Direct Monitoring of the Inhibition of Protein–Protein Interactions in Cells by Translocation of PKCδ Fusion Proteins

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Abstract

In the field of drug discovery and development, the increasing use of cell-based assays has resulted in an increased demand for novel cellular bioassays. Such bioassays are expected to detect a wide variety of signaling molecules in real time.[1] In this study, we demonstrate that direct monitoring of the inhibition of protein-protein interactions (iPPI) using a small-molecule inhibitor is possible using a redistribution approach. We used a PKCδ-fused bait protein to co-translocate the target protein and monitor iPPI by chemical inhibitors. PKC is known to translocate from the cytoplasm to the plasma membrane in response to physiological stimuli, as well as exogenous ligands such as phorbol esters. A study using green fluorescent protein (GFP)-tagged PKC revealed that the dynamics of PKC translocation in response to different stimuli can be monitored in real time in live cells.[2] Furthermore, we demonstrated that our assay can be applied to different protein pairs. p53-MDM2 was used to study interaction and inhibition, FKBP-FRB was used to study induction and competitive inhibition, and p90RSK-ERK2 was used to study blockage of signaling pathways. Our technique is robust and widely applicable in analysis of new interaction partners such as chemical compounds, peptides, and proteins using library screens. The approach provides good spatial resolution with a high signal-to-noise ratio and a low false-positive ratio across a wide range of protein expression levels. In this context, we believe that our assay is particularly well-suited for drug discovery applications that are based on high-content and high-throughput cell-based assays.

Reference
