away from the catalytic cleft, and the cleft itself has opened up, allowing substrates to bind. Furthermore, Thr 160 is now much more accessible to phosphorylation by CAK, explaining why CAK only phosphorylates CDKs complexed with a cyclin. The inhibitory sites of phosphorylation on the CDK, Thr 14 and Tyr 15, have moved further into the catalytic cleft in the cyclin–CDK complex, which would enhance their ability when phosphorylated to hinder phosphate transfer from ATP to a bound substrate.

How does cyclin achieve these changes when it binds to a CDK? Alanine scanning and point-mutation analysis had indicated that the critical CDK regions involved are around the T-loop, as well as a highly conserved region of 16 amino acids called PSTAIRE[5,6]. The PSTAIRE region is also important in determining the specificity of which cyclin binds to which CDK. The cyclin A–CDK2 crystal structure shows that cyclin binds primarily to the amino-terminal lobe of CDK, and, in particular, that two cyclin α-helices clamp to the middle of the PSTAIRE helix by hydrophobic interactions, and to either end of PSTAIRE by hydrogen bonds. This has several effects — it rotates the PSTAIRE helix through about 90°, moving it into the catalytic site; it changes the packing of the amino-terminal lobe; and it melts the αL12 helix. Most importantly, the reorientation of the PSTAIRE helix brings the side chain of the catalytic residue Glu 51 into the active site. In this way the ATP-binding site is reconfigured, and the β–γ phosphate bond of ATP moved into a position favourable for attack from a bound substrate (see figure).

Cyclin also strongly interacts with the amino-terminal portion of the T-loop, which, allied with the melting of the αL12 helix, causes the T-loop to move away from the active site and take up a similar, though not identical, position to that seen for this region in PKA. As already mentioned, Thr 160 is phosphorylated in the fully active kinase and this would stabilize the T-loop in this position through ionic interactions with a basic patch of residues in the carboxy-terminal lobe of the protein, which may alter the conformation of the T-loop to resemble more exactly that seen in PKA. Phosphorylation of Thr 160 also seems to stabilize the cyclin–CDK complex itself, so there may also be ionic interactions with the cyclin.

This brings us to the structure of cyclin itself, and here there is quite a surprise. The region of cyclin in this crystal structure is an amino-terminal helix followed by a repeat of two sets of five α-helices. Each repeat consists of a three-helix bundle with the other two helices packed against the side, and the two sets of α-helices can be superimposed on one another. One repeat of α-helices is formed by the 'cyclin box', the 100-amino-acid region that is most conserved between cyclins and had been shown by mutational analysis to be essential for binding to CDKs. The second repeat extends from the end of the cyclin box to about 40 amino acids from the carboxy terminus of the protein, yet it bears only 12 per cent sequence identity with the cyclin box. Interestingly, this repeated structure was first noted by Gibson *et al.*, who also pointed out a similar repeat in TFIIB and the retinoblastoma family of proteins[7]. When the crystal structures of these proteins are solved it will be fascinating to discover whether this is a conserved structural motif.

The structure of the cyclin box means a variety of observations now make sense. Of the five most conserved residues[8,9] (which have also been shown to be important for CDK binding), Arg 211 and Asp 240 form a buried salt bridge that stabilizes the interaction between the α1 and α2 helices. Two others, Lys 266 and Glu 295, hydrogen-bond with the PSTAIRE region of CDK2 and with each other. The presence of the second repeat extending close to the carboxy terminus of the cyclin may explain why even small deletions from this terminus disable cyclin binding to a CDK. Similarly, deletions extending into the helix amino-terminal to the cyclin box prevent CDK binding[8,10], and this helix wraps around both α-helical repeats, stabilizing the buried salt bridge and interacting with the carboxy-terminal lobe of CDK2. The extensive interdigitation of cyclin A and CDK2 also makes it possible that cyclins contribute to the substrate specificity of the cyclin–CDK complex.

However, all is not yet solved. Apart from the unanswered effect of Thr 160 phosphorylation, this crystal structure is not of a full-length cyclin. A fundamental characteristic of cyclin A is its rapid degradation in mitosis by an ubiquitin-dependent pathway. The region recognized by the ubiquitination machinery is the 'destruction box'[11], which is in the amino-terminal part of the cyclin removed in this crystal structure. Nor does the structure tell us what the cyclin looks like before it binds to the CDK. If cyclin changes its conformation upon binding a CDK, this may be important for interactions with other proteins, such as the CDK inhibitors p21 and p27, and indeed components of the cyclin destruction machinery[9,12–15]. □

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Cultural developments

LICHENS, such as *Usnea articulata* seen festooning this tree in Saudi Arabia, are partnerships between fungi and algae. The fungi concerned are potential sources of novel, pharmacologically active compounds, but the problem is getting at them: lichen-forming fungi are reputedly hard to culture on their own, away from their algal partner. However P. D. Crittenden *et al.* have managed to isolate an impressive 431 lichen-forming fungi from no fewer than 1,021 phylogenetically diverse species, a success rate of 42 per cent (*New Phytologist* 130, 267–297; 1995). Their survey included notoriously tough subjects such as the so-called 'lichenicolous' fungi which, although not symbionts, are obligate inhabitants of lichens; lichen-forming fungi with cyanobacterial (as opposed to simply algal) partners; and fungi of the order Peltigerales, a group of exclusively lichen-forming fungi thought to be phylogenetically ancient, and therefore hard to tease from their symbionts. The most promising subjects were lichens of shrubby habit, such as *U. articulata* (although this species was one of the 590 in which the fungal symbiont remained obdurately faithful to its partner). H.G.