

**P041** Identifying sites of phosphorylation on the eukaryotic initiation factor -2B

**SS Mohammad-Qureshi and GD Pavitt**

*Faculty of Life Sciences, the University of Manchester, UK*

In eukaryotic translation initiation, eukaryotic initiation factor-2 (eIF2), a G-protein, delivers initiator-tRNA<sub>i</sub><sup>Met</sup> to the 40S ribosomal subunit prior to mRNA binding. Upon AUG recognition, eIF2 is released as an eIF2:GDP complex. eIF2B then re-activates eIF2, removing GDP and promoting association of GTP. eIF2B activity can be regulated indirectly, by phosphorylation of its substrate eIF2, or through pathways directly targeting eIF2B nucleotide exchange activity, such as the negative effect observed with butanol. Protein phosphorylation can also down- or up-regulate the activity of eIF2B. Previous literature has identified sites of phosphorylation within mammalian eIF2B $\epsilon$ .

In this study we exploit different approaches to identify the phospho-sites that regulate yeast eIF2B GEF activity and identify kinases controlling these phosphorylation events. Through mass spectrometry, yeast genetics and protein biochemistry we identify phospho-residues on both eIF2B $\gamma$  and  $\epsilon$ . To determine the impact of eIF2B phosphorylation on eukaryotic translation and control *in vivo*, we have targeted these for site-directed mutagenesis to both non-phosphorylatable Ala and acidic Asp and Glu. To identify the kinases responsible we are screening a yeast kinase library and aim to expand our knowledge of the eIF2B regulation network.