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Report:

Sequential activation of the CDKs directs progression through the eukaryotic cell cycle (reviewed in (1)). CDKs are heterodimeric complexes composed of a catalytic protein kinase subunit and an essential activatory cyclin partner. Both protein families contain multiple members. The order of activation of the different CDKs and their specific association with different cyclins are important for ensuring that critical steps are completed before cells enter the next cell cycle stage. The CDKs are regulated by multiple mechanisms that include additional association with activatory and inhibitory molecules, modification by phosphorylation and programmed proteolysis.

Datasets were collected on crystals of four proteins involved in cell cycle control: (i). *CDK2.* CDK2 complexes with cyclin E in Gl and Gl/S. Throughout late Gl and S-phase, CDK2 is also found in complexes that contain cyclin A. (ii). $p13^{suc1}$. $p13^{suc1}$ is a member of the essential CKS (cyclin-dependent kinase subunit) protein family that binds to CDC2 (CDKl). X-ray crystallographic analysis has shown that CKSs can be isolated in two oligomeric states: one a compact monomeric fold, the second a strand-exchanged

dimer. Analysis of the conservation of residues between CKS molecules across species reveals two highly conserved surfaces, one dominated by hydrophobic residues which has been shown to be involved in the the contact to CDK2 (2), and a second cluster of charged residues whose function is unknown (3). (iii). *GroEL/p25* and (iv). *GroEL/CDK-5*. CDK5/p25 activity is detectable in terminally differentiated neuronal tissue suggesting that CDK activity is not restricted to the control of the cell cycle.

Results

CDK2 crystals were grown under different conditions from those previously reported (4). The crystals tested were rather small (typically 20 x 60 x 60 μm) but a complete dataset was collected to 2.8 Å resolution. Subsequent data processing confirmed that the crystal form was identical to that previously reported (5), unit cell dimensions a=53.2 Å, b=71.7 Å, c=72.4 Å, $\alpha=\beta=\gamma=90^{o}$, space group $P2_12_12_1$ and a molecular replacement solution using the published CDK2 coordinates (5) was successful.

p13suc1 strand-exchanged dimer crystals were grown under different conditions from those previously reported. Unfortunately, the "charged cluster site" in our p13suc1 monomer fold crystals is occupied by residues from the second molecule in the asymmetric unit and is not available for binding studies. A second crystal form was grown in the hope that the site might be accessible for analysis. The crystals grew as arrowheads of circa 200μm in length, but only 40μm in width and were variable in quality, but diffracted to 3.5 Å. The unit cell was characterised.

Both GroEL/CDK5 and GroEL/p25 crystals were tested. The crystals appeared substantially more disordered in one dimension, and the diffraction quality was poor to 8-10 A. However the unit cell was characterised and shown to be very similar to that previously reported for GroEL complexes (6). This suggests that the (partially unfolded) substrates are largely buried within the chaperonin.

References.

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5. De Bondt, H.L., et al., (1993), Nature 363: 592-602.
6. Boisvert, D.C., (1996) Nature Struct. Biol. 3: 170-7.