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Stepwise improvements in the crystallization of a complex of human Cdk6 and virus-encoded cyclin from herpesvirus saimiri

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Cyclin-dependent kinases (CDKs) play a central role in cell cycle control (Norbury & Nurse 1992; Reed 1992) and are activated by complex formation with positive regulatory proteins called cyclins and by phosphorylation (Morgan 1995). The overexpression and mutation of cyclins and CDKs have been associated with tumorigenesis and oncogenesis. A virus-encoded cyclin from herpesvirus saimiri (vcyclin) has been shown to exhibit highest sequence homology to type D cyclins and specifically activates CDK6 of host cells to a very high degree (Jung et al. 1994). Structure determination of a Cdk6-vcyclin complex will provide the first view of the activated form of human Cdk6. In addition, details of the activation process such as cyclin specificity for different CDKs and the contribution of cyclins to substrate specificity of the kinase complex may be derived from the structure of a CDK6-vcyclin complex.

A complex of human CDK6 and vcyclin from herpesvirus saimiri was purified to very high homogeneity and crystallized. Successful crystallization involved trying slightly different constructs for recombinant protein expression, very efficient purification procedures because of low yields of protein expression, removal of His-tags by highly specific proteases, thorough screening for crystallization conditions that yielded microcrystals, and refinement of crystallization conditions. Crystallization of the complex proved to be difficult due to the need for very narrow concentration ranges of certain ions and additives. Under optimized conditions, large single crystals that diffract to 3.2 Å can be grown. Structure determination from these crystals is currently under way.

Norbury, C. & Nurse, P. (1992). Animal cell cycles and their control. *Annu. Rev. Biochem.* **61**, 441-470.

Reed, S.I. (1992). The role of p34 kinases in the G1 to S-phase transition. *Annu. Rev. Cell Biol.* **8**, 529-561.

Morgan, D.O. (1995). Principles of CDK regulation. *Nature* **374**, 131-134.

Jung, J.U. et al. (1994). Virus-encoded cyclin. *Mol. Cell. Biol.* **14**, 7235-7244.