Regulation of Cdk5 unveiled by its structure with the p25 neuronal activator

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The enzymatic activity of cyclin-dependent kinases (CDKs) consists in the addition of a phosphate group to target proteins, leading to a temporary modofication of their activities. In order to deliver their activity, CDKs rely on the association with regulatory subunits, whose best characterised examples are known as cyclins. CDK5 is a highly conserved member of the CDK family (Lew et al., 1992; Meyerson et al., 1992), but its expression and regulation are quite distinct. CDK5 activation requires the selective association with proteins known as p35 and p39, whose expression is restricted to the central nervous system (CNS) (Delalle et al., 1997; Humbert et al., 2000; Ishiguro et al., 1994; Lew et al., 1994; Tang et al., 1995; Tsai et al., 1994). Consistently, CDK5 activity is most abundant in the CNS.

Functional alterations in the biochemical machinery involved in neuronal organisation may lead to severe neuro-degenerative manifestations in adult animals (reviewed in (Rice and Curran, 1999)). Alzheimer's disease (AD), a degenerative disorder caused by progressive loss of neurons, is the principal cause of senile dementia in humans. A recent survey of CDK5 and p35 expression profiles in human brain tissues revealed that the accumulation of p25, a 25 kDa C-terminal proteolytic product of p35, strongly correlates with AD affection (Patrick et al., 1999). Proteolytic processing of p35 is operated by the cysteine-protease calpain under a variety of neurotoxic conditions (Kusakawa et al., 2000; Lee et al., 2000; Nath et al., 2000). p35 is a high turnover protein that localises to cellular membranes by virtue of an N-terminal myristoylation motif (Patrick et al., 1999). p25 retains unaltered ability to bind and activate CDK5, but it partitions to a soluble fraction, and it is a stable protein (Patrick et al., 1999). The combination of increased steady-state levels of p25 with the mis-localisation of the p25-associated kinase activity leads to CDK5-mediated tau hyper-phosphorylation, cytoskeletal disruption, and apoptotic death of neurons (Patrick et al., 1999).

In order to understand the structural mechanisms of CDK5 activation by p25, we have raised crystals of a complex of these proteins. X-ray diffraction data were collected on beamline BW7A at the European Molecular Biology Laboratory (EMBL). Using a native dataset in the monoclinic spacegroup, we were able to determine the structure of the CDK5/p25 complex by Molecular Replacement, using a previoulsy reported CDK2 structure as a search model. Protein kinases (the molecule on the left-hand side of the picture) share a characteristic bilobar structure, with a small N-terminal domain rich in β-strands, and a C-terminal α-helical domain. The catalytic cleft and the ATP binding site are located at the interface between the two lobes (Knighton et al., 1991). Ligand access to the active site is regulated by a flexible segment, the activation loop (Endicott et al., 1999; Johnson et al., 1996; Pavletich, 1999). p25 appears as a globular domain, that binds on one side of the kinase. p25 binding results in the activation of CDK5 activity, but the regulatory model revealed by the interaction is unprecedented. We hope to be able to use this structure to understand the basis of selective binding of potential CDK5 inhibitors, to be used as anti-Alzheimer's drugs.

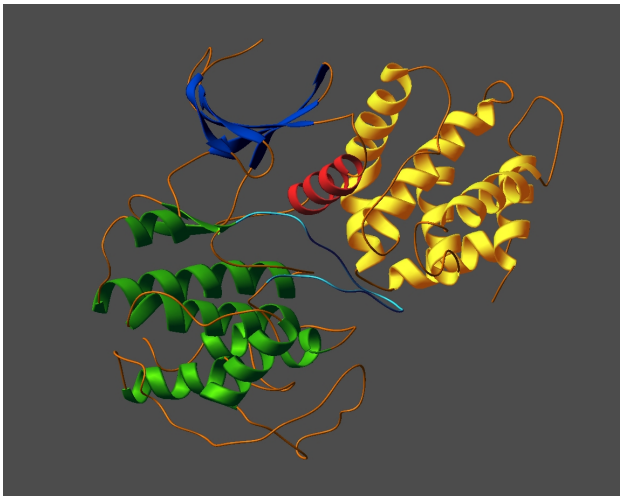

Figure 1: Structure of the CDK5/p25 protein complex

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