

P030 Localization-dependent activation of Ras isoforms
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Recent studies indicate that differential subcellular localization of Ras isoforms plays a role in creating isoform-specific signalling outputs. H- and N-Ras show different activation kinetics depending on their location in the cell: plasma membrane localized H- or N-Ras is activated rapid and transient, Golgi localized H- or N-Ras is activated delayed but sustained.

We investigated if these activation patterns are dependent on the Ras isoform or if different signalling environments in the cell cause the observed activation profiles. To rule out any influence of cellular heterogeneity and to observe correlated activities, activation kinetics had to be measured in the same cell. We established a FRET-based method that allows imaging of activation profiles of two different Ras proteins simultaneously. As a probe for plasma membrane localized Ras wildtype K-Ras was used, as a probe for plasma membrane and Golgi localized Ras wildtype H-Ras and as probe that is predominantly localized to the Golgi H-Ras C181S. K-Ras showed the same fast and transient activation as plasma membrane localized H-Ras whereas H-Ras C181S showed delayed and sustained activation like Golgi localized wildtype H-Ras. This demonstrates that the activation profile of a Ras protein is determined rather by its subcellular localization and not by its isoform.