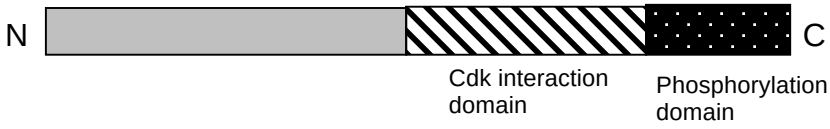
The key: 7.013 Recitation 12 – Spring 2018

1. The cyclin D protein has the following structure.



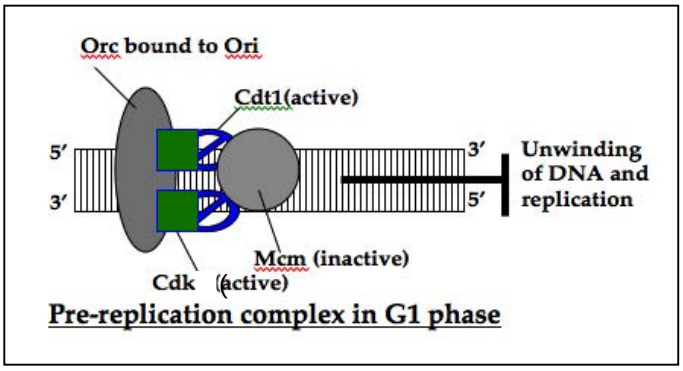
a) What effect would the deletion of the Cdk-binding domain of Cyclin D have on cell proliferation? Explain.*The cyclin will not be able to bind to and activate the CDK. Hence the CDK will not be available to control the regulatory effect of proteins such as RB, p53 on cell cycle. Hence no cell division will be observed.*

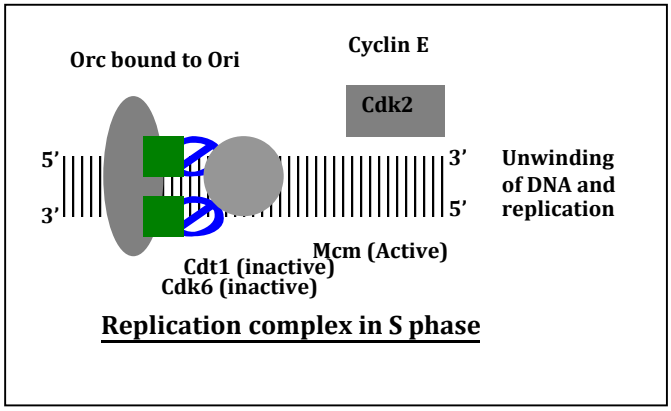
b) Using a temperature sensitive variant of Cyclin D, you find that the cells fail to divide at the non-permissive temperature. The arrested cells have a diploid DNA content (2n) in a single nucleus. At what stage of cell cycle is cyclin D likely to act? Explain. *At G1/S phase since the DNA content is still 2n, meaning that it has not undergone DNA duplication/ replication (4n).*

c) How would the Cdk protein levels vary during the normal cell cycle?
The level of CDK protein remains the same through all the phases of cell cycle. However it becomes functional only when it binds to and is activated by its specific Cyclin.

2. The origin recognition complex (ORC) is a multi- subunit protein complex that binds to the **ori site(s)** and serves as a platform for the assembly of kinases like Cdk6 and Cdt1.

During the G1 phase of the cell cycle in yeast, ORC forms a pre- replication complex by recruiting Cdk6 and Cdt1 that bind to both strands of DNA. These factors bind and inhibit the Mcm protein that functions as a helicase as is shown in the schematic below. ***Note:*** *The activation is shown by an → and inhibition by ⊥ sign.*





a) Activation of the pre-replication complex occurs during the S phase and this requires its interaction with Cdk2 and Cyclin E proteins that degrade Cdk6 and Cdt1. This results in the replication of DNA. Draw a schematic, similar to the one above, to show the regulatory interactions between ORC, Cdk6, Cdt1, Mcm, Cdk2 and Cyclin E proteins. ***Note:*** *Please indicate the activation by an → and inhibition by ⊥ sign.*

b) In a cell showing a Cdk2 **loss-of–function** mutation, in which phase (*choose from G1, S, G2, M, all or none*) would the cell arrest? **Explain** why you selected this option.
In this mutant, since the Mcm protein is never activated the cell will not replicate its DNA and instead remain arrested in the G1 phase.

c) If the cdk-2 gene encodes Cdk-2 protein, in which phase (*choose from G1, S, G2, M, all or none*) will the cdk-2 gene be expressed? **Explain** why you selected this option.
The cdk-2 gene will be expressed in all the phases of cell cycle but the synthesized Cdk-2 protein will be activated only in G1/ S interphase by the Cyclin E protein.

d) If the cyclin E gene encodes Cyclin E protein, in which phase (*choose from G1, S, G2, M or all*) will the cyclin E gene be **optimally expressed**? **Explain** why you selected this option.
Each cyclin shows a transient/ cyclic expression in a specific phase of cell cycle. Therefore, the cyclin-E gene will be expressed only in the G1/ S interphase where it encodes Cyclin E protein, which can bind to and activate Cdk6 protein.

e) You further create two mutant cells each having a mutation in Cdk-2 genes as described below.

- **Mutant cell- type 1:** The Cdk-2 protein lacks its kinase domain.
- **Mutant cell- type 2:** The Cdk-2 lacks its Cyclin E binding domain.

Predict what would happen to the **cell cycle** in…

iii. **Mutant 1:** *The cells will arrest in the G1 phase.*

iv. **Mutant 2:** *The cells will arrest in the G1 phase.*

3. Proteasomal complex can degrade any misfolded protein.

a) What is the highest order of protein structure for the proteasomal complex?

Primary **Secondary** **Tertiary** **Quaternary**

b) Circle the correct option. Proteasome mediated degradation requires ATP. The hydrolysis of ATP is example of …

Exergonic reaction **Endergonic reaction**

c) If, within a cell, the proteasome complex has a mutation as a result of which it cannot bind to the ubiquitin binding site, would you expect the cell to survive and have normal functions? Why or why not?
No, this will result in accumulation of misfolded proteins, which are the causes of multiple neurological disorders.

7.013 Introductory Biology

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