*Morgan Lecture 2: Cdk substrates*

**Key words and terms**

Cyclin-dependent kinase, kinase substrates, kinase specificity, evolution.

**Review Questions**

1. Review the molecular mechanisms that generate waves of Cdk activity during the cell cycle.

2. Review how protein kinases work, and explain how the analog-sensitive kinase mutant system allows identification of kinase substrates in a crude cell extract containing many different kinases.

3. Explain how the analog-sensitive kinase mutant system allows the construction of a cell line in which a single protein kinase can be specifically inhibited by addition of a chemical to the medium.

4. Describe some of the cellular processes that Cdks regulate as the cell proceeds through the various stages of the cell cycle.

5. Explain how cyclins might influence the physiological function of the associated Cdk.

6. Discuss some of the mechanisms by which phosphorylation changes protein function.

**Facilitator Questions**

1. Discuss the basic features of a mass spectrometer, with an emphasis on the methods used to identify Cdk1 substrates in the experiments described in this lecture.

2. Most phosphorylation events in the cell are not permanent: protein kinases are generally opposed by protein phosphatases that remove phosphates. Why are phosphatases critical for the success of the mass spectrometry approach to finding Cdk targets?

3. Imagine that you have discovered a protein kinase that you suspect helps regulate an essential cellular process such as mitotic spindle assembly. (a) Discuss the methods you might use to assess the importance of your protein kinase in mitotic spindle assembly. (b) Discuss some of the approaches you might use to identify the relevant targets of this kinase.

4. Imagine that you have identified a candidate target for your favorite protein kinase. How do you prove that this candidate is a physiologically relevant substrate of your protein kinase in the cell?

5. Imagine that your studies reveal that your favorite protein kinase has hundreds of potential substrates, as in the case for the Cdks described in this lecture. How do you assess the importance of phosphorylation of so many candidates?

6. The experiments described in this lecture led to the identification of several Cdk substrates that are phosphorylated more rapidly by one cyclin-Cdk complex (Clb5-Cdk1) than another (Clb2-Cdk1). Describe some experiments that would allow you to test if Clb5 specificity for these targets is important in the cell.

7. Fission yeast cells are able to survive reasonably well when they contain just a single cyclin that drives both S phase and M phase. It has been proposed that early eukaryotes also controlled their cell cycle with a single cyclin-Cdk complex. What are the potential problems that might arise when the cell cycle is controlled by a single cyclin-Cdk complex, and how did the evolution of multiple cyclins help solve these problems?

8. Cyclins are destroyed in mitosis (as discussed in Lecture 1), leading to Cdk inactivation; this allows phosphatases to dephosphorylate Cdk substrates. Dephosphorylation of Cdk substrates is essential for the completion of many late mitotic events. Interestingly, all cyclins are not destroyed at the same time in mitosis: Clb5 is destroyed earlier (in metaphase) than most Clb2 (in late anaphase). How might the timing of destruction of different cyclins influence the time at which different Cdk substrates are dephosphorylated?

9. Several Cdk substrates contain clusters of phosphorylation sites that seem randomly scattered in poorly conserved regions. What experiment might you do to test whether the positioning of phosphates in these regions is important for their regulatory function?

**Explain/teach these concepts to a friend**

1. Explain how the analog-sensitive mutant system allows identification of kinase substrates in a crude cell lysate containing many different kinases.

2. Explain the mechanism underlying the high activity of certain cyclins for specific targets.

3. Explain the mechanisms by which phosphorylation can change the function of a protein.

**Research the literature on your own**

1. Learn about the basic principles underlying mass spectrometry.

2. Several Clb5-specific substrates (Orc2, Orc6, Cdc6, Mcm3) form a large protein complex (called the pre-replicative complex or pre-RC) at origins of replication. Why is it important for these proteins to be phosphorylated by Clb5-Cdk complexes at the onset of S phase?

3. One Clb5-specific substrate (Sld2) is a component of a large 'pre-initiation' complex of proteins that forms at origins of replication. Learn about how phosphorylation of this protein (and a related protein called Sld3) is important for the initiation of chromosome duplication.

4. One of the Clb5-specific proteins is called Fin1. What is the function of Fin1, and why is Fin1 a better substrate for Clb5 than it is for Clb2?

5. Phosphatases reverse the effects of Cdks in the cell. In the budding yeast, the phosphatase Cdc14 is the key regulator of Cdk substrates in late mitosis. Learn how Cdc14 is regulated in late mitosis, and how it is thought to promote various late mitotic events.

6. The proteins Sic1 and Cdh1 are excellent examples of proteins that are regulated by phosphorylation by Cdk1 at multiple sites. Learn how the multi-site phosphorylation of these proteins regulates their function.

**Papers for journal club**

The following papers illustrate the usefulness of the analog-sensitive kinase method. The first paper documents the use of analog-sensitive yeast strains to allow specific inhibition of protein kinases in the cell; the second paper illustrates the use of these mutants to identify Cdk1 substrates, as described in the lecture.

Bishop, A.C., Ubersax, J.A., Petsch, D.T., Matheos, D., Gray, N.S., Blethrow, J., Shimizu, E., Tsien, J.Z., Schultz, P.G., Rose, M.D., Wood, J.L., Morgan, D.O., and Shokat, K.M. (2000) A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* **407**, 395-401.

Ubersax, J.A., Woodbury, E.L., Quang, P.N., Paraz, M., Blethrow, J.D., Shah, K., Shokat, K.M., and Morgan, D.O. (2003) Targets of the cyclin-dependent kinase Cdk1. *Nature* **425**, 859-864.

The following papers illustrate the importance of cyclin specificity in Cdk function. The first paper provides genetic evidence in yeast that the S-phase cyclin Clb5 has greater

S-phase promoting activity than the mitotic cyclin Clb2. The second paper includes the identification of Clb5-specific Cdk1 substrates as described in the lecture.

Cross, F.R., Yuste-Rojas, M., Gray, S., and Jacobson, M.D. (1999). Specialization and targeting of B-type cyclins. *Mol. Cell* **4**, 11-19.

Loog, M., and Morgan, D.O. (2005) Cyclin specificity in the phosphorylation of cyclin-dependent kinase substrates. *Nature* **434**, 104-108.

The following paper describes the mass spectrometric approach to the identification of Cdk1 substrates, plus discussions of general issues in the function and evolution of phosphorylation sites.

Holt, L.J., Tuch, B.B., Villén, J., Johnson, A.D., Gygi, S.P., and Morgan, D.O. (2009) Global analysis of Cdk1 phosphorylation sites provides insights into evolution. *Science* **325**, 1682-1686.