

1. Jane A. Endicott

Cell Cycle Control

The cyclin-dependent protein kinases (CDKs) are a family of well-conserved enzymes that play multiple roles within the cell. They were first discovered as key enzymes that control eukaryotic cell cycle progression, but recently family members have been shown to be essential for other cellular processes, notably transcription. CDK activities are tightly regulated by multiple mechanisms that include phosphorylation, reversible association with regulatory proteins and location within the cell. Loss of CDK regulation has been genetically linked to the development of human cancers and there is considerable interest in the development of selective CDK inhibitors as novel therapeutics. The aims of our research are to provide a structural understanding of the molecular events that control cell cycle progression and to help to develop methods to identify and characterise molecular interactions with the aim of improving our current protocols for producing proteins and protein complexes amenable to crystallisation.

CDK regulation

Marc Morgan, Tim Johnson, Sarah Major, Christiane Riedinger and Keiko Yata

Our work in this area aims to elaborate the structural principles underlying the regulation of CDK activity. For example, Cdk2 is inhibited by phosphorylation on Tyr15 and the activity of Cdk1 towards certain substrates is modulated by association with Cks1. Structure determination of Cdk2/cyclin A phosphorylated on T160 and Y15 and phosphorylated on T160 and bound to Cks1 (Figure 1) provide a starting point to elaborate the mechanisms by which CDK activity is modulated by phosphorylation and protein association. In this study we are also focussing on selected checkpoint pathways that regulate CDK function. For example, Cdk2 phosphorylation on Tyr15 is a response to DNA damage. The components of the pathways that signal to Cdk2 are known and so there is an opportunity to elaborate their interactions by integrating the results of biochemical, biophysical and structural studies. This knowledge will be essential to ultimately build up a picture of how information flows through the networks of protein interactions within a cell so that we can understand at molecular resolution how a stimulus applied to a cell results in a particular cellular response.

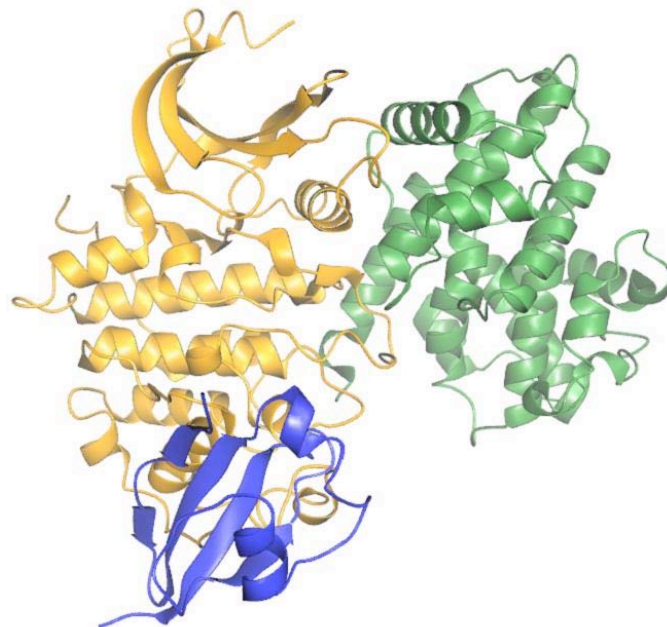


Figure 1 T160pCdk2/cyclin A/Cks1 structure. Cdk2 phosphorylated on T160, cyclin A and Cks1 are drawn in ribbon representation and coloured gold, green and purple respectively.

The *P. falciparum* CDK family

Anais Merckx and Alex Zawaira

Unlike higher eukaryotic CDKs, the CDK family members of lower eukaryotes have not been extensively studied. *Plasmodium falciparum*, the causative agent of human malaria has a cell cycle, the overall organisation of which differs considerably from that in mammalian cells. CDKs have been identified, but it appears that their modes of regulation differ considerably from their higher eukaryotic counterparts. In collaboration with Dr. Christian Doerig (Wellcome Centre for Molecular Parasitology, Glasgow), we are currently determining the structures of *P. falciparum* CDK-related proteins and using biochemical and biophysical approaches to characterize *P. falciparum* CDK macromolecular assemblies. We hope that this approach will allow us to identify unknown components of the *P. falciparum* cell cycle proteome to elaborate the mechanisms controlling their CDK activity. Ultimately we hope that a detailed knowledge of the regulation of the *P. falciparum* cell cycle will reveal opportunities for clinical intervention ([1], Figure 2).

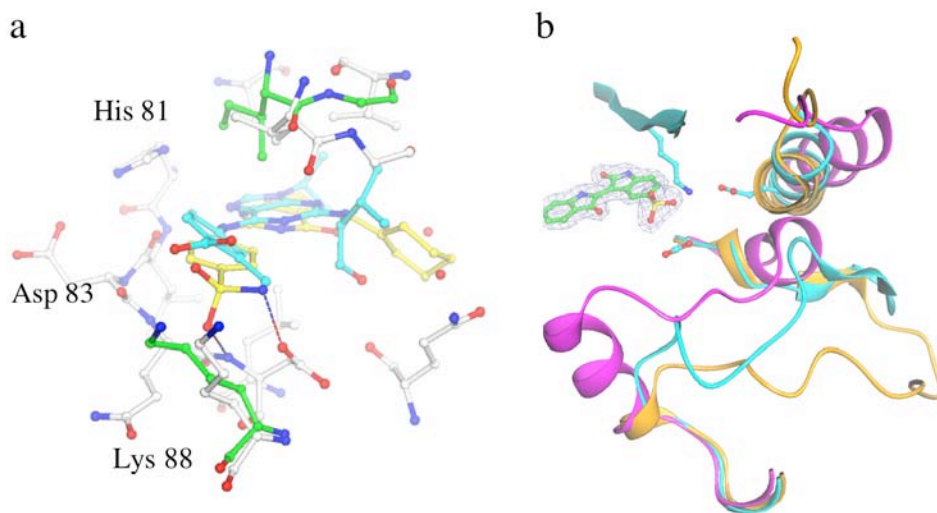


Figure 2 ATP-competitive inhibitor binding to PfPK5.

(a). NU6102 and purvalanol B bound within the PfPK5 active site. All residues in the PfPK5 structure bound to NU6102 within a 7Å sphere of the inhibitor are drawn. The carbon atoms of PfPK5, NU6102 and Purvalanol B are coloured white, yellow and cyan respectively. To illustrate the minor differences between the PfPK5 structures in the glycine loop and at residue Lys88, residues Ile10 and Lys 88 from the PfPK5/purvalanol B structure have also been drawn with carbon atoms coloured white. **(b). Indirubin-5-sulphonate binding to PfPK5.** The structures of monomeric PfPK5 (magenta), T160-phosphorylated CDK2/cyclin A (gold/green) and PfPK5/indirubin-5-sulphonate (cyan) were superimposed. The carbon atoms of the side chains of PfPK5 residues Lys 32, Glu 50 and Asp 143, and indirubin-5-sulphonate are coloured cyan and green respectively. The view highlights the different orientations of the C-helix relative to the rest of the PfPK5 fold in the PfPK5 and PfPK5/indirubin-5-sulphonate structures.

CDK inhibitor design

Aude Echali er

Over the past five years, together with Dr. M. Noble and Professor L. Johnson and in separate collaborations with the ADDI group at Newcastle University (co-ordinated by Professor D.R. Newell) and AstraZeneca Pharmaceuticals, and with Dr. Laurent Meijer (CNRS, Roscoff), we have been working to develop potent and selective small molecule CDK inhibitors ([2-4]). These compounds have excited considerable interest in the pharmaceutical industry as potential lead compounds for cancer therapeutics. In the context however of other ongoing projects they may also offer important leads for developing small molecule probes for dissecting the roles of the CDKs in controlling the *P. falciparum* lifecycle and if validated, a route to novel anti-malarials.

CDK-related kinases

Julie Welburn

CDK-related proteins in higher eukaryotes are also an area of study. In collaboration with the group of Dr. T. Hunt (CRUK, South Mimms), Dr. R. Graeser (Proqinase, Freiburg) and Dr. J. Joore (Pepsan) we are characterising members of the PCTAIRE family. These enzymes are predominantly expressed in terminally differentiated cells in the brain and testis, a profile that suggests that they may not function in cell cycle control. Unlike the CDKs that encode little more than a protein kinase catalytic domain, the PCTAIREs have an N-terminal extension of 127-198 residues that has been implicated in the regulation of their function. Notably, their activity appears cyclin-independent, they are protein kinase A substrates, and they bind members of the 14-3-3 family. These characteristics are not shared with members of the CDK family and suggest their modes of regulation are distinct.

Ubiquitination and cell cycle control

Jean-Francois Trempe

Timely destruction of cell cycle regulators via the ubiquitin (Ub) mediated proteolytic pathway is essential for cellular homeostasis and is linked to cell cycle progression by CDK activity. As a result of the sequential action of Ub-activating (E1), Ub-conjugating (E2) and Ub-ligase (E3) enzymes a polyUb chain is attached to a protein that acts as a signal to direct it to the proteasome. SCF (Skp1/Cul-1/F-box) E3 Ub ligase complexes recognise phosphorylated substrates, specificity being conferred by the F-box protein present in the complex. The SCF complex containing Skp2 (SCF^{Skp2}) mediates degradation of the CKIs p27^{Kip1} and p21^{Cip1}, as well as p130 (a pRB family member), and Orc1, a component of the origin recognition complex. The importance of timely degradation of cell cycle regulators is highlighted by the observation that altered patterns of protein degradation underlie many cancers and several neurodegenerative diseases including Parkinson's disease. Mutations in the F-box protein Skp2 have been shown to be oncogenic and it can collaborate with ras to transform cells. In various tumours, increased Skp2 expression has been shown to correlate with decreased levels of p27^{Kip1}, an indicator of poor patient prognosis.

Destruction of the CKIs p21^{Cip1} and p27^{Kip1} is signalled by phosphorylation on Ser130 and Thr187 respectively, catalysed by Cdk2/cyclin A or /cyclin E. The phosphorylated CKI is presented to SCF^{Skp2} as a substrate as a subunit of a trimeric CKI/Cdk2/cyclin complex. Association of phosphop27^{Kip1} with SCF^{Skp2} also requires an accessory factor Cks1. We are currently characterising various Skp1 and Skp2-containing complexes with the aim of understanding how p27^{Kip1}, bound to Cdk2/cyclin A is presented to the SCF^{Skp2} complex as a substrate and in what structural context it is ubiquitinated by SCF^{Skp2}.

It is becoming apparent that ubiquitinated proteins are targeted to the proteasome by multiple pathways. The contribution of each pathway to the degradation of a specific protein has been most extensively studied in yeast but is currently poorly understood. One pathway involves the direct binding of polyUb chains to the proteasome subunit Rpn10/Pus1 (*S. cerevisiae/S. pombe*) via its Ubiquitin Interacting Motif (UIM). A second pathway is indirect and

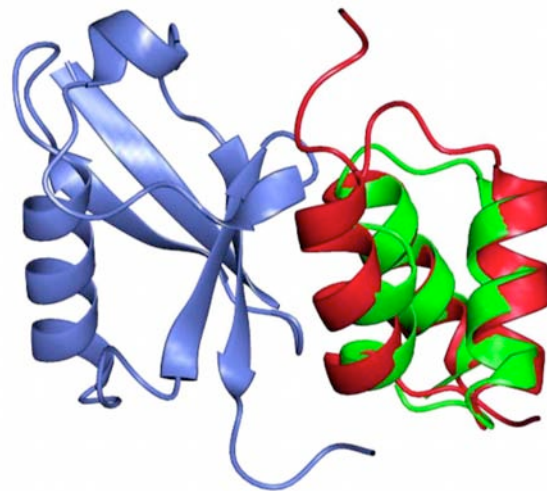


Figure 3 Comparison of the Mud1 UBA domain and CUE domain bound to Ub. Ribbon representation of the structures of Mud1UBA (green) and CUE2-1 bound to Ub (PDB code 1OTR, CUE2-1 coloured red, Ub coloured blue). The Figure highlights the similarities and differences in the folds of the UBA and CUE domains, particularly with respect to the position

is mediated by proteins that contain both a Ub-like (UBL) domain that recognises the proteasomal subunit Rpn1/Mts4 (a subunit of the base region of the 19S proteasomal particle) and a Ub-associating domain (UBA) domain that binds poly-ubiquitinated chains.

In collaboration with Dr. Colin Gordon (MRC Human Genetics Unit, Edinburgh) we are aiming to elaborate the molecular details of the pathways that target ubiquitinated proteins to the proteasome. We have determined the structure of the *S. pombe* Mud1 UBA domain by sulphur SAD at 1.8 Å resolution. The structure shows a three-helix bundle with a well-defined hydrophobic core (Figure 3). HSQC NMR experiments have identified residues important for Ub binding and comparison with a CUE2/Ub complex (Kang *et al.*, (2003), Cell 113:621-30) has given indications of the preference of Mud1 for polyUb chains. We are also using NMR and SPR methods to quantify the enhancement of UBA affinity with Ub chain length and the differences in affinity for Ub that characterise different UBA domains.

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