P. 33

CHARACTERIZATION OF THE MOLECULAR MECHANISM OF ACTION OF A NEW INHIBITOR OF THE COMPLEX CYCLIN-DEPENDENT KINASE 2-CYCLIN A

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Eukaryotic cell cycle progression requires the coordinated interaction and activation of cyclins and cyclin-dependent kinases (CDKs). Since deregulation of cell cycle has frequently been observed in several cancer types, cyclin and cyclin/CDK complexes are considered as anticancer target with a high therapeutic index. Cyclin A binds selectively to CDK2 and CDK1 promoting G1/S and G2/M transitions respectively. A new highly selective class of CDK2/cyclin A inhibitor has been reported recently with a promising therapeutic potential (Canela, N. et al. (2006) JBC 281: 35942-35953). It is a hexapeptide (NBI1) that, in contrast to other existing CDKs peptide inhibitors, binds specifically to a new binding site on cyclin A and inhibit the formation of the cdk2-cyclin A. The characterization of its molecular mechanism of action is of vital importance in its development toward a useful pharmacological compound.

Human cyclin A is a very unstable protein whose structure is only partially known as a complex of a truncated form (173-432) with CDK2. In recent years, mass spectrometry in conjunction with other protein chemistry strategies such as chemical modifications, has emerged as a sensitive tool for probing tertiary structures of proteins and protein-protein interactions. This methodology is specially useful in cases were protein stability and concentration prevent the use of other structure determination techniques.

The main goal of the work is to determine the molecular bases of the interaction between cyclin A and CDK2 complexes with NBI1. We are using a proteomic approach combining protein surface labelling, cross-linking reagents as well as limited proteolysis with mass spectrometry. We have also developed a strategy to determine the exact cleavage sites using fluorous-based labelling of the newly created N-terminal residues.