mRNA and then degrading it if it is not needed. Therefore, with transcriptional control, no excess work or regulation is being done by the cell.

14. Describe in detail how proteins recognize a specific sequence of DNA. Basically how do proteins "read" the DNA?
    a. Proteins typically bind to the major groove because it has a more complex code due to the more unique interactions possible. Consensus sequences in DNA promoters are binding sites for specific proteins that regulate gene expression. The farther the DNA sequence is from the consensus sequence, the weaker the interaction and the farther the binding between the two is. INTERACTIONS OF R GROUPS BETWEEN R GROUPS AND ATOMS ON NUCLEOTIDES
15. Define consensus sequence and give an example of how different proteins interact with the same consensus sequence with differing degrees of affinity.
    a. A consensus sequence is the highest average nucleotide sequence in DNA in a functional region of the DNA. Different proteins interact with the same consensus sequence with differing degrees of affinity BECAUSE AMINO ACIDS CHANGE; MORE NON-COVALENT INTERACTIONS WITH SAME AMINO ACIDS.
16. Describe the various DNA binding motifs and how they function to interact with the DNA. Include the complexity of protein-DNA interaction that can occur. UNDERSTAND THAT TF'S CAN DIMERIZE
    a. One of the DNA binding motifs is the Helix-Turn-Helix motif, which is where there are two helices with a turn and the helix fits into the major groove it fits into the major groove because there are more interactions there. These proteins can function as a dimer, which have one transcription factor for two proteins. Since they are homodimers, two major grooves are bound and need to be similar since same protein. Also, if one of the proteins binds very well the other protein doesn't have to bind perfectly because it is helped to remain in place by the other protein that is also bound.
    b. Another DNA binding motif is the Zinc Finger Motif, which requires a cofactor. The Zinc Finger Motif can show how multiple DNA binding domains can be found in a single transcription factor. Zn fingers can be linked together to form heterodimers, heterotrimers, etc. because they are not all the same and can bind different consensus sequences. BETA SHEET 2 of them with helix.
    c. The Leucine Zipper Motif must have a leucine at every fourth amino acid, which holds together the Leucine zipper at the hydrophobic core.
    d. Transcription factors can have more than one type of DNA binding domain as well, such as a HTH motif and a Leucine Zipper motif.
    e. Mostly we see a-helices interacting with DNA in major groove (because more interactions); rare to see beta sheets in major grooves or loops used in DNA binding domains interacting with minor groove. This is because loops more flexible and consensus sites tend to have more N's in them.
17. Draw and Describe the structure and function of a Eukaryotic Gene
    a. draw it
18. Explain what it means for a Gene Regulatory Protein (GRP) to be modular. What experimental evidence was there to support this conclusion that GRPs are

one of these, it will bind to the KDEL signal sequence and COPI vesicle and bring it back to the ER. High affinity in Golgi and low in ER because of pH difference.

29. BE able to answer also the questions posed in lecture.
30. Describe the 2 models of how proteins move through the Golgi.
    a. One model is the vesicular transport model where COP 1 vesicles move through the cisternae of the Golgi from cis to trans. The other model is the cisternal maturation model where vesicles from the ER becomes a vesicular cluster and become the cis golgi and the golgi shifts down so that the trans golgi blebs off completely that go various places. Retrograde transport brings proteins back to cis that are needed in cis, etc.
31. Know how proteins get sorted to the various compartments in the cell and how proteins are retrieved if they are mistakenly sent to the wrong compartment.

ENZYME FUNCTION: forgot to add to last study guide.

32. Describe in detail how proteins interact with one another. Use appropriate terminology
    a. Proteins interact by non-covalent interactions, multiple specific interactions (e.g. a nucleotide bound to a specific R group), and binding sites for specific ligands.
33. Explain why a loss of 2-3 non-covalent interactions can dramatically decrease the association between two proteins?
    a. Small changes in non-covalent interaction have big changes in the equilibrium constant which indicated how often proteins are associated with each other. A loss of 2-3 hydrogen bonds can make the associated proteins from most commonly associated to most commonly not associated.
34. Define the equilibrium constant (K). In math mathematical terms as well as functional terms.
    a. The equilibrium constant is the ratio of how often certain proteins are associated with each other to how often they are disassociated. Therefore, if the equilibrium constant (K) is large they are more often together, and if small they are often found apart. [AB]/[A][B] = Keq.
35. What does K_m tell you?
    a. Km is ½ V max and tells the affinity the substrate has for the enzyme. If Km is small then the V max will be reached quicker, so higher affinity for the substrate.
36. How is V_{max} reached?
    a. V max is reached when the maximum enzyme-substrate concentration is reached, and then is determined on how fast the enzyme can process the substrate.
37. Be able to describe the 3 ways proteins interact.
    a. One way is surface-string interaction, where a linear protein binds in the cleft of a protein. Another way is helix-helix binding such as in tendons. The third way is surface-surface where both proteins fit together well like puzzle pieces.
38. What is the ligand of an Enzyme called?
    a. Substrate
39. What is a typical enzyme reaction? Be able to write the reaction equation and

17. Know the GPCR signaling pathways in detail that deal with activating PKA and PKC.
    a. Signaling molecule binds to GPCR which acts as GEF to activate trimeric G protein. This activates phospholipase C-beta which cleaves PIP2 into IP3 and diacylglycerol. Diacylglycerol recruits PKC and IP3 opens a ion-gated Ca 2+ channel that passively releases Ca2+ which binds to PKC, activating it. PKC now active phosphorylates dependent proteins.
18. Understand how Calmodulin monitors the Calcium levels in the cell and how CaM kinase is activated.
    a. The kinase domain is held inactive by the bound inhibitory domain. Calmodulin (protein) without calcium can not bind, and when there is calmodulin with calcium it has a binding domain in which now it associates with the inhibitor domain, which frees the kinase domain (weakly active). Then, the kinase domain phosphorylates the inhibitory domain so if calmodulin falls off the inhibitory domain can't bind to the kinase domain. The phosphate group must be removed before negatively regulated because no protein-protein interactions can occur until it is. The calmodulin is no longer dependent on calcium and phosphatase removes phosphate at some point.
19. Describe a basic RTK signaling pathway.
    a. A signal molecule binds that brings the inactive tyrosine kinases together. Induced proximity causes them to cross-phosphorylate each other activating the kinase domains and having phosphorylated docking sites that acts as a scaffold protein which increases the speed and efficiency of the downstream signal.
20. Know RTK singling pathways in detail that deal with activating Ras and Rho.
    a. First, Boss binds to an RTK in the membrane which activates it allowing for an adaptor protein to dock on one of the RTKs phosphorylated docking sites. A domain of the adaptor protein binds to Ras-GEF which activates Ras. Then a downstream signaling cascade occurs where Ras activates Raf kinase. Raf then phosphorylates and activates Mek, which does the same thing to Erk. Erk then either activates (by phosphorylation) proteins (changing their protein activity) or acts as a transcription factor to change gene expression.
    b. First, if the growth cone binds the incorrect muscle, ephrin A1 will bind to the EphA4 receptor which will activate a tyrosine kinase which will in turn activate the Rho-GEF that will activate RhoA. The sustained activation of RhoA causes the growth cone to collapse and will continue to look for the correct muscle.
21. Understand the JAK/STAT pathway.
    a. The binding of cytokine cross-links the tyrosine kinase receptors and the JAKs cross-phosphorylate each other on the tyrosines. The activated JAKs phosphorylate receptors on tyrosines and then STATs dock on the phospho-tyrosines. The JAKs phosphorylate the STATs and they disassociate, dimerize, and are translocated to the nucleus. Other GRPs are recruited and the complex activates gene expression.
22. Describe how GPCRs and RTK can cross-talk to elicit a specific cellular response.
    a. Any time two or more signaling pathways converge. If you want specific

starts the formation of a branch in the middle of a made actin filament at a 70 º angle.

14. What is the role of γTuRC in microtubule polymerization?
    a. The γTuRC (gamma-tubulin ring complex) forms a ring that caps the - end so they are protected and don't lose monomers while the + ends extent toward the peripheries. It also nucleates the microtubules.
15. Describe the MTOC.
    a. The MTOC is the microtubule organization center which contains all of the - ends of microtubules and keeps them capped while the + ends extend to the peripheries of the cell. A MTOC is always required (even without centrioles if they are severed) because the - ends need to be capped in the middle.
16. What are the proteins that regulate microtubule stabilization and destabilization?
    a. Tau family of proteins and MAP2 are regulated by phosphorylation and when activated tau proteins bind to the side of the microtubule and regulate their spacing. +Tip is another MAP (microtubule associated protein) that helps regulate microtubule length (gamma tubulin binding to + end) and stabilizes it so no catastrophe. While attachment is regulated by End binding 1 (promotes attachment to organelles and cell membranes; decreases dynamic instability).
17. Negative Regulation of Microtubules
    a. Kinesin-13 proteins bind the + end GTP-tubulin and converts it to GDP-tubulin thus promoting catastrophe.
    b. Stathmin binds to two dimers of GDP-tubulin and forces the tubulin filament to bend, thus preventing assembly.
    c. Katanin proteins cuts microtubules from the MTOC, thus promoting - end tubulin loss.
18. What are the motor proteins of microfilaments and microtubules?
    a. The motor protein of microfilaments (actin motors) is myosin. Myosin contains 6 polypeptides, 2 light chains (stock/legs) and 4 heavy chains (head/neck).The motor proteins of microtubules are kinesins and dyneins.
19. Describe the functions of the various types of myosin proteins.
    a. Class 1 of myosin proteins are membrane associated and regulate endocytosis. Class 2 of myosin proteins regulate contraction and have long stocks and two heads. Class five of myosin proteins function in organelle transport and have 2 head groups with a long neck that walks along the F-actin.
    b. The head of myosin converts ATP hydrolysis to do mechanical work in cell. Neck length dictates how long each "step" is in their "walk" down the actin filament. The tail co-evolved with the head to function together in moving specific types of cargo.
20. Draw/Describe the mechanism of myosin movement. (i.e. How Myosin converts the chemical energy of ATP hydrolysis into mechanical work).
    a. Myosins tend to move in one direction: toward the + end of the actin filament (except class VI which moves toward the - end to move endocytotic vesicles into the cell).
    b. When no ATP is bound, myosin is in a conformation that binds very tightly to the F-actin filament. When ATP is bound, myosin changes conformation and loses affinity for the F-actin filament. ATPase activity

own environment for what to do than to continuously go into cell division (cancer).

10. How does DNA damage block cell cycle progression? Why is this important?
    a. DNA damage activates p53 (98% tumors mutate p53) via ATM/ATR then Chk1/Chk2 kinase activation that phosphorylates the p53 which causes the Mdm2 to fall off. The active p53 binds to the regulatory region of the p21 gene and activates transcription of p21 that is transcribed and translated into p21 (Cdk inhibitor protein). p21 inhibits both G1/S and S phase Cdk-cyclin complexes, which allows for DNA repair to occur, or if the damage is too severe, for p53 to trigger apoptosis.
11. How does too much Myc activate p53?
    a. Too much Myc activates Arf, which will negatively regulate Mdm2, which will leave p53 uninhibited and therefore activated. The p53 will then lead to cell-cycle arrest or apoptosis.
12. Describe two examples of apoptosis in development.
    a. One example is in WBCs and some things you don't want to attack, such as yourself, so you program the WBCs to die that would attack the self, which helps you have a safe immune system. Neurons, if you don't use a pathway over time and practice, those neurons will undergo apoptosis.
13. How are apoptotic cells biochemically recognized?
    a. TUNEL staining for ends of cleaved DNA. Endonucleases during apoptosis cleave DNA into fragments and create new DNA ends that can be labeled by immunofluorescence. Also, live healthy cells have PS (phosphatidylserine) on the inside, while apoptotic cells have it on the outside. Therefore, if cells are stained with PI and FITC is added, it is possible to see what cells are alive, what cells are alive and apoptotic (membrane has not been compromised because PI can only get inside if membrane broken), and if they cells are dead/compromised.
14. Caspases are the main intracellular signaling molecules that regulate if the cell will respond to an apoptotic signal. Explain how caspases are activated and what their end targets are.
    a. Inactive caspases are activated by cleavage and they fold and become an active caspases and lose their cleaved pro-domains. Initiator caspases start the cascade and continue to activate downstream executioner procaspases and eventually cleave target proteins (cytosolic/ nuclear lamin).
15. Understand how the caspase cascade is activated via the Extrinsic and Intrinsic Pathway.
    a. In the extrinsic pathway, an immune cell has a Fas ligand and binds to the Fas death receptor on the target cell (contact-dependent). The binding of the ligand recruits the FADD adaptor proteins and the procaspases which are activated by induced proximity. The procaspases are cleaved and form active caspases that lead to activation of executioner caspases and an apoptotic target cell.
    b. In the intrinsic pathway, an apoptotic stimulus causes the release of cytochrome c from the mitochondria and bind to Apaf1 and the bound dATP is hydrolyzed to dADP. This releases the CARD domain of Apaf1 and the assembly of the apoptosome is triggered by the released of the dADP for dATP. 7 Procaspase-9 binds to the apoptosome and caspase-9 cleaves and activates executioner procaspases, which leads to a caspase

16. Know the names of the pro-apoptotic and anti-apoptotic regulators and how they function.
    a. Anti-apoptotic Bcl2 proteins: The active Bcl2 proteins inhibit the ability of BH123 proteins to aggregate and form a pore to allow cytochrome c to escape the mitochondria.
    b. Pro-apoptotic BH123 proteins: An apoptotic signal recruits BH123 proteins together that form a pore that allows Cytochrome C to get out.
    c. Pro-apoptotic BH3-only proteins: An apoptotic stimulus causes activated BH3-only protein to inhibit the Bcl2 protein, therefore allowing for a pore of BH123 proteins to form, allowing cytochrome c to be released from the mitochondria.
17. IAPs inhibit apoptosis by blocking activated caspases. However, DIABLO produces anti-IAPs that block IAPs and allow for caspases to form and continue their function.
18. Explain how survival signals block apoptosis signals.
    a. Survival factors bind to extracellular signaling receptors and activate intracellular signaling pathways that suppress apoptosis. They activate proteins like IAPs and Bcl2 or inhibit proteins like BH123, BH3-only proteins, and anti-IAPs (DIABLO).