20 Regulating the Eukaryotic Cell Cycle

Review the Concepts

1. The unidirectional and irreversible passage through the cell cycle is brought about by the degradation of critical protein molecules at specific points in the cycle. Examples are the proteolysis of securin at the beginning of anaphase, proteolysis of cyclin B in late anaphase, and proteolysis of the S-phase CDK inhibitor at the start of S phase. The proteins are degraded by a proteasome, a multiprotein complex. Proteins are marked for proteolysis by the proteasome by the addition of multiple molecules of ubiquitin to one or more lysine residues in the target protein. Securin and cyclin B are both polyubiquitinylated by the APC/C complex. The S-phase CDK inhibitor is polyubiquitinylated by SCF.

2. When fused with a cell in S phase, a cell in G_1 will immediately enter S phase and begin DNA replication because S-phase promoting factors (SPFs), which are the S-phase CDK complexes, can activate prereplication complexes on replication origins in G_1 nuclei. However, prereplication complexes have already initiated replication by G_2 and M phase, and cannot be reassembled until the activity of B-type cyclin-CDK complexes falls in late anaphase, ensuring that DNA is replicated once and only once per cell cycle. Therefore, G_2- and M-phase cells will not initiate DNA replication when fused to an S-phase cell. Instead, the M-phase cell will induce the S-phase cell to begin chromatin condensation prematurely, since the S-phase cell is susceptible to the mitotic cyclin-CDK complexes, which function as mitosis-promoting factor (MPF). When G_1- and G_2-phase cells are fused, each will enter S phase according to its own timetable since the G_1 nucleus is licensed for replication and the G_2 nucleus will not be licensed until that cell progresses through M phase and forms prereplication complexes early in G_1.

3. Murray and Kirschner performed a classic set of experiments in frog egg extracts to reveal the essential role of cyclin B synthesis and degradation in cell-cycle progression. In one experiment, extracts were treated with RNase to destroy all endogenous mRNAs. These extracts arrested in interphase, suggesting that an essential protein needed to be translated to drive the cell cycle into mitosis. When RNase-treated extracts were supplemented with a single exogenous mRNA encoding wild-type cyclin B, the extract progressed into mitosis, indicating that cyclin B was the essential protein that had to be synthesized to drive entry into mitosis. When RNase-treated extracts were supplemented with mRNA encoding a nondegradable form of cyclin B, the extract entered mitosis and arrested there with high MPF activity instead of eventually destroying MPF and exiting mitosis. This experiment revealed that degradation of cyclin B was necessary for mitotic exit.

4. The inhibitory tyrosine-phosphate in the T-loop of mitotic CDK is the substrate of Cdc25. MPF activates Cdc25 (by phosphorylating it) and Cdc25, in turn, activates MPF (by dephosphorylating it). Therefore, when a small amount of active MPF is injected into an immature egg, it will phosphorylate and activate the endogenous Cdc25, which can then dephosphorylate and activate MPF. This explains the autocatalytic nature of MPF.

5. The wee phenotype in *S. pombe* displays smaller than usual cells. Premature entry into mitosis, before the cell has grown to the size that normally signals cell division, is the cause of this phenotype. Wee cells result from the excess activity of Cdc2, the cyclin-dependent kinase of *S. pombe* MPF. The wee phenotype can result from a mutation in the *wee1* gene, which encodes the Wee1 protein kinase responsible for catalyzing the addition of phosphate to tyrosine 15 of Cdc2, which inhibits Cdc2 function and prevents premature entry into

mitosis. The wee phenotype also results when a mutation renders Cdc2 insensitive to Wee1 or a mutation in which Cdc25, the phosphatase that opposes Wee1, is overexpressed. Discovery of the wee phenotype and the characterization of the *wee1* gene revealed the intimate link between cell size and cell-cycle progression as well as the important role that tyrosine phosphorylation plays in regulating the activity of CDKs.

6. Phosphorylation of the nuclear lamins by MPF causes their depolymerization, contributing to the breakdown of the nuclear envelope during mitosis. Phosphorylation of nucleoporins causes dissociation of nuclear pore complexes into soluble subcomplexes and subcomplexes with transmembrane proteins that remain associated with the nuclear envelope as it retracts into the ER. Phosphorylation of condensin by MPF or a kinase regulated by MPF promotes chromatin condensation.

7. To initiate sister chromatid segregation at anaphase, the APC/C polyubiquitinylates securin, targeting it for degradation by the proteasome. Degradation of securin releases the enzyme separase, which cleaves kleisin, a component of the cohesin complexes that hold sister chromatids together.

8. The fusion of membranes around chromosomes requires Ran·GTP. The conentration of Ran·GTP is highest in the microvicinity of chromosomes because the Ran-GEF is bound to chromatin. This results in the fusion of extensions of the ER along the surface of chromosomes, forming the double nuclear membrane around each chromosome that results in a karyomere. High Ran·GTP concentration also promotes the fusion of karyomere membranes, which form a single nuclear envelope around all the chromosomes segregated to the same spindle pole during anaphase.

9a. CDKs are active as kinases only when bound to a cyclin. Cyclin-binding exposes the active site of the CDK and also helps to form the substrate-binding pocket.

9b. CAK is a kinase that phosphorylates cyclin-CDKs on a threonine residue in the T loop. This phosphorylation induces a conformational change that increases affinity of CDKs for their substrates, thereby greatly enhancing the catalytic activity of the CDK.

9c. Wee1 is a kinase that phosphorylates CDKs on tyrosine 15 in the ATP-binding region. This phosphorylation interferes with ATP binding and thereby inhibits the catalytic activity of the CDK.

9d. p21 is a stoichiometric inhibitor that binds and inhibits the activity of cyclin-CDKs, usually in response to damaged DNA.

10. G_1 cyclin-CDKs phosphorylate and target Sic1 for degradation, which releases active S-phase cyclin-CDK complexes. They also inactivate the APC/C by phosphorylation of Cdh1, which allows B-type cyclins to accumulate. G_1 cyclin-CDKs promote the synthesis of B-type cyclins by activating their transcription factor, MBF.

11. In *S. cerevisiae*, S-phase cyclin-CDKs become active at the beginning of S, when the CDK inhibitor Sic1 is degraded. These S-phase cyclin-CDKs, as well as other B-type cyclin-CDKs synthesized later in S and in G_2, remain active until late anaphase. Prereplication complexes can assemble on origins of replication only during G_1, when B-type cyclin-CDK activity is low. However, initiation of replication requires the phosphorylation of components of the prereplication complex by S-phase cyclin-CDKs. Once an origin has "fired" (i.e., replication has been initiated), the persistence of B-type cyclin-CDK activity during S, G_2 and M prevents reassembly of prereplication complexes on that origin until the cell has completed the segregation of chromosomes in late anaphase and B-type cyclins are degraded. Therefore, each origin initiates replication once and only once per cell cycle because of the oscillating activity of B-type cyclin CDKs.

12. The restriction point is the place in the cell cycle beyond which cells are committed to completing DNA replication and mitosis even if growth

factors, or mitogens, are removed. To enter the cell cycle, quiescent cells in G_0 require growth factors, which bind to cell-surface receptors and trigger a signaling cascade that leads to the transcription of early-response genes and then delayed-response genes. Among the delayed-response genes is the cyclin D gene, which partners with CDKs 4 and 6, and this mid-G_1 cyclin-CDK complex phosphorylates the Rb protein. When Rb is phosphorylated by the mid-G_1 cyclin-CDK, it can no longer bind the transcription factor E2F. When E2F is released from Rb, then it induces transcription of the genes that promote entry into S phase.

12a. High levels of cyclin D bypass the requirement for growth factors, which normally induce synthesis of cyclin D.

12b. If Rb is not functional, then growth-factor induced synthesis of cyclin D is not required to promote phosphorylation and inactivation of Rb by CDK 4/6. E2F will be constitutively active.

12c. Virally-encoded Fos and Jun bypass the requirement for growth factors to induce expression of cellular *fos* and *jun*, which are early-response genes.

13. Unphosphorylated Rb protein binds to E2F proteins, repressing transcription of genes for various proteins required for the S phase. When Rb is phosphorylated by the mid-G_1 cyclin-CDK, E2F is liberated. E2F activates transcription of genes required for entry into S phase. Two of these genes code for cyclin E and CDK2, the late G_1 cyclin-CDK which further phosphorylates Rb in a positive feedback loop. In this way, Rb remains phosphorylated throughout the S, G_2 and early M phases.

14. A cell-cycle checkpoint is a place in the cell cycle where a cell's progress through the cycle is monitored, and, if the current process has not been completed properly, further progression through the cell cycle is inhibited; the cell cycle is arrested at this checkpoint until the process in question is completed successfully. Checkpoints exist at G_1 and S phases to assess DNA damage, at G_2 to assess DNA damage and to determine whether DNA replication is complete, and at M phase to identify any problems with assembly of the mitotic spindle or chromosome segregation. Because these checkpoints identify problems with the genome (unreplicated, damaged, or improperly segregated DNA) and arrest the cell cycle so that these problems can be fixed, checkpoints can prevent the propagation of mutations into the next cell generation and thereby preserve the fidelity of the genome.

15. In a normal cell, p53 is rapidly degraded as a result of polyubiquitination by Mdm2, a ubiquitin-protein ligase. DNA damage activates ATM and ATR, which phosphorylate p53, blocking the interaction with Mdm2 and therefore interfering with p53 degradation. As a consequence, the p53 concentration increases in cells with DNA damage. p53 acts as a transcription factor for several genes involved in protecting cells from DNA damage. One of these genes codes for $p21^{CIP}$, a cyclin/CDK inhibitor. $p21^{CIP}$ inhibits all mammalian cyclin-CDK complexes and, as a result, the cell cycle cannot be completed until the DNA damage is repaired and phosphorylation of p53 by ATM and ATR ceases.

16. Besides p53, ATM also phosphorylates Chk1 and Chk2 kinases (as well as several additional substrates). Chk1 and Chk2 phosphorylate the phosphatases Cdc25A and Cdc25C, targeting Cdc25A for degradation and inactivating Cdc25C. In the absence of Cdc25 phosphatases, CDKs are maintained with inhibitory phosphorylations, thereby arresting the cell cycle.

17a. Ime2 replaces the G_1-CDK function of phosphorylating Sic1, allowing the cell to enter S phase. The cell cannot use the normal G_1 cyclin-CDKs for this, since diploid yeast cells are induced to enter meiosis by starvation. In the absence of nutrients, the mid- and late-G_1 cyclins are not expressed and consequently cannot function. In contrast, Ime2 is induced by starvation. Since Ime2 is expressed during meiosis I but not meiosis II, DNA replication is prevented during meiosis II, allowing for reduction to 1n chromosome content in the resulting gametes.

17b. Rec8, a homolog of the mitotic cohesin subunit kleisin, maintains centromeric cohesion of sister chromatids during meiosis I. Centromeric Rec8 is protected from degradation by separase during meiosis I, so that sister chromatids remain attached. Rec8 is degraded during meiosis II when sister chromatids must separate.

17c. Monopolin is required for the formation of specialized kinetochores during meiosis I that co-orient sister chromatids of synapsed homologous chromosomes so that they bind to microtubules emanating from the same spindle pole.

Analyze the Data

a. Cyclin B is degraded more quickly when Xnf7 is depleted from the extracts. Thus, these studies suggest that Xnf7 functions in some way to delay cyclin degradation and perhaps the onset of anaphase. Because Xnf7 binds to APC/C, it is possible that Xnf7 normally inhibits APC/C. If so, depletion of Xnf7 would allow APC/C to be activated and target cyclin B for destruction sooner, as observed here.

b. In untreated extracts, cyclin B is polyubiquitinylated 10 minutes after release from metaphase arrest, whereas addition of exogenous Xnf7 delays the onset of cyclin polyubiqutination to 16 minutes. These data reinforce those in (a), suggesting that the presence or absence of Xnf7 affects the timing of cyclin B ubiquitinylation and subsequent destruction. These data suggest that Xnf7 affects the activity APC/C, the ligase responsible for ubiquitinylating cyclin B.

c. Cells proceeding normally through mitosis (see text figure on page 901, top panel) degrade cyclin at 40 minutes after addition of Ca^{2+}. When the spindle checkpoint is activated, as in the presence of nocodazole (middle panel), cells are checked and cyclin B is not degraded as it is in control cells (top panel). However, if the extracts are depleted of Xnf7 (bottom panel), then cyclin B is degraded even though the cells are in nocodazole and should be checked at a stage prior to cyclin degradation. These data suggest that Xnf7 is required for maintenance of the spindle checkpoint.