ESRF	**Experiment title: Structural studies on signal transduction pathways and cell cycle control.**	**Experiment number**: LS-1382 (LS-912)
Beamline: ID14-3 BM14	**Date of experiment**: from: 5th March to: 6th March, 1999 from: 30th April to: 1st May, 1999	**Date of report**: February 17th, 2000
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Report: Cyclin dependent kinase specificity: crystal structures of CDK2-cyclin A peptide complexes.

(Nature Cell Biol., (1999), 1: 438-443).

Progression through the eukaryotic cell cycle is driven by the orderly activation of cyclin dependent kinases (CDKs). For activity, CDKs require association with a cyclin and phosphorylation by a separate protein kinase at a conserved threonine (Thr160 in CDK2). Each cell cycle phase is characterised by the expression of different CDK-cyclin complexes that phosphorylate appropriate substrates. The canonical CDK recognition sequence is S/TPXK/R where S/T is the phosphorylatable serine or threonine and X is any amino acid.

The crystal structure of a phospho-CDK2-cyclin A3 substrate peptide complex has been determined at 2.2 Å resolution (Table 1). The peptide substrate HHASPRK, binds in an extended conformation across the catalytic site on the surface of the kinase contacting only the C-terminal lobe of CDK2 and especially the activation segment (Figure 1).

The serine at the P(0) position is directed towards the γ phosphate of the ATP analogue, AMPPNP. The specificity for a proline at the P(+1) position is explained by the contacts with, and conformation of, the activation segment. In both the uncomplexed and peptide complexed phospho-CDK2-cyclin A3 structures, residue Val164 has unusual ϕ,ψ angles (72.5°, 130.8°) that result in the carbonyl oxygen atom being directed away from the substrate. The unfavourable conformation is compensated by a hydrogen bond from Val164 main chain carbonyl O to Arg169. Binding of any residue except proline at the P(+1) site would be disfavoured because of an uncompensated hydrogen bond from the substrate's main chain nitrogen. The differences do not affect Val164 but do affect Val163 whose main chain carbonyl oxygen blocks the P(+1) site in the unphosphorylated complex, thus

indicating the importance of the difference in the phosphorylated activation segment conformation for substrate binding. Phosphorylation results in an increase in activity of CDK2-cyclin A from a basal 0.3% to 100%.

The arginine at the P(+2) site makes no contacts with the protein and is directed to the solvent. A major interaction occurs with the lysine in the P(+3) position that explains the specificity for basic residues. The lysine side chain is within hydrogen bonding distance of the Thr160-phosphate and the main chain oxygen of residue Ile270 on cyclin A3. The contact to Ile270 on cyclin A3 is the only contact to the cyclin. The surface presented by the binary complex indicates that further residues of the substrate would contact sites formed predominantly by cyclin A.

The conformation of the peptide explains the specificity for proline at P(+1) and lysine at P(+3). The restricted conformational angles of a proline residue (ϕ = -62.9°, ψ = 135.0°) direct the side chain of the P(+2) site to the solvent and allow the side chain of the P(+3) site to be directed towards the Thr160-phosphate. MAPK also has specificity for a proline residue in the P(+1) position. In the structure of doubly phosphorylated MAPK, the activation segment has an almost identical conformation to the activation segment of the phospho-CDK2-cyclin A3 substrate complex including the unusual ϕ,ψ angles for the corresponding residue to Val164.

The substrate peptide makes fewer interactions with phospho-CDK2-cyclin A3 on the N-terminal side of the serine. Histidine in P(-3) contacts Trp167, a residue from the activation segment while the histidine at position P(-2) is external and makes one hydrogen bond from NE2 to the main chain of Gly205, the glycine of the conserved CDK motif GDSEID. This structure elucidates the key events required for CDK substrate recognition.

TABLE 1 Statistics for the refined phospho-CDK2-cyclin A3 peptide complex.

Cell dimensions (Å) (Space group $P2_12_12$)	152.6 163.7 73.3
Maximal Resolution (Å)	2.2
Observations	146344
Unique reflections, completeness (%)	51615 (95.4)
R_{merge}	0.12
Mean I / σ(I)	8.1
Highest resolution bin (Å):	2.32-2.20
Completeness (%)	95.4
R_{merge}	0.51
Mean I / σ(I)	2.5
Protein atoms	9062
Residues	1124
Other atoms	827 (62 AMPPNP, 2 Mg, 763 H_2O)
Resolution range (Å)	20.0-2.2
R_{conv}[2] /R_{free}[3]	0.22/0.28
Mean protein main chain temperature factor $(Å)^2$	34.3
Mean peptide temperature factor $(Å)^2$	53.3
RMS deviation bond lengths (Å)/ angles(^{0})	0.019/2.7

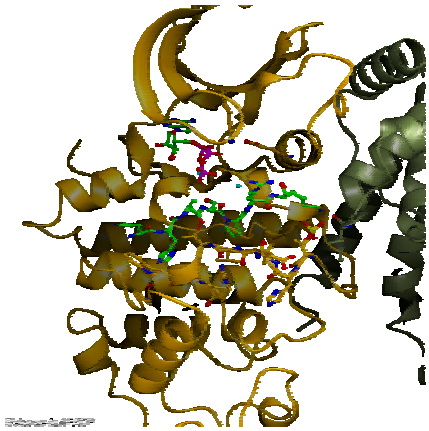

FIGURE 1: Contacts between CDK2 and the substrate peptide.