

**P011** Ras knockdown reveals isoform-specific signalling coupled to receptor activation

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The ubiquitously expressed major Ras isoforms, H-, K- and N-Ras are highly conserved, except for a hyper variable C-terminal region that confers differing fatty acid modifications and micro-localisation. We have analysed the efficiency of activation for each Ras isoform following acute stimulation of HeLa cells with either hepatocyte growth factor (HGF) or epidermal growth factor (EGF). For each stimulus, the rank order of activation efficiency was conserved. In both cases, inhibition of receptor endocytosis led to reduced H-Ras and N-Ras activation, but K-Ras was unaffected. RNAi specific to each isoform, resulted in efficient knockdowns that allowed estimation of the actual abundance of each isoform in HeLa cells ( $N \approx K \gg H$ ). Thus we are able to quantitatively profile the contribution of each Ras isoform to the activated Ras pool for the first time. We have analysed Ras-dependent signalling events following specific knockdown of Ras isoforms. Most remarkably, Akt/PKB phosphorylation is highly sensitive to N-Ras knockdown and relatively independent of other Ras isoforms. We propose that this represents a preferential coupling of N-Ras to PtdIns 3-kinase, which cannot simply be accounted for by mass action.