

2. Jane A. Endicott

Cell Cycle Proteins

Sequential activation of members of the cyclin-dependent protein kinase family (CDKs) orders the events required for DNA replication and cell division. CDKs are controlled through mechanisms that include inhibitory phosphorylation, association with regulatory proteins, and sub-cellular localisation. Our research continues to combine a variety of biochemical and biophysical techniques together with structure determination by X-ray crystallography to elucidate the structure/function relationships of members of the CDK family and their associated regulators. 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.

Current research areas include (i) Elaboration of the structural consequences of CDK phosphorylation; (ii) Characterisation of the interactions between CDKs and their regulatory molecules; (iii) Characterisation of molecular complexes responsible for targeted degradation of CDK regulatory proteins; and (iv) Structural characterisation of the *Plasmodium falciparum* cell cycle proteome. We are also continuing our collaboration with the groups of Louise Johnson and Martin Noble on the development of small molecule CDK inhibitors.

2.1 The structural consequences of CDK phosphorylation

J. Welburn

CDKs are inhibited by phosphorylation within the conserved glycine-loop motif (CDK2 residues 11 to 16) that forms part of the ATP binding site. All CDKs except CDK7 contain a tyrosine residue equivalent to CDK2 Tyr15, and only three members of the family (CDKs 4, 6 and 7) do not contain an adjacent threonine. The extent of active-site phosphorylation is regulated by the opposing activities of the Wee1 (which phosphorylates Tyr15 only) and Myt1 (which phosphorylates both Thr14 and Tyr15) families of protein kinases and the Cdc25 family of dual-specificity phosphatases (reviewed in (1)). Following on from our determination of the structure of a CDK2/cyclinA complex phosphorylated on both Tyr15 (Y15p) and Thr160 (T160p), we are now employing a number of approaches to characterise the effect of Tyr15 phosphorylation on CDK2 activity. CDK active-site phosphorylation is important for regulation both of the unperturbed cell cycle, and of cell cycle arrest in response to checkpoint signaling. For example, sustained active site phosphorylation of CDK1 in yeast, and of CDK1, CDK2 and CDK4 in higher eukaryotes is a response to DNA damage. From this starting point, we plan to use biochemical, biophysical and structural approaches to further understand the macromolecular complexes involved in DNA damage checkpoint signaling to inhibition of CDK/cyclin complexes.

2.2 CDK complexes

N. Schueller, S. Holton, S. Major, and J. Welburn

CDK activity is tightly controlled through protein association and we are currently characterising a number of CDK complexes. This work on full-length proteins is complemented by peptide binding studies to map protein-protein interaction sites. As described in last year's report we have developed a strategy to purify a Y15pT160pCDK2/cyclin A/Cdc25A complex. CDK2/cyclin A also associates with Skp1 and Skp2 in a complex that is present in elevated levels in human transformed cell lines compared to their non-transformed counterparts (2). Cks proteins bind to CDKs and have been proposed to regulate CDK activity by modulating their ability to recognise substrates (3). This may in certain cases be through the ability of the proposed Cks anion-binding site (4) to selectively recognise phosphoamino acids. To elaborate the role of Cks1 in modulating CDK function we currently have a CDK2/cyclin A/Cks1 complex in crystallisation trials.

2.3 Ubiquitin-mediated protein degradation and cell cycle control

N. Schueller and J. Gruber and in collaboration with N. Brown

The ubiquitin (Ub)-mediated protein degradation pathway acts to selectively target proteins to the proteasome (reviewed in (5)). Through the sequential action of Ub-activating (E1), Ub-conjugating (E2) and

Ub-ligase (E3) enzymes, a polyUb chain is specifically attached to target proteins as a signal for their degradation. The molecular interactions by which the Ub-conjugated protein is specifically recognised by the protein degradation machinery and transferred from the E3 complex to the proteasome are beginning to be elucidated. Our interest is in E3 Ub ligases that control cell cycle progression. In particular SCF^{Skp2} is a complex of at least five subunits that is required for S-phase entry and the anaphase promoting complex/cyclosome (APC/C) is essential for cell passage through mitosis (reviewed in (6)).

SCF^{Skp2} is a member of a large family of SCF complexes which all contain the proteins Rbx/Roc1, Cul-1, Skp1 and Cdc34 (the E2 or ubiquitin-conjugating enzyme). SCF complexes are distinguished by the identity of their F-box protein the component that mediates the complex's substrate specificity (7). Two important SCF^{Skp2} targets during G1 phase are the cyclin-dependent kinase inhibitor p27^{Kip1} and the transcription factor E2F-1. Recent research has shown that Cks1 may have a second CDK-independent function to assist Skp2 in substrate recognition (8). We are characterising various components of the SCF^{Skp2} complex and a number of proteins that selectively bind poly-ubiquitinated proteins to elaborate the molecular details of how selected cell cycle regulatory proteins are so efficiently targeted for destruction.

2.4 Structural Characterisation of the *P. falciparum* cell cycle proteome

S. Holton and D. Burgess

Plasmodium falciparum is the causative agent of human malaria which affects 400 million people every year. The identification of novel anti-malarial drugs is urgently needed as the disease is responsible annually for 1-2 million deaths, mostly among sub-Saharan children. The overall organisation of the cell cycle in malaria parasites differs considerably from that in mammalian cells and available data indicate that structural and functional peculiarities of the regulatory molecules involved underlie this observation (reviewed in (9)).

At the sequence level, members of the *P. falciparum* CDK family (PfCDKs) display 40-60% identity with CDKs from other Eukaryotes over the catalytic domain. Although sufficient to recognise these proteins as members of the CDK family, this leaves ample room for structural divergence. Furthermore, several PfCDKs carry large extensions and even insertions within the catalytic domain.

We have initiated a collaborative project with C. Doerig (Wellcome Trust Centre for Parasitology/INSERM, Glasgow) to apply X-ray crystallographic approaches to determine structures for *P. falciparum* CDKs and cyclins, both alone and in complexes and to use biochemical and biophysical approaches to characterize *P. falciparum* CDK macromolecular assemblies. This work may identify unknown components of the cell cycle proteome to elaborate the mechanisms controlling CDK activity and to identify novel targets for structural studies. However, most importantly, we hope that we will be able to use this information to identify and validate targets to develop potent and selective small molecule inhibitors as potential anti-malarial agents.

We have determined the structure of monomeric *P. falciparum* protein kinase 5 (PfPK5), a member of the *P. falciparum* CDK family that shows 60% sequence identity with human CDK1. It is the second example of a monomeric CDK fold and the first from a non-human species and provides an opportunity to compare the conservation of CDK fold and function between two evolutionarily distinct organisms. All aspects of the monomeric CDK2 architecture are well conserved (Figure 1) and the two proteins have an rmsd of 0.936Å calculated over all main-chain atoms. The two deletions in the PfPK5 sequence (equivalent to CDK2 residues Lys24 and Ala93) are both accommodated at the apex of β -turns resulting in very little perturbation to the overall structure. The CDK2 C-terminal tail that wraps back along the rear of the protein is absent from PfPK5.

We have also determined structures for two PfPK5 mutants (Thr158Ala and Thr198Ala). In one of the two copies of the PfPK5Thr198Ala mutant within the asymmetric unit, the activation loop adopts an α -helical structure of 2.5 turns that protrudes across the ATP binding cleft (Figure 1). This activation loop conformation is stabilised by crystal lattice contacts and could not be accommodated within the wild-type PfPK5 crystals. These results suggest that the PfPK5 activation loop can be stabilised into a distinct preferred conformation, but that it is inherently flexible. Monomeric PfPK5 is inactive, and PfPK5T158A is activated by binding cyclin A. Taken together, these results suggest that PfPK5 requires cyclin binding to induce rearrangement of the activation loop to create the protein-substrate binding site and that it does not require activation loop phosphorylation for activity.

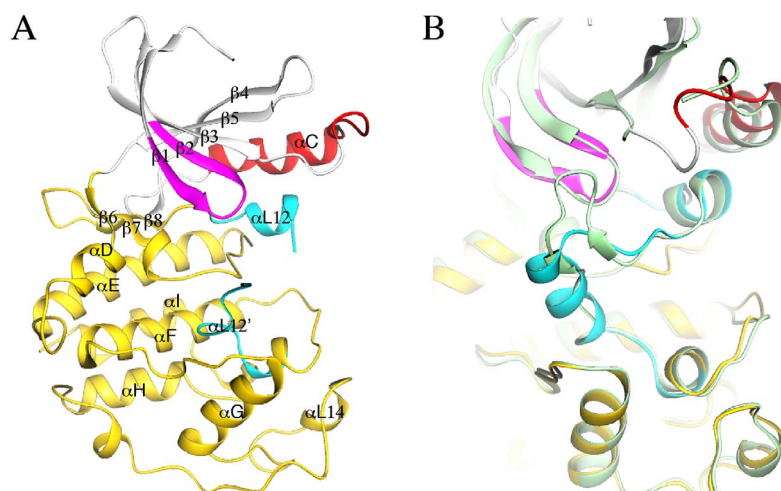


Figure 1. (A) The monomeric PfPK5 fold. The N-terminal domain (residues 1-82) is coloured white and the C-terminal domain (residues 83-288) gold. The PfPK5 N- and C-termini are labelled. The glycine-rich loop (residues 10-19), the C-helix (residues 39-56) and the activation loop (residues 143-170 from the conserved DFG to APE motifs) are coloured magenta, red and cyan respectively. **(B) Overlay of the structures of monomeric PfPK5Thr198Ala and CDK2 in the vicinity of the activation loop.** CDK2 has been superimposed, coloured pale green. The CDK2 activation loop forms a β -hairpin that turns across the end of the glycine-loop, while the activation loop of PfPK5Thr198Ala adopts an extended structure as it stretches away from α L12 into a short α -helix of 2.5 turns.

We have co-crystallised PfPK5 with four inhibitors that together probe regions of the CDK active site that have been shown by studies on CDK2 to mediate potency and selectivity (reviewed in (10)). The PfPK5/Nu6102 and PfPK5/purvalanol B structures show that these potent purine-based inhibitors adopt a similar binding mode within the PfPK5 active site as they do when bound to CDK2 and that the interactions between the inhibitors and CDK2 are conserved in PfPK5 (Figure 2). Sequence differences between PfPK5, CDK1, 2 and CDK4/6 at positions adjacent to the ATP binding site suggest that developing PfPK5-selective ATP-competitive inhibitors may be a realistic goal.

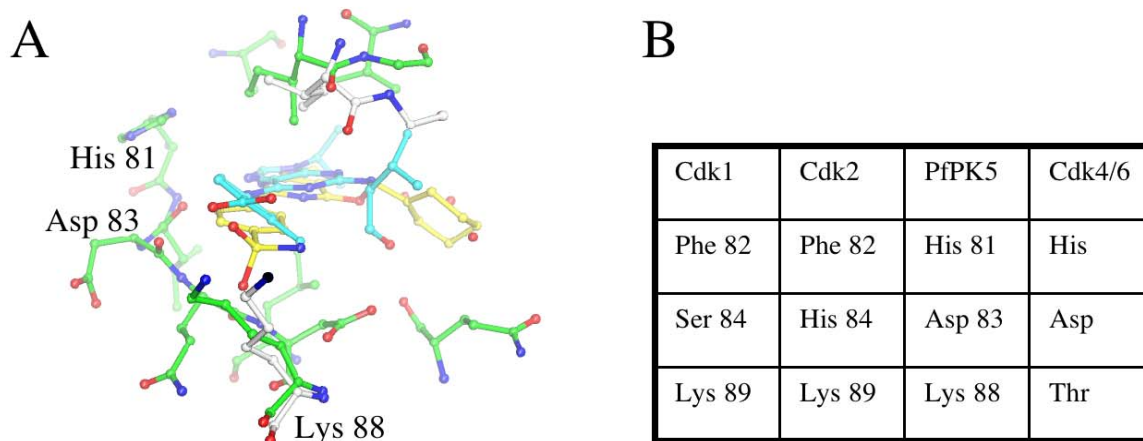


Figure 2. Inhibitor binding to PfPK5. (A) NU6102 and purvalanol B bound within the PfPK5 active site. All residues in the PfPK5 structure bound to purvalanol B within a 7 Å sphere of the inhibitor are drawn. The inhibitor and PfPK5 structures are drawn in ball and stick mode with carbon atoms of PfPK5, NU6102 and purvalanol B coloured green, yellow and cyan respectively. To illustrate the minor differences between the PfPK5 structures in the glycine loop and at residue Lys89, residues Ile10 and Lys 89 from the PfPK5/NU6102 structure have also been drawn with carbon atoms coloured white. **(B) Sequence differences between CDK1, CDK2, CDK4/6 and PfPK5 close to the ATP nucleotide-binding site.**

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