

Lecture 5. Cell cycle regulation is necessary for normal cell division. Protein kinases that regulate the cell cycle and alter targets are themselves regulated by activators and inhibitors as we learned in Discussion paper 4. What controls the inhibition of the inhibitor, p27? It is modified by adding ubiquitin proteins which target p27 for destruction by proteolysis.

The Discussion paper reveals protein interactions that show how phosphorylation of p27, by its own kinase, causes assembly of SCF-Skp2:p27:Cks complex which is how ubiquitin is added through presentation to E2, the ubiquitin conjugation enzyme. In this assembly Cks can be thought of as an adaptor, and it recognizes the phosphorylation.

Discussion paper

Hao B, Zheng N, Schulman BA, Wu G, Miller JJ, Pagano M, Pavletich NP. Structural basis of the Cks1-dependent recognition of p27(Kip1) by the SCF(Skp2) ubiquitin ligase. *Mol Cell*. 2005 Oct 7;20(1):9-19.

Gibbs

Summarize the E1-E2-E3 system and the proteasome degradation.

What comprises a typical SCF E3 molecule and what are the functions of the components?

What do Cks proteins do? What is special about Cks1 in this case, and how might it function?

How do Cks1 and Skp2 interact?

Sanger

What fragments make up the 4 member complex- Fig 1?

What are the Kd's and how were they determined?

What is an LRR? What secondary structure elements of LRR are used to bind Cks1?

Do Skp2 or Cks change conformation on assembly?

In Fig 2-3, how does the p27 chain bind? How does Thr phosphate of p27 bind? From buried surface guess the interaction energy of the p27 contacts. What is the role of Gly 185 of p27 in the complex stability?

Pauling

How did the authors phosphorylate p27, since it is an inhibitor of the kinase?

Discuss the results in Figure 4.

Which side chains become ubiquitinated?

Discuss the family of F box proteins? How can they be specific?

Franklin

How might SCF Cks be stimulated by CDK2-cyclin A?

Does the Cks 1 region that interacts with Skp2 relate to that that binds the kinase, its common partner?

How did the authors support the idea that the complex in Figure 6B exists?