

P006 Characterization of the association between kinesin-1 and GRIF-1

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GABA- A receptor interacting factor (GRIF-1) is a member of a coiled-coil family of proteins thought to function as adaptors in anterograde trafficking of organelles to synapses utilizing molecular motor proteins. Here we report the mapping of the GRIF-1 binding domain of kinesin-1 using yeast two-hybrid interaction assays and immunoprecipitation following expression of tagged GRIF-1 and kinesin-1 constructs in HEK 293 cells. GRIF-1 was shown to associate with kinesin-1 heavy chains (KHCs), KIF5A and KIF5C, but not with kinesin-1 associated light chain (KLC). KHCs contain three functional domains, an N-terminal motor domain, an alpha helical stalk domain and a globular C-terminal tail cargo binding domain. Truncation KIF5C constructs generated were:- the motor domain, the non-motor domain (alpha helical stalk and globular C-terminal tail), two individual coil domains within the stalk region and the cargo binding domain. Yeast two-hybrid interaction assays and immunoprecipitation showed that GRIF-1 bound to the KIF5C non-motor region and within that to the cargo binding domain. It has been suggested that kinesin-1 cargoes bind to either KHCs which direct cargoes to dendrites or, to KLCs which direct cargoes to axons. Since we have shown that GRIF-1 associates directly with KHC, this suggests that GRIF-1 is an adaptor involved in anterograde transport in dendrites. Supported by the BBSRC (UK).