomous containers capable of performing cellular signaling reactions in individual vesicles opens the door for downscaling bioanalysis of reactions thus functioning like minimal cells. We measured in vesicles derived by optical tweezers and they comprise parts of the plasma membrane, or by optical tweezers and they comprise parts of the plasma membrane vesicles represent subsequent G protein activation and finally receptor deactivation by protein-coupled receptor mediated signalling like ligand binding to receptors. Cell-derived plasma membrane vesicles represent the smallest autonomous containers capable of performing cellular signaling reactions thus functioning like minimal cells. Observing cellular signalling reactions in individual vesicles opens the door for downscaling bioanalysis of cellular functions to the attoliter range for multiplexing single cell analysis and investigating receptor mediated signalling in multiarray format.

Monitoring receptor diffusion and ligand binding

(A) Confocal micrograph of a native vesicle showing the fluorescence of the YFP-A2AR (green, left) and the fluorescence of a receptor-bound antagonist XAC-Atto655 (red, right); scale bar: 1 µm. FCS measured autocorrelation curve of (D) YFP-A2AR (diffusion time t = 22.4 ms) and (E) XAC-Atto655 (t = 32.3 ms). Competition with non-fluorescent XAC confirmed specific binding of XAC-Atto655. (E) Receptor binding of XAC-Atto655 at different concentrations yielded a dissociation constant of Kd = 67±11 nM.

Monitoring receptor silencing

(A) Binding of agonists to GPCRs initiates receptor phosphorylation (by GRKs, PKA or PKC), which in turn leads to binding of arrestin to the GPCRs preventing further activation of G proteins. Here we monitor in an individual native vesicle interaction between GFP-arrestin (in vesicle lumen) and Neurokinin-1 receptor (NK1R, in vesicle membrane) by confocal microscopy. (B) After adding the agonist Substance P to the bulk, it binds to the NK1R and induces rapid recruitment of GFP-arrestin at the vesicle membrane (scale bar: 2µm) as shown in (C) as a time course of the cytosolic fluorescence of GFP-arrestin.

Monitoring G protein activation

(A) FRET was measured between CFP-tagged Gγ subunits and YFP-tagged receptors. Upon agonist binding to the GPCR the heterotrimetric G protein forms a complex with the GPCR leading to FRET between CFP and YFP. (B) Change in FRET depends on the agonist concentration added to the bulk solution (concentrations are indicated at the bars in nM). (C) A dose-dependent increase of the FRET yields IC50 = 104±51 nM.

Conclusion

We show that cell-derived vesicles function as autonomous containers (minimal cell) capable of performing cellular signalling reactions. We measured the different steps of G protein-coupled receptor mediated signalling in individual vesicles. The observation of cellular signalling reactions in individual (sub)micrometer sized vesicles opens the door to downscale the analysis of cellular functions to the attoliter range for multiplexing single cell analysis or investigating receptor mediated signalling in multiarray format.

Vesicles production by optical tweezers

(A-C) Optical micrographs showing the production of a vesicle (arrow) which is pulled off from the plasma membrane of HEK expressing GFP-tagged BK1 receptor with an optical tweezer. (D) Fluorescence micrograph of the vesicle (arrow) selected with an optical tweezer from HEK cells expressing GFP-tagged BK1 receptor.

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(V-E) Sequence of optical micrographs showing the isolation of a preformed vesicle (arrow) selected with an optical tweezer from HEK cells expressing arrestin-GFP and NK1R. Fluorescence micrographs of the vesicle before (I) and after (K) the perfusion with the agonist SP; (J) and (L) are the respective fluorescence profiles of the vesicles.