

P001 Activation of endogenous Ras is predominant at the plasma membrane

**Martin Augsten¹, Rico Pusch², Reinhard Wetzker²,
Karlheinz Friedrich¹, Ignacio Rubio²**

¹Institute of Biochemistry, Medical Faculty, Friedrich-Schiller-University Jena, Nonnenplan 2, 07743 Jena, Germany. ²Research Unit Molecular Cell Biology, Medical Faculty, Friedrich-Schiller-University Jena, Drackendorfer Str.1, 07747 Jena, Germany

Owing to its preferential binding to active Ras-GTP versus inactive Ras-GDP, the RBD of c-Raf fused to GFP has proven a useful live-cell reporter of Ras activity. However, this approach fails to report endogenous Ras activation but, instead, requires Ras overexpression to increase fractional probe recruitment and raise signal over background fluorescence. We report that RBD oligomerization provides probes that enable visualisation of endogenous Ras-GTP, obviating Ras overexpression. RBD oligomerization results in tenacious binding to Ras-GTP with one-to-one stoichiometry and interruption of Ras signalling. A trimeric RBD fused to GFP reported agonist-sparked endogenous Ras activation exclusively at the plasma membrane (PM) of COS-7 and Jurkat cells. PM illumination was independent of the stimulus' action on the actin cytoskeleton and its sensitivity to dominant-negative RasS17N matched Ras-GTP formation assessed biochemically. Ras overexpression exacerbated agonist-induced probe recruitment to the PM and caused agonist-independent accumulation of the probe at a perinuclear compartment. Our data illustrate differences in the subcellular localisation of endogenous versus overexpressed Ras-GTP and argue for the PM as the predominant site of growth factor-induced Ras activation.