

3-1 Cyclin-Dependent Kinases

The cyclin-dependent kinases are a small family of enzymes that require cyclin subunits for activity

The cyclin-dependent kinases (Cdks) are a family of serine/threonine protein kinases whose members are small proteins (~34–40 kDa) composed of little more than the catalytic core shared by all protein kinases. By definition, all Cdks share the feature that their enzymatic activation requires the binding of a regulatory cyclin subunit. In most cases, full activation also requires phosphorylation of a threonine residue near the kinase active site.

Although originally identified as enzymes that control cell-cycle events, members of the Cdk family are involved in other cellular processes as well. Animal cells, for example, contain at least nine Cdks, only four of which (Cdk1, 2, 4 and 6) are involved directly in cell-cycle control (Figure 3-2). Another family member (Cdk7) contributes indirectly by acting as a *Cdk-activating kinase* (CAK) that phosphorylates other Cdks, as we discuss in section 3-3. Cdks are also components of the machinery that controls basal gene transcription by RNA polymerase II (Cdk7, 8 and 9) and are involved in controlling the differentiation of nerve cells (Cdk5).

We will focus on the small number of Cdks for which there is clear evidence of a direct role in cell-cycle control (see Figure 3-2). In the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* (see section 2-1), all cell-cycle events are controlled by a single essential Cdk called Cdk1. Cell-cycle events in multicellular eukaryotes are controlled by two Cdks, known as Cdk1 and Cdk2, which operate primarily in M phase and S phase, respectively. Animal cells also contain two Cdks (Cdk4 and Cdk6) that are important in regulating entry into the cell cycle in response to extracellular factors.

Cdk function has been remarkably well conserved during evolution. It is possible, for example, for yeast cells to proliferate normally when their gene for Cdk1 is replaced with the human one. This and other evidence clearly illustrates that Cdk function, and thus the function of the cell-cycle control system, has remained fundamentally unchanged over hundreds of millions of years of eukaryotic evolution.

Cdks exert their effects on cell-cycle events by phosphorylating a large number of proteins in the cell. During mitosis in particular, when many aspects of cellular architecture and metabolism are altered, Cdks phosphorylate hundreds of distinct proteins. These Cdk substrates are phosphorylated at serine or threonine residues in a specific sequence context that is recognized by the active site of the Cdk protein. In most cases, the target serine (S) or threonine (T) residue is followed by a proline (P); it is also highly favorable for the target residue to have a basic amino acid two positions after the target residue. The typical phosphorylation sequence for Cdks is [S/T*]PX[K/R], where S/T* indicates the phosphorylated serine or threonine, X represents any amino acid and K/R represents the basic amino acid lysine (K) or arginine (R).

The active site of cyclin-dependent kinases is blocked in the absence of cyclin

All protein kinases have a tertiary structure comprising a small amino-terminal lobe and a larger carboxy-terminal lobe. ATP fits snugly in the cleft between the lobes, in such a way that the phosphates are oriented outwards, toward the mouth of the cleft. The protein substrate

Cyclin-Dependent Kinases

Species	Name	Original name	Size (amino acids)	Function
<i>S. cerevisiae</i>	Cdk1	Cdc28	298	all cell-cycle stages
<i>S. pombe</i>	Cdk1	Cdc2	297	all cell-cycle stages
<i>D. melanogaster</i>	Cdk1	Cdc2	297	M
	Cdk2	Cdc2c	314	G1/S, S, possibly M
	Cdk4	Cdk4/6	317	G1 promotes growth
<i>X. laevis</i>	Cdk1	Cdc2	301	M
	Cdk2		297	S, possibly M
<i>H. sapiens</i>	Cdk1	Cdc2	297	M
	Cdk2		298	G1/S, S, possibly M
	Cdk4		303	G1
	Cdk6		326	G1

Figure 3-2 Table of cyclin-dependent kinases that control the cell cycle

Definitions

L12 helix: small alpha helix adjacent to the **T-loop** in the active site of Cdk2 (residues 147–151), which changes structure to a beta strand upon cyclin binding.

PSTAIR helix: alpha helix in the amino-terminal lobe of Cdks (also known as the $\alpha 1$ helix), which interacts with cyclin and is moved inward upon cyclin binding, resulting in reorientation of key active-site residues. The name of this helix comes from its amino-acid sequence, which is conserved among all major Cdks.

T-loop: flexible loop adjacent to the active site of Cdks, named for the threonine whose phosphorylation is required for maximal activity. Sometimes called the activation loop.

binds at the entrance of the cleft, interacting mainly with the surface of the carboxy-terminal lobe. Nearby residues catalyze the transfer of the terminal γ -phosphate of ATP to a hydroxyl oxygen in the protein substrate.

Cdks have the same two-lobed structure as other protein kinases (Figure 3-3), but with two modifications that make them inactive in the absence of cyclin. These modifications have been revealed by detailed crystallographic studies of the structure of human Cdk2. First, a large, flexible loop—the **T-loop** or activation loop—rises from the carboxy-terminal lobe to block the binding of protein substrate at the entrance of the active-site cleft. Second, in the inactive Cdk several important amino-acid side chains in the active site are incorrectly positioned, so that the phosphates of ATP are not ideally oriented for the kinase reaction. Cdk activation therefore requires extensive structural changes in the Cdk active site.

Two alpha helices make a particularly important contribution to the control of Cdk activity. The highly conserved **PSTAIRE helix** of the upper kinase lobe (also known as the $\alpha 1$ helix) interacts directly with cyclin and moves inward upon cyclin binding, causing the reorientation of residues that interact with the phosphates of ATP. The small **L12 helix**, just before the T-loop in the primary sequence, changes structure to become a beta strand upon cyclin binding, also contributing to reconfiguration of the active site and T-loop. We discuss the structural basis of Cdk activation in more detail in section 3-4. First, we will describe the cyclins and other regulators that influence activation.

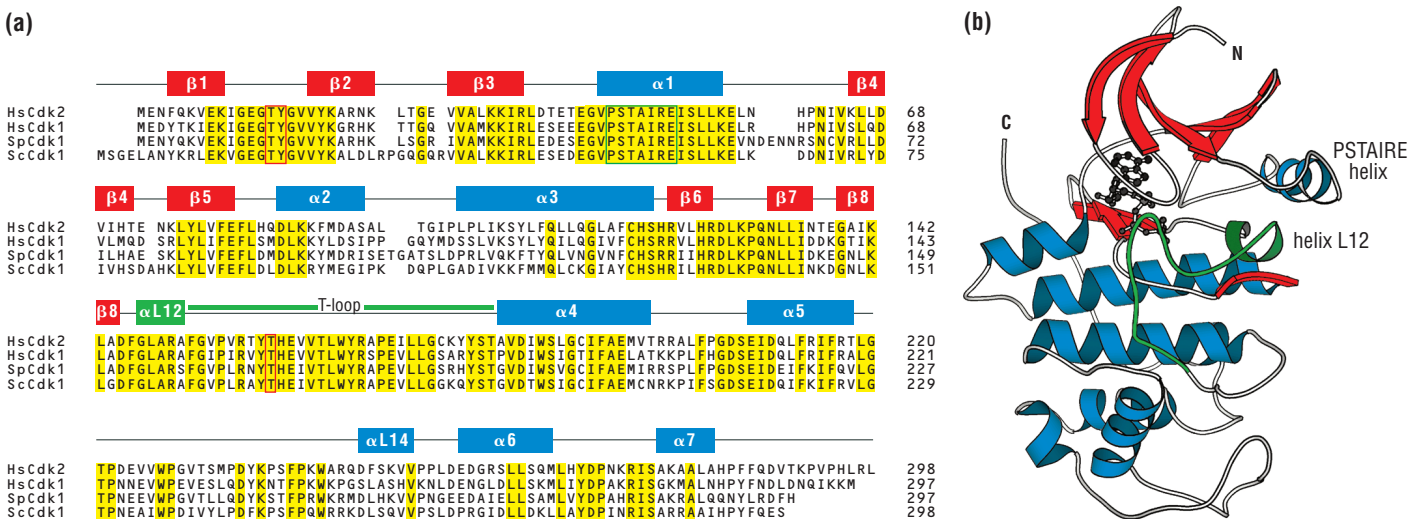


Figure 3-3 Cyclin-dependent kinase structure (a) Amino-acid sequences of major Cdks controlling the cell cycle in humans (*H. sapiens* (Hs) Cdk1 and Cdk2) and yeast (*S. pombe* (Sp) Cdk1 and *S. cerevisiae* (Sc) Cdk1). Yellow residues are identical in all four kinases. Above the alignment, secondary structure elements in human Cdk2 are shown for comparison with the tertiary structure in panel (b). Key landmarks are highlighted, including the PSTAIRE or $\alpha 1$ helix, the inhibitory phosphorylation sites Thr 14 and Tyr 15, the activating phosphorylation site (Thr 160 in human Cdk2) and the T-loop or activation loop where Thr 160 is found. (b) Tertiary structure of human Cdk2, determined by X-ray crystallography. Like other protein kinases, Cdk2 is composed of two lobes: a smaller amino-terminal lobe (top) that is composed primarily of beta sheet and the PSTAIRE helix, and a large carboxy-terminal lobe (bottom) that is primarily made up of alpha helices. The ATP substrate is shown as a ball-and-stick model, located deep within the active-site cleft between the two lobes. The phosphates are oriented outward, toward the mouth of the cleft, which is blocked in this structure by the T-loop (highlighted in green). (PDB 1hck)

References

- De Bondt, H.L. et al.: **Crystal structure of cyclin-dependent kinase 2.** *Nature* 1993, **363**:595–602.
- Morgan, D.O.: **Cyclin-dependent kinases: engines, clocks, and microprocessors.** *Annu. Rev. Cell Dev. Biol.* 1997, **13**:261–291.
- Ubersax, J.A. et al.: **Targets of the cyclin-dependent kinase Cdk1.** *Nature* 2003, **425**:859–864.