

PROTEIN KINASE SUBSTRATE RECOGNITION AND REGULATION

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In this Symposium we celebrate 50 years of signalling. In 1955 Edmond Fischer and Edwin Krebs observed that the activation of glycogen phosphorylase was catalysed by the enzyme phosphorylase kinase resulting in the phosphorylation of a single serine residue (1). For many years, until the discovery of cyclic AMP dependent kinase, signalling by phosphorylation was thought to be an idiosyncrasy related to control of glycogen metabolism. We now know that the process is ubiquitous throughout eukaryotes and that de-regulation of protein kinase signalling pathways is a hallmark of the majority of human cancers.

Glycogen phosphorylase and phosphorylase kinase. Several years ago crystallographic studies on glycogen phosphorylase defined the changes that are promoted by the phosphorylation of Ser14 and the allosteric mechanism that linked phosphorylation to the catalytic site (2, 3). The addition of a single phosphate group to this large enzyme (a dimer of individual subunits molecular weight 97,400) is sufficient to induce strong subunit/subunit contacts that mediate conformational changes at the catalytic site some 66 Å away, showing the power of phosphorylation to influence proteins. Later we determined the structure of the catalytic subunit of phosphorylase kinase and were able to demonstrate how the kinase recognised the local epitope surrounding the phosphorylated serine (4, 5). One problem was never solved however. It is known that phosphorylase kinase is a very much better enzyme against glycogen phosphorylase than against short peptides. (Phosphorylase is characterised by a $k_{cat} = 67 \text{ s}^{-1}$ and $K_m = 9 \text{ }\mu\text{M}$ while for peptide substrates the $k_{cat} = 1 \text{ s}^{-1}$ and $K_m = 2 \text{ mM}$). The explanation appears to be that phosphorylase kinase recognises other regions on phosphorylase remote from the catalytic site and that these sites help enhance the catalytic efficiency.

Remote site substrate recognition in CDK2/cyclin A. Remote recognition sites are a feature of many protein kinases including the cell cycle protein kinase CDK2/cyclin A, the MAPK family of protein kinases, GSK3 β , and polo-like kinase. Such sites help substrate selectivity and improve catalytic efficiency. They also offer alternative sites for anti-cancer therapy to the more widely exploited ATP binding site of protein kinases (6). CDK2/cyclin A promotes progression through S phase of the cell cycle. It phosphorylates several important substrates that include CDC6, pRb and related proteins such as p107. These substrates contain a sequence RxL (using the single letter code) that binds to a hydrophobic region on the cyclin at a site, which is some 40 Å from the catalytic site (7, 8). Through structural studies we have attempted to investigate whether there is a direct stereochemical route between the recruitment site and the catalytic site or whether the recruitment site operates as a localisation site effectively increasing the local concentration of the substrate. The most recent results with peptides that span the two sites and with a bisubstrate molecule indicated that the latter mechanism is most likely.

Kinase Associated Phosphatase and CDK7. However there is one example in the cell cycle field where there is a direct stereochemical relationship between a remote substrate recognition site and the catalytic site. The Kinase Associated Phosphatase (KAP) dephosphorylates phospho-CDK2 at the end of mitosis, thus ensuring complete inactivation of the kinase before the start of the next cell cycle. Structural studies (9) on the KAP/phospho-CDK2 complex showed that the phosphatase recognised the phospho-Thr160 residue at the catalytic site but other interactions in this region were relatively sparse. The main site of recognition is over 20 Å away where part of the α -G helix and the L14 loop of the kinase interacted with residues from the C-terminal helix of KAP. CDK7, the master kinase that in complex with cyclin H and Mat1 phosphorylates the other CDKs of the cell cycle, is not dephosphorylated by KAP. Structural studies show two amino acid changes in the L14 loop from CDK2. Engineering these two changes into CDK2 prevents CDK2 from being dephosphorylated by KAP, thus demonstrating the power of remote sites to effect catalysis (10). In seeking inhibitors of the cell cycle that might prove beneficial as therapeutic agents for cancer, much attention has been focussed on CDK2 as a target. Recent work that has shown ablation of CDK2 does not prevent cell cycling suggests that such a

strategy may not be as productive as hoped (11, 12). CDK7 offers a possible alternative target. The structural studies have shown that the ATP site of CDK7 has much in common with CDK2, but there are significant differences that could be exploited to engineer specificity. CDK7 plays important roles in transcription through its activities as part of the TFIIF transcription factor where it phosphorylates the C-terminal domain of RNA polymerase II to initiate transcription. Any inhibitor programme would need to take these other activities into account.

Cyclin E. Cyclins play a key role in the orderly progression of the cell division cycle through their timed expression and their ability to bind, activate and enhance substrate affinity of their associated cyclin dependent protein kinases (CDKs). Cyclin E, a G1 cyclin and activator of phospho-CDK2 (pCDK2), is important for cell cycle progression in metazoans. It is essential for entry to the cell cycle from G0 quiescent phase, for the assembly of pre-replication complexes and for endoreduplication in megakaryotes and giant trophoblast cells. Levels of cyclin E decline during S phase in response to auto- and other phosphorylation events that target cyclin E for ubiquitination by the Skp1/cullin/hCdc4 F-box protein (SCF^{hCdc4}) complex and subsequent degradation by the proteasome. Unusually high and persistent levels of cyclin E have frequently been observed in human tumour cells, especially in the most aggressive cancers (13). Cyclin E deregulation is frequently associated with defects in the cyclin E degradation pathway resulting from mutations in the F-box protein Cdc4 (14). We have recently determined the crystal structure of pCDK2 in complex with a truncated cyclin E1 (residues 81-363) at 2.15 Å resolution (15). The N-terminal cyclin box fold of cyclin E1 is very similar to that of cyclin A and promotes identical changes in pCDK2 that lead to kinase activation. The C-terminal cyclin box fold shows significant differences to cyclin A and makes additional interactions with pCDK2, especially in the region of the activation segment. The results will be discussed with reference to the essential roles of cyclin E in cells (16-18) and possible indications for cancer therapy.

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