

HIGHLY PARALLEL SINGLE CELL MONITORING OF RECEPTOR-TRIGGERED MEMBRANE TRANSLOCATION OF A CALCIUM SENSING PROTEIN MODULE

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ABSTRACT

The dynamic behavior of signaling systems can only be explored by measuring signaling time-courses in individual cells since critical cell-to-cell differences are lost in averaged bulk cell measurements [1]. A prominent dynamic behavior of signaling proteins with SH2- PH-, C1-, C2- and related domains [2] is their translocation from the cytosol to the plasma membrane in response to receptor stimuli. In particular, C2-domains that can be found in kinases, lipases and many other enzymes and regulatory proteins [3,4] are intriguing examples of translocation domains since they can target signaling proteins to lipid membranes in response to ubiquitously triggered Ca²⁺-signals [5].

We have simultaneously measured translocation time-courses of fluorescently-conjugated proteins to the plasma membrane in thousands of individual living cells. We show that the C2-domain, a calcium-sensing, lipid binding protein module that is an essential regulator of Protein Kinase C and numerous other proteins, can target proteins to the plasma membrane transiently if calcium is released from internal stores and persistently with

extracellular calcium entry. The temporal C2-domain translocation patterns of individual cells clustered into distinct differentiating and integrating modes, demonstrating that simultaneous recruitment of signaling proteins from one cellular compartment to another is one of the earliest mechanism for inducing discrete states of cellular signaling networks.

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