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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 polyubiquitinated by the APC/C complex. The S-phase CDK inhibitor is polyubiquitinated by SCF.

2. Three of the most important systems employed to study the cell cycle are the single-celled yeasts *Saccharomyces cerevisiae* (budding yeast) and *Schizosaccharomyces pombe* (fission yeast) and the oocytes and early embryos of the frog *Xenopus laevis*. (Studies using mammalian tissue culture cells and model organisms like *D. melanogaster* are also used.)

Genes critical for virtually all aspects of cell division have been described using genetic experiments in yeast. This is done by studying yeast mutants that are blocked at specific steps in the cell cycle or that exhibit altered regulation of the cycle. Researchers can isolate temperature-sensitive mutants whose genes encode proteins that are functional at one temperature but become inactive at a different, often higher temperature, and use this approach to map the cell cycle genes. This can be done in large-scale genetic screens.

Yeast cells are less amenable to biochemical analysis, and the eggs and early embryos of amphibians and marine invertebrates are used to prepare extracts for such analyses. Researchers can obtain extracts from cells at specific points in the cell cycle for biochemical analysis of proteins and enzymatic activities.

3. Hunt and colleagues used a variety of marine invertebrate cell culture systems in their discovery of cyclin proteins. The experimental strategy employed was primarily a biochemical one, in which radiolabeled amino acids were added to the cell culture media under controlled conditions. Isolation of cell extracts at various time points under various conditions enabled the researchers to differentiate between proteins expressed in both the pre- and post-fertilization states of development. Through correlating the presence or absence of several proteins with the cell cycle stage identified through light microscopy in several cell culture systems, Hunt and colleagues were able to identify the cyclin protein family.
4. Both strains of yeast are eukaryotic and can (although do not always) exist in the haploid state, meaning that they carry only one copy of their genome. Both can be easily cultured and utilized to develop mutant strains that contain alterations in single genes. In addition, the genomes of both strains have been sequenced and characterized. The two strains differ from each other in how they divide (by budding or dividing in the middle). Unlike budding yeast, fission yeast have very short G1 and S phases of the cell cycle, and instead spend most of the cell cycle in G2. The molecular mechanisms governing G2 and entry into mitosis in fission yeast is more similar to metazoan cells.
5. The inhibitory threonine 14 and tyrosine 15 of mitotic CDK is the substrate of Cdc25. Cdc25 and mitotic CDKs form a positive feedback loop, and this drives the rapid rise in mitotic CDK activity as cells enter mitosis.
 - a. 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.
 - b. CAK is a kinase that phosphorylates cyclin-CDKs on Thr-160. This phosphorylation induces a conformational change that increases affinity of CDKs for their substrates, thereby greatly enhancing the catalytic activity of the CDK.
 - c. 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.
 - d. p21 is a stoichiometric inhibitor that binds and inhibits the activity of cyclin-CDKs, usually in response to damaged DNA.
6. Mitogen-dependent progression through the first gap phase (G1) and initiation of DNA synthesis (S phase) during the eukaryotic cell division cycle are cooperatively regulated by several classes of cyclin-dependent kinases (CDKs) whose activities are in turn constrained by CDK inhibitors (CKIs). CKIs inhibit the function of CDKs. CKIs that govern these events have been assigned to one of two families based on their structures and CDK targets. The first class includes the INK4 proteins (inhibitors of CDK4), so named for their ability to specifically inhibit the catalytic subunits of CDK4 and CDK6. The second class of CKIs inhibit G1/S-CDKs and S-Phase CDKs, and must be degraded before DNA replication can begin. The activity of CKIs represents an additional layer of cell-cycle regulation over and above that mediated by temporal cyclin synthesis and degradation.

8. START represents a point in the cell cycle beyond which cells are irrevocably 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.
 - a. High levels of cyclin D bypass the requirement for growth factors, which normally induce synthesis of cyclin D.
 - b. If Rb is not functional, then growth-factor induced synthesis of cyclin D is not required to promote phosphorylation and inactivation of Rb by CDK4/6. E2F will be constitutively active.
 - c. p16 (INK4A) is an inhibitor of G_1 /S-CDK complexes. If p16 function is lost, this promotes activation of G_1 /S-CDK complexes and entry into S phase.
 - d. E2F proteins are transcription factors that stimulate expression of genes whose products are required for entry and progression through S phase. E2F activity also stimulates its own expression, and E2F hyperactivity in this regard would further promote entry into S phase.
9. 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.
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 mitotic 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 mitotic 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 mitotic cyclin-CDK activity is low. Origins are activated by recruitment of other initiation factors to the pre-RC to form the pre-initiation complex (pre-IC) and this event requires CDKs and Dbf4-dependent Kinase, Dbf4-Cdc7 (DDK). These events lead to activation of the MCM helicase and recruitment of DNA synthesis machinery. The S-phase kinase, DDK, phosphorylates the MCM helicase, and is thought to be required either for helicase activation or for recruitment of pre-IC factors (or both). Once an origin has “fired” (i.e., replication has been initiated), the persistence of mitotic 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 mitotic cyclins are degraded. Therefore, each origin initiates replication once and only once per cell cycle because of the oscillating activity of mitotic cyclin-CDKs.

12. Phosphoserine/threonine-binding proteins bind to CDK substrates or proteins that control CDK activation and can do one of the following: change the target protein activity, alter its 3D conformation, target it for specific modifications, such as phosphorylation or ubiquitination (and ubiquitin-mediated destruction), or target the protein to a specific subcellular localization. These proteins are also parts of phospho-binding modules that form the positive and negative feedback loops that control progression through the cell cycle and play a role in surveillance mechanisms that stop the cell cycle in response to catastrophic events. The transition from G₂ into M phase involves 14-3-3, Pin1, and Plk1. In G₂, 14-3-3 inactivates the Cdc25 phosphatase. CyclinB-CDK phosphorylation of Cdc25 leads to Pin1 and Plk1 binding and release from 14-3-3 inhibition, leading to progression into M phase. F-box protein Skp2 binds to the SCF ligase, which controls entry from G₁ to S phase. At the G₁/S transition, G₁/S phase CDKs and S phase CDKs reach high levels and begin to phosphorylate p27, targeting it for ubiquitinylation by SCFSKP2. Degradation of p27 causes activation of G₁/S phase and S phase CDKs which then initiate S phase.
13. Monitoring of sister kinetochore attachment to the mitotic spindle is accomplished through a mitotic spindle assembly checkpoint (SAC). The spindle assembly checkpoint (SAC) is an active signal produced by improperly attached kinetochores, and is highly conserved in all eukaryotes. The SAC stops the cell cycle by negatively regulating Cdc20, thereby preventing the activation of the polyubiquitylation activities of anaphase promoting complex (APC). The proteins responsible for the SAC signal compose the mitotic checkpoint complex (MCC), which includes SAC proteins and Cdc20.
14. The regulation of Rho, centralspindlin, and Ect2 provides the mechanisms to ensure that cell cleavage only occurs after chromosome separation. RhoA is a small GTPase which is required for the formation of the actomyosin-based contractile ring that creates a cleavage furrow and divides the cell in half. The centralspindlin complex recruits and activates the RhoGEF Ect2 to the central spindle during anaphase. As cytokinesis begins, these colocalize with and activate RhoA.
15. The function of centralspindlin is inhibited by the phosphoserine/threonine-binding protein 14-3-3. During anaphase, when cyclin B-CDK1 activity is extinguished but Aurora and Polo kinases are still active, Aurora B phosphorylates centralspindlin and displaces the 14-3-3 inhibitor, allowing the MKLP1 component of centralspindlin to acquire catalytic motor activity and compact the spindle midzone. Ect2 is auto-inhibited during prior stages of mitosis but, at the end of anaphase, Plk1 phosphorylation recruits Ect2 to form an active centralspindlin-Ect2 complex. This catalyzes the GTP-dependent activation of RhoA, leading to cleavage furrow formation and cytokinesis.

16. 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. 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 checkpoint pathways identify problems with the genome (unreplicated, damaged, or improperly segregated DNA) and arrest the cell cycle so that these problems can be fixed, checkpoint pathways can prevent the propagation of mutations into the next cell generation and thereby preserve the fidelity of the genome.
17. 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.
18. 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.
19.
 - a. 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 because 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.
 - b. 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.
 - c. 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.
20. In vertebrates, premeiotic DNA replication and recombination occur in the female embryo. The oocytes then become arrested in G_2 until the oocyte is ovulated. The oocytes that enter the meiotic division in a 40 year old women had been arrested in G_2 for 40 years, during which time chiasmata close to the end of chromosomes can slip off or cohesins that hold the homologous chromosomes together during this very long G_2 phase deteriorate causing the homologous chromosomes to dissociate from each other. Both events can cause the meiosis I non-disjunction leading to the formation of eggs with too few or too many chromosomes. Eggs that contain two copies of chromosome 21 instead of one will, upon fertilization produce a person with Down Syndrome.

