

**P035** Employing live-cell imaging to visualise the control of Ras signalling by the Ras GTPase-activating proteins GAP1<sup>m</sup> and GAP1<sup>IP4BP</sup>.

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In addition to p120<sup>GAP</sup>, mammalian Ras GTPase-activating proteins (RasGAPs) include NF1, the neuronal SynGAPs and the GAP1 family. Within these RasGAPs there are many different arrangements of modular domains indicative of a diverse array of cellular interactions and regulations. Our work on RasGAPs has focused on the GAP1 family, which comprises: GAP1<sup>IP4BP</sup>, GAP1<sup>m</sup>, CAPRI and RASAL. Each protein has a common architecture comprising amino-terminal C<sub>2</sub> domains, a carboxy-terminal PH domain adjacent to a Bruton's tyrosine kinase (Btk) motif, and a central RasGRD. Whereas CAPRI and RASAL are regulated by increases in intracellular free Ca<sup>2+</sup>, GAP1<sup>IP4BP</sup> and GAP1<sup>m</sup> are regulated by phosphoinositides. GAP1<sup>m</sup> is a cytosolic protein that undergoes a rapid plasma membrane association following activation of Class I PI 3-kinases; an event that arises as a result of the GAP1<sup>m</sup> PH domain-binding phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>). In contrast, GAP1<sup>IP4BP</sup> is constitutively associated with the plasma membrane through a complex interaction of its PH domain with phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>). In this state, GAP1<sup>IP4BP</sup> is inactive as a RasGAP, but becomes activate following receptor-mediated elevation in the second messenger inositol 1,3,4,5-tetrakisphosphate (IP<sub>4</sub>). Currently - employing various live cell imaging techniques coupled with Ras biosensors - we are examining the regulation of Ras activation, focusing on how endogenous GAP1<sup>IP4BP</sup> and GAP1<sup>m</sup> modulate the spatial and temporal dynamics of Ras activation following receptor stimulation.