Molecular biology of the cell
BIO 207

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Example question

3: Phosphatidyl inositol (PI) is an important signaling lipid. Which of the following statements are true about this important molecule.

A: PI can be phosphorylated on the sugar group (Inositol) to create 8 different kinds of PI's

B: Phosphorylation of PI's is irreversible.

C: PI can serve as a docking complex for proteins to associate with phospholipid membrane

D: The distribution of phosphorylated PI's in the phospholipid membranes of the cell is random

E: Thanks to the extracellular location of the functional headgroup, PI can interact with AKT

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Example question

22: A nuclear localization signal is always located at the N-terminus of a protein

True / False

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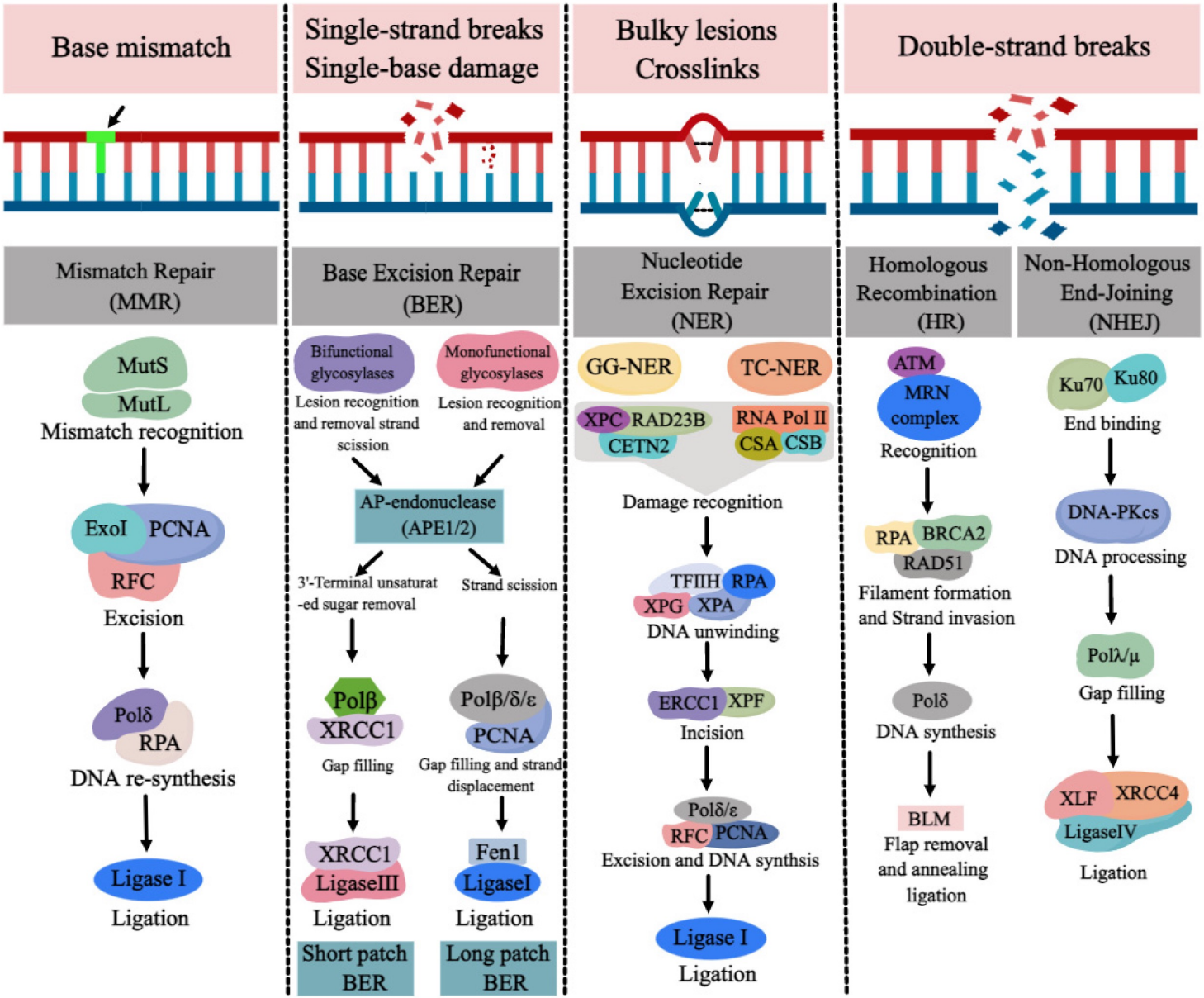
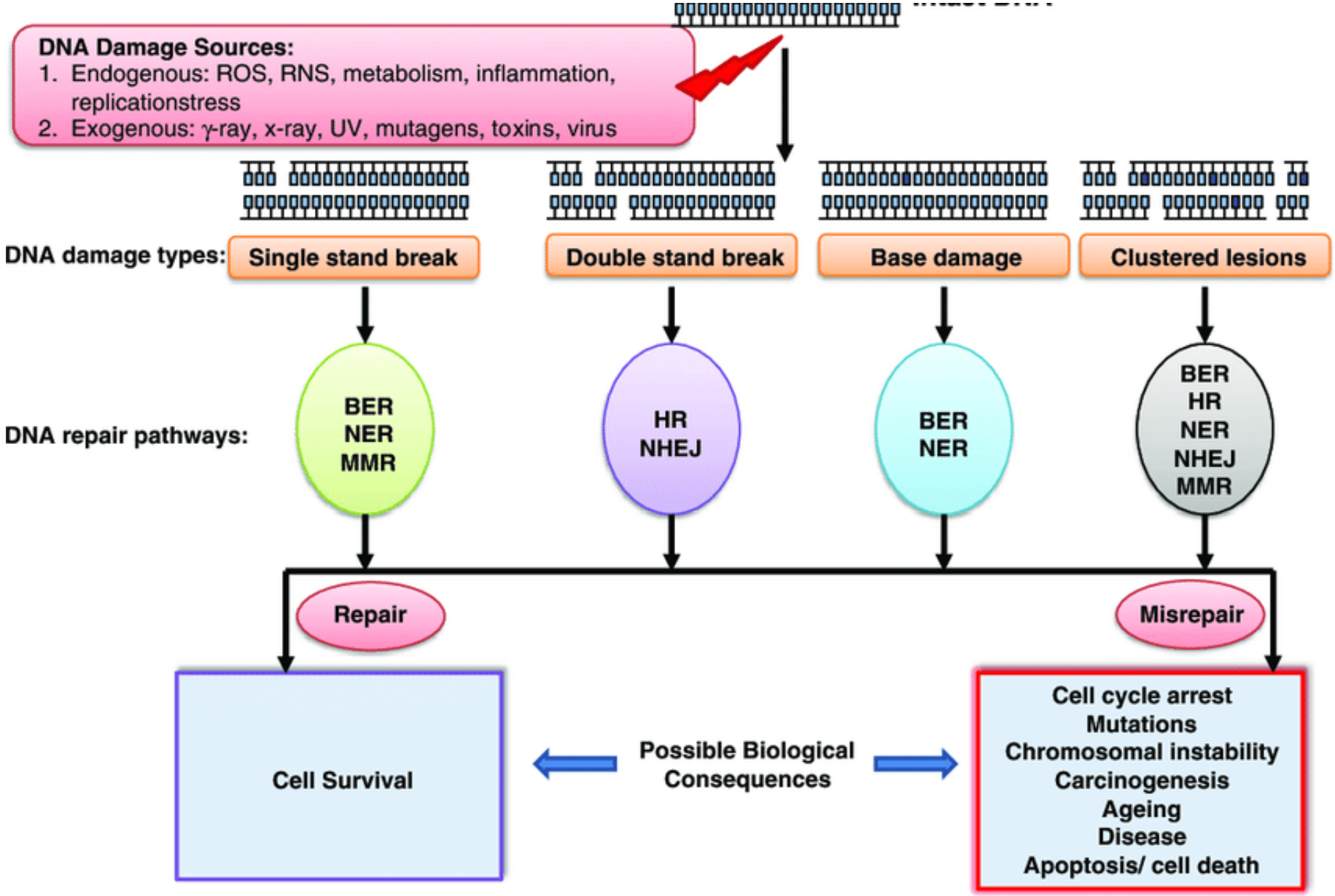
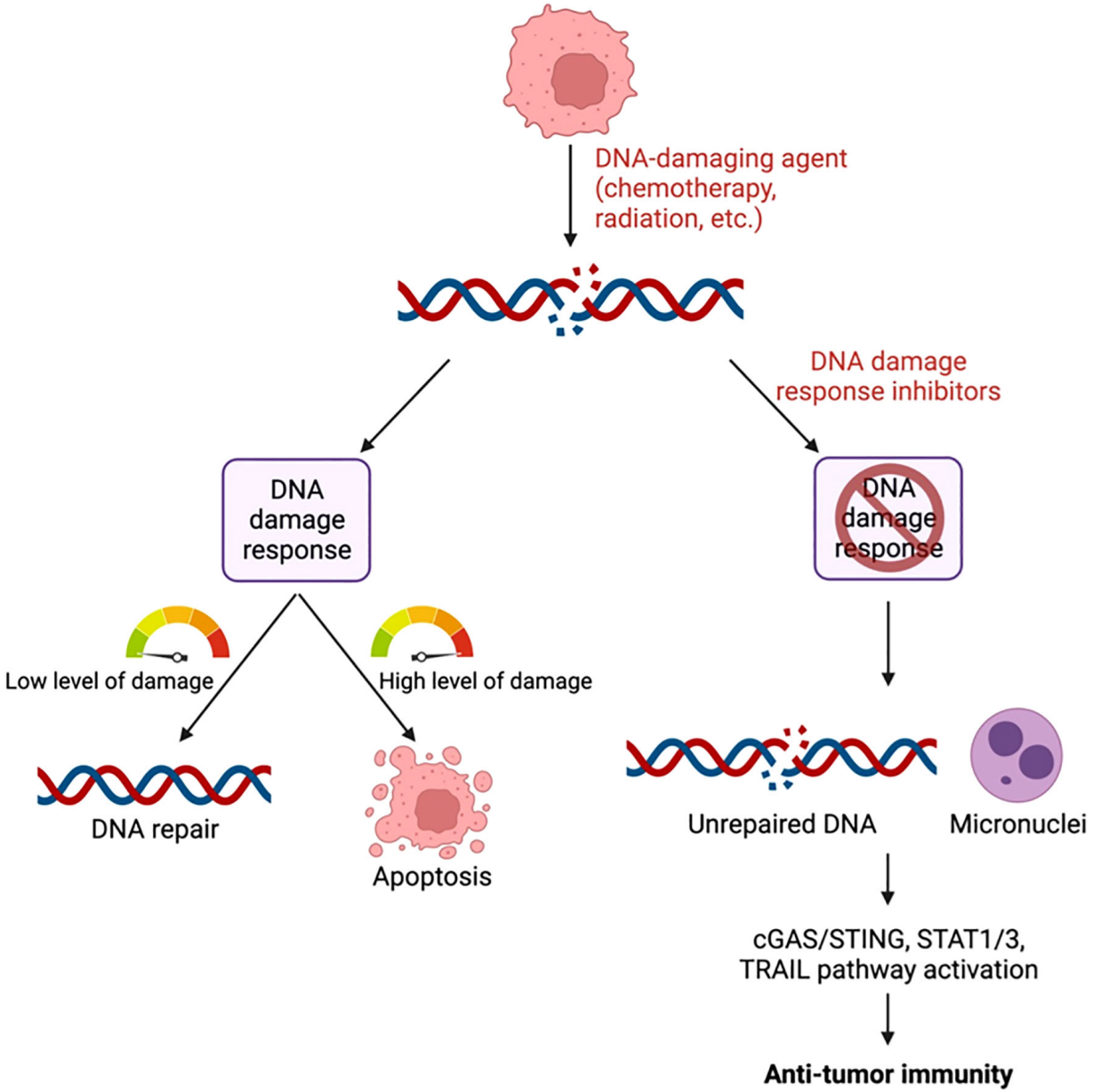
True / False

Q&A

In slide 46, it is mentioned that with DNA repair, the cell death could be avoided even if a death signal has already been received. How is this regulated? What gives the cell enough time to repair its DNA?


Base mismatch
Single-strand breaks
Single-base damage
Bulky lesions
Crosslinks
Double-strand breaks
Mismatch Repair
(MMR)
Base Excision Repair
(BER)
Nucleotide
Excision Repair
(NER)
Homologous
Recombination
(HR)
Non-Homologous
End-Joining
(NHEJ)
MutS
MutL
Mismatch recognition
ExoI
PCNA
RFC
Excision
Polδ
RPA
DNA re-synthesis
Ligase I
Ligation
Bifunctional
glycosylases
Lesion recognition
and removal strand
scission
Monofunctional
glycosylases
Lesion recognition
and removal
AP-endonuclease
(APE1/2)
3'-Terminal unsaturat
-ed sugar removal
Strand scission
Polβ
XRCC1
Gap filling
Polβ/δ/ε
PCNA
Gap filling and strand
displacement
XRCC1
LigaseIII
Ligation
Fen1
LigaseI
Ligation
Short patch
BER
Long patch
BER
GG-NER
TC-NER
XPC
RAD23B
CETN2
RNA Pol II
CSA
CSB
Damage recognition
TFIIH
RPA
XPG
XPA
DNA unwinding
ERCC1
XPF
Incision
Polδ/ε
RFC
PCNA
Excision and DNA synthsis
Ligase I
Ligation
ATM
MRN
complex
Recognition
RPA
BRCA2
RAD51
Filament formation
and Strand invasion
Polδ
DNA synthesis
BLM
Flap removal
and annealing
ligation
Ku70
Ku80
End binding
DNA-PKcs
DNA processing
Polλ/μ
Gap filling
XLF
XRCC4
LigaseIV
Ligation


DNA Damage Sources:
1. Endogenous: ROS, RNS, metabolism, inflammation, replicationstress
2. Exogenous: γ-ray, x-ray, UV, mutagens, toxins, virus
DNA damage types:
Single stand break
Double stand break
Base damage
Clustered lesions
DNA repair pathways:
BER NER MMR
HR NHEJ
BER NER
BER HR NER NHEJ MMR
Repair
Misrepair
Cell Survival
Possible Biological Consequences
Cell cycle arrest
Mutations
Chromosomal instability
Carcinogenesis
Ageing
Disease
Apoptosis/ cell death


DNA-damaging agent
(chemotherapy,
radiation, etc.)
DNA damage
response inhibitors
DNA
damage
response
DNA
damage
response
Low level of damage
High level of damage
DNA repair
Apoptosis
Unrepaired DNA
Micronuclei
cGAS/STING, STAT1/3,
TRAIL pathway activation
Anti-tumor immunity

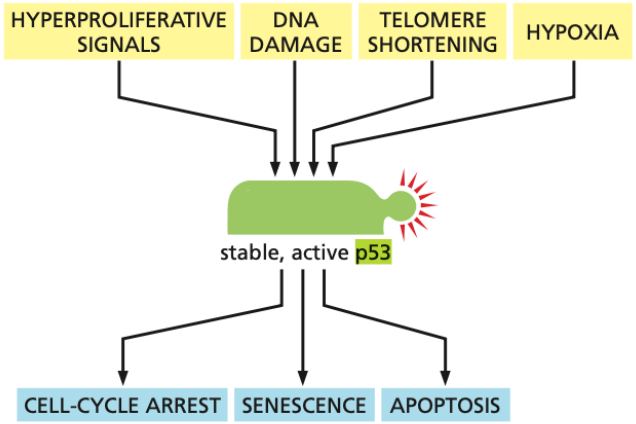


Figure 20–27 Modes of action of the p53 tumor suppressor. The p53 protein is a cellular stress sensor. In response to hyperproliferative signals, DNA damage, hypoxia, telomere shortening, and various other stresses, the p53 levels in the cell rise. As indicated, this may either arrest cell cycling in a way that allows the cell to adjust and survive, trigger cell suicide by apoptosis, or cause cell "senescence"—an irreversible cell-cycle arrest that stops damaged cells from dividing.

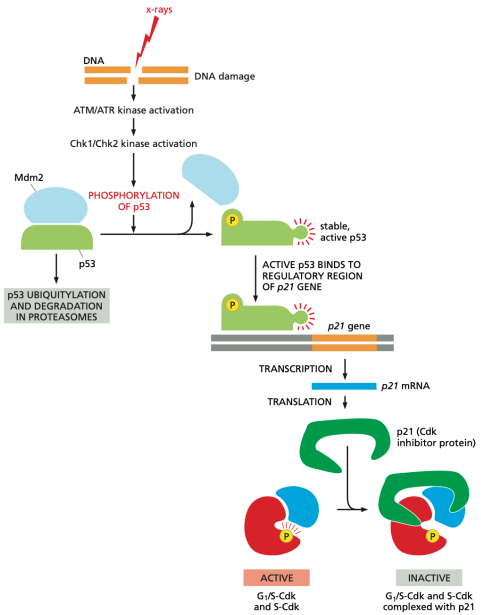


Figure 17–62 How DNA damage arrests the cell cycle in G_1. When DNA is damaged, various protein kinases are recruited to the site of damage and initiate a signaling pathway that causes cell-cycle arrest. The first kinase at the damage site is either ATM or ATR, depending on the type of damage. Additional protein kinases, called Chk1 and Chk2, are then recruited and activated, resulting in the phosphorylation of the transcription regulatory protein p53. Mdm2 normally binds to p53 and promotes its ubiquitylation and destruc in proteasomes. Phosphorylation of p5 blocks its binding to Mdm2; as a result p53 accumulates to high levels and stimulates transcription of numerous ge including the gene that encodes the CI protein p21. The p21 binds and inactiv G_1/S-Cdk and S-Cdk complexes, arres the cell in G_1. In some cases, DNA dan also induces either the phosphorylatior Mdm2 or a decrease in Mdm2 product which causes a further increase in p53 shown).

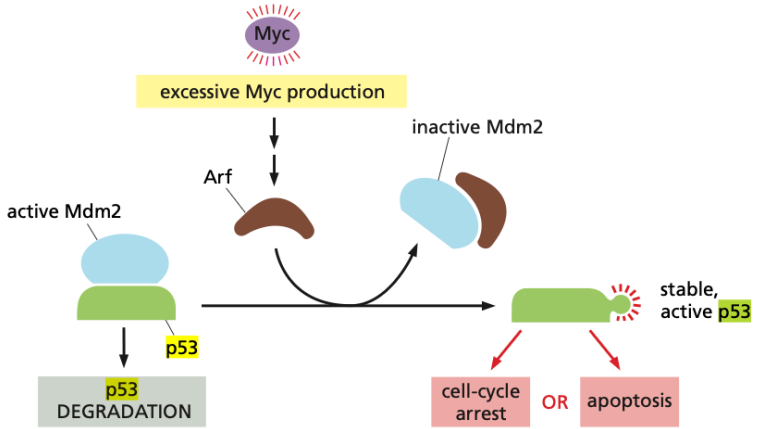
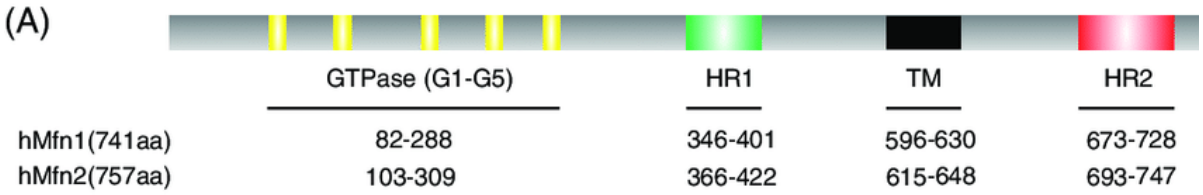


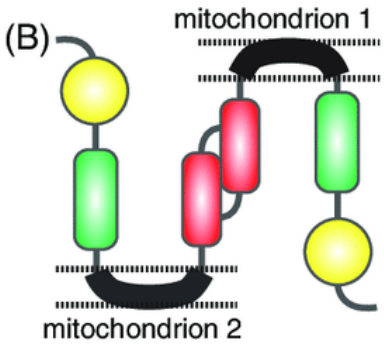
Figure 17–63 Cell-cycle arrest or apoptosis induced by excessive stimulation of mitogenic pathways. Abnormally high levels of Myc cause the activation of Arf, which binds and inhibits Mdm2 and thereby increases p53 levels (see Figure 17–62). Depending on the cell type and extracellular conditions, p53 then causes either cell-cycle arrest or apoptosis.

Are the Mitofusins 1 and 2 interchangeable? We saw this is a possibility in the exercises, but is that also the case in reality?

Association with the ER

2. Are the Mitofusins 1 and 2 interchangeable? We saw this is a possibility in the exercises, but is that also the case in reality?

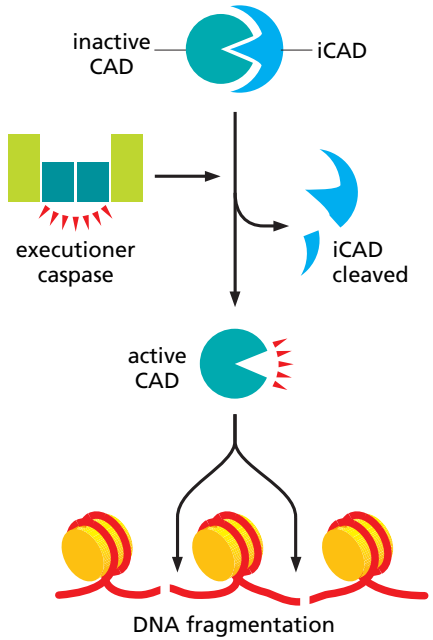




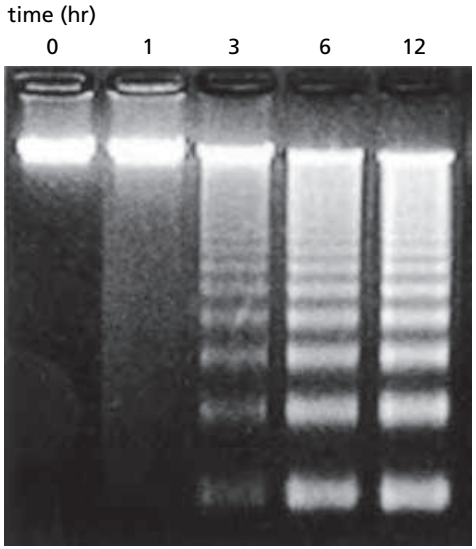
Yes, Mfn1 and Mfn2 have high conservation in the domains necessary for mitochondrial fusion. Thus, they have some functional redundancy.

3. In slide 49, there is a gel electrophoresis image showing how the DNA is fragmented by CAD. It is described that, due to CAD cutting the linker DNA, a characteristic ladder pattern appears. However, this would suggest that the fragments are of different sizes. How is this possible if nucleosomes with linker DNA all have ~180bp of DNA (slide 62)?

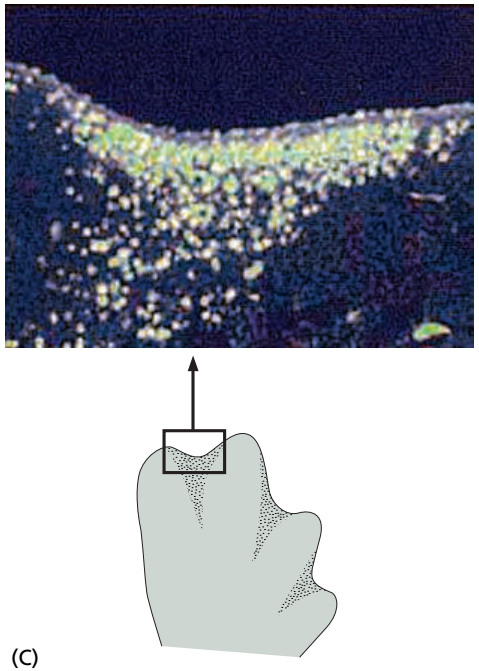
DNA fragmentation during apoptosis by an endonuclease CAD



(A)



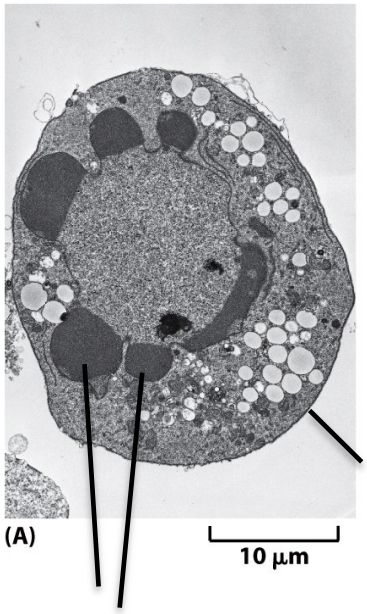
(B)



(C)

Figure 18–4 DNA fragmentation during apoptosis. (A) In healthy cells, the endonuclease CAD associates with its inhibitor, iCAD. Activation of executioner caspases in the cell leads to cleavage of iCAD, which unleashes the nuclease. Activated CAD cuts the chromosomal DNA between nucleosomes, resulting in the production of DNA fragments that form a ladder pattern (see B) upon gel electrophoresis. (B) Mouse thymus lymphocytes were treated with an antibody against the cell-surface death receptor Fas (discussed in the text), inducing the cells to undergo apoptosis. DNA was extracted at the times indicated above the figure, and the fragments were separated by size by electrophoresis in an agarose gel and stained with ethidium bromide. Because the cleavages occur in the linker regions between nucleosomes, the fragments separate into a characteristic ladder pattern on these gels. Note that in gel electrophoresis, smaller molecules are more widely separated in the lower part of the gel, so that removal of a single nucleosome has a greater apparent effect on their gel mobility. (C) Apoptotic nuclei can be detected using a technique that adds a fluorescent label to DNA ends. In the image shown here, this technique was used in a tissue section of a developing chick leg bud; this cross section through the skin and underlying tissue is from a region between two developing digits, as indicated in the underlying drawing. The procedure is called the TUNEL (TdT-mediated dUTP nick end labeling) technique because the enzyme terminal deoxynucleotidyl transferase (TdT) adds chains of labeled deoxynucleotide (dUTP) to the 3′-OH ends of DNA fragments. The presence of large numbers of DNA fragments therefore results in bright fluorescent dots in apoptotic cells. (B, from D. McIlroy et al., *Genes Dev.* 14:549–558, 2000. With permission from Cold Spring Harbor Laboratory Press; C, from V. Zuzarte-Luís and J.M. Hurlé, *Int. J. Dev. Biol.* 46:871–876, 2002. With permission from UBC Press.)

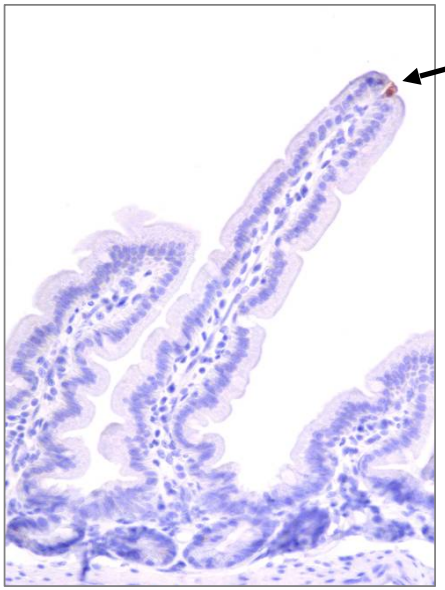
Transmission electron microscopy



Intact plasma membrane

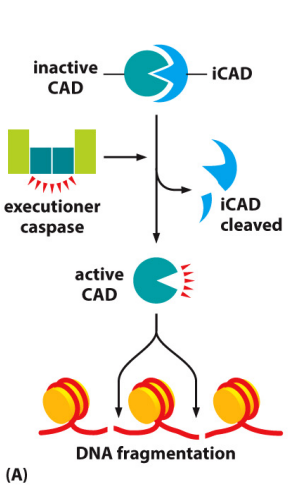
condensed chromatin

Immunohistochemistry



cleaved caspase3

DNA fragmentation assay



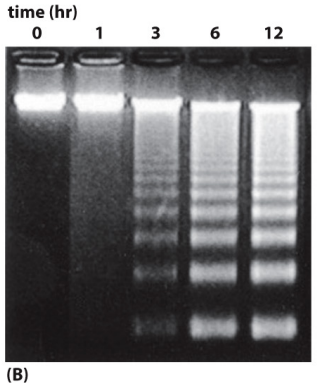
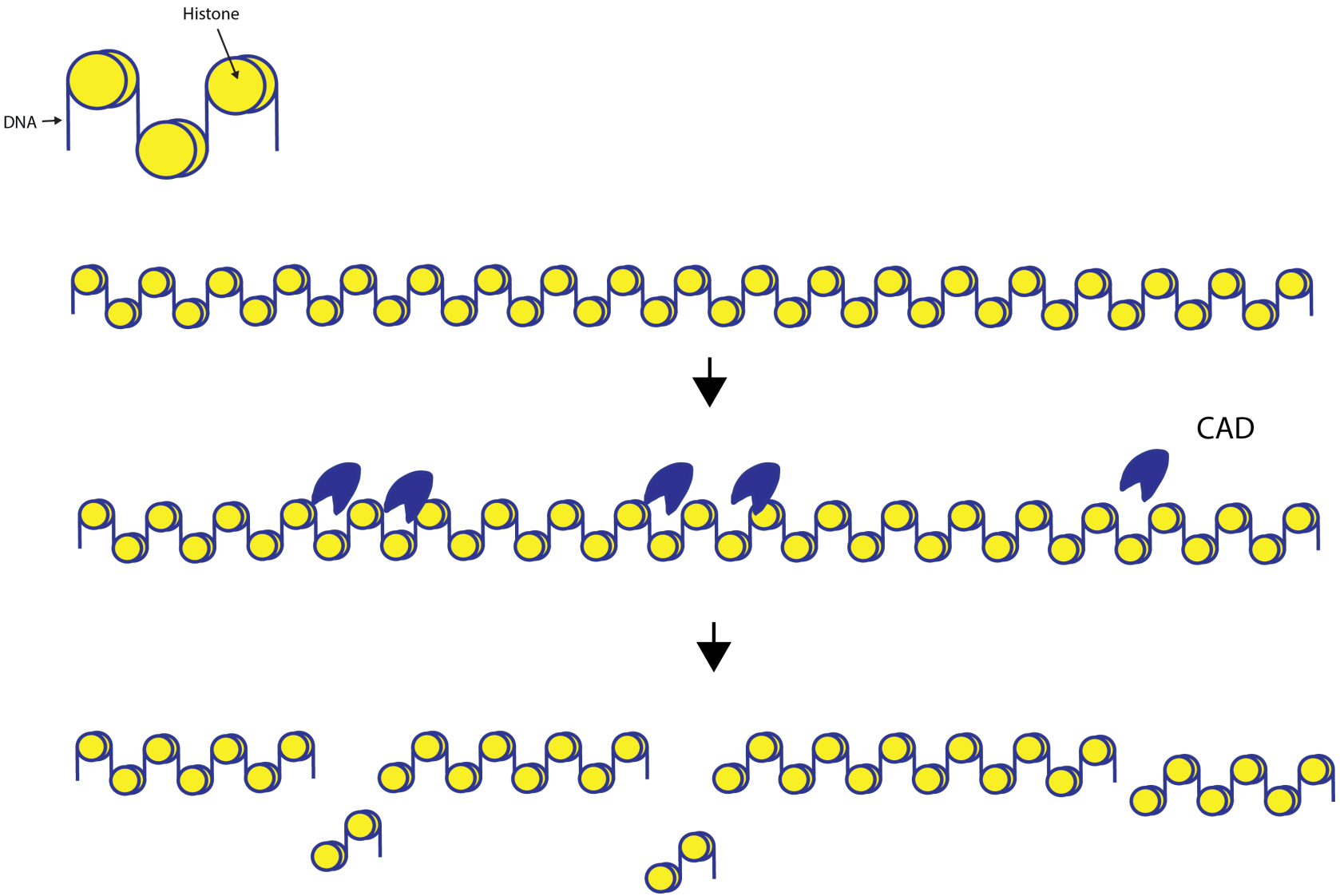


Figure 18-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

≈ 180 nt

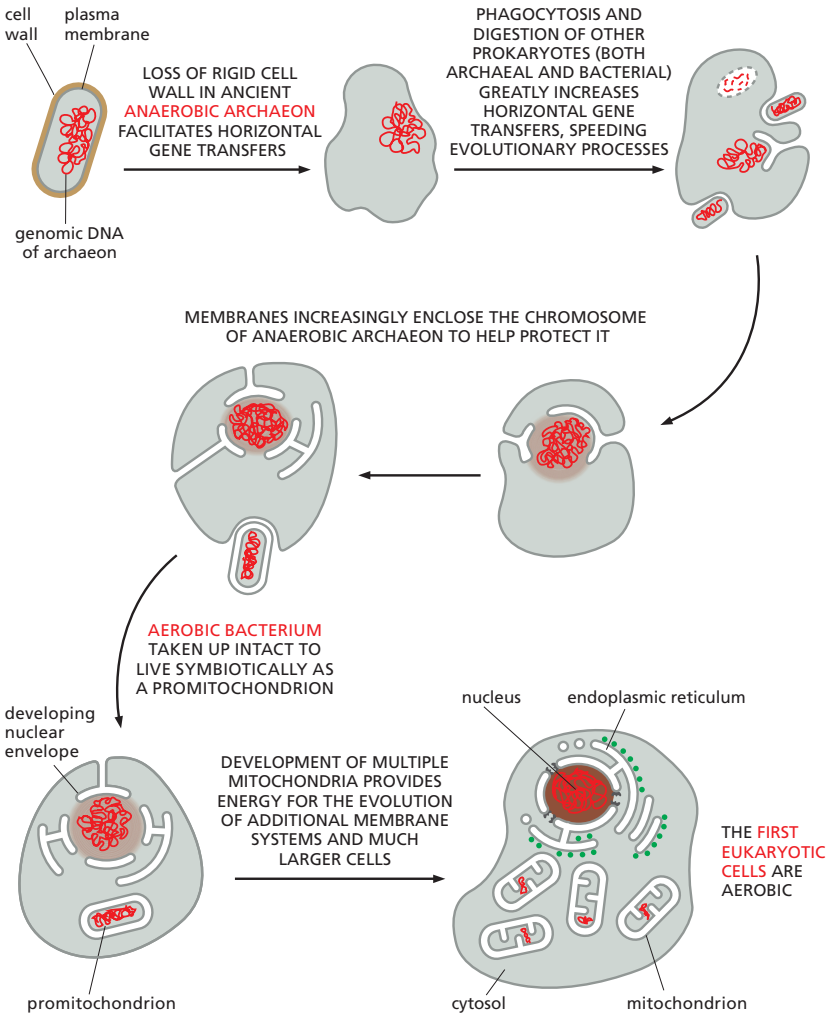


CAD cuts randomly creating the distinct banding pattern

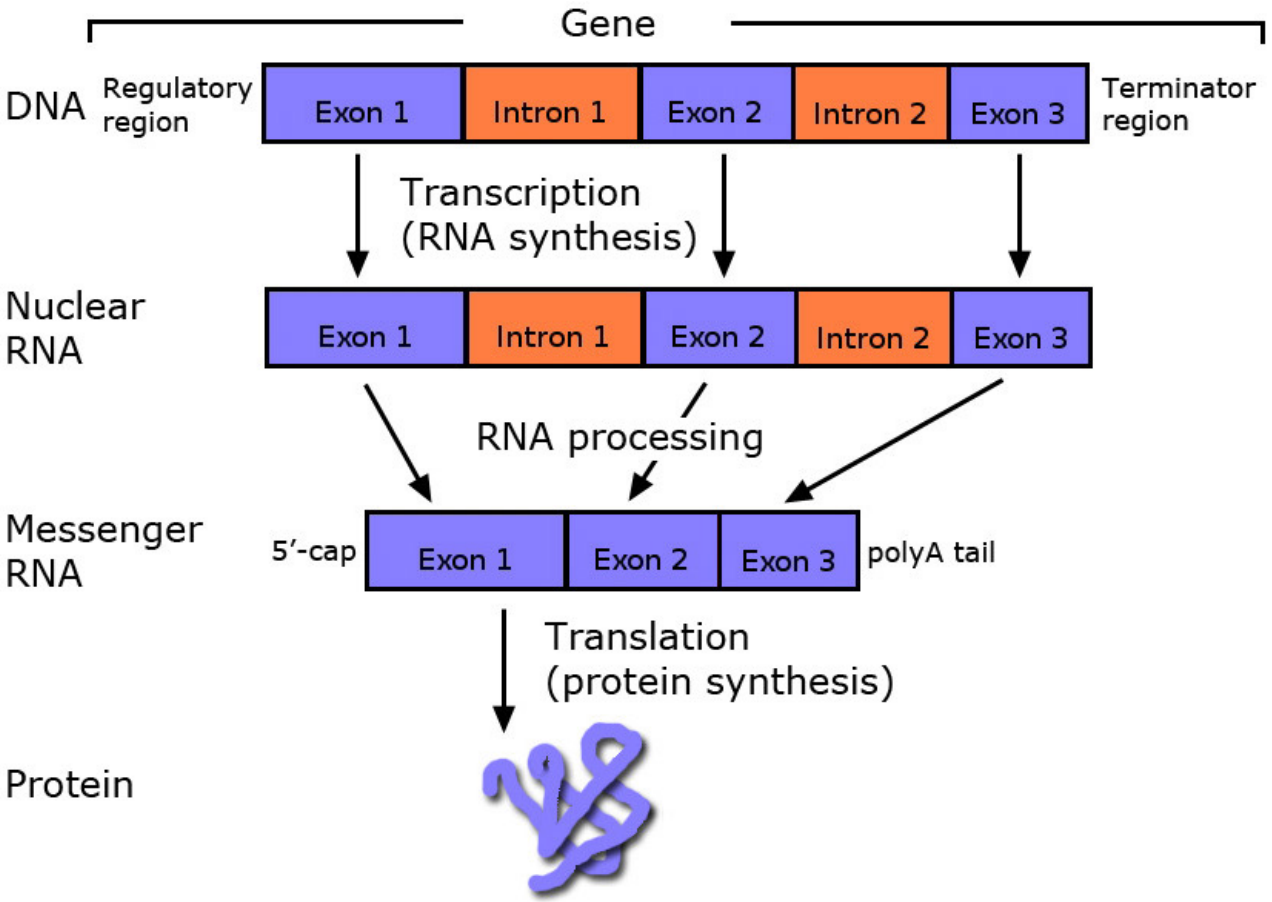
I have a few questions for Wednesday's session:
1.Last week, we discussed question 7.B, but I'm not sure I fully understood the correction. Would it be possible to go over it again in more detail?

The presence of introns in organellar genes is not surprising since similar introns have been found in related genes from bacteria whose ancestors are thought to have given rise to mitochondria and chloroplasts. (it was a True or False question) and here is the answer : False. The presence of introns in organellar genes is surprising precisely because corresponding introns are so uncommon in related bacterial genomes.

How did these compartments come to be?



Eukaryotic Transcription/Translation



Bacterial genomes are quite devoid of introns, so there is very little/no splicing.

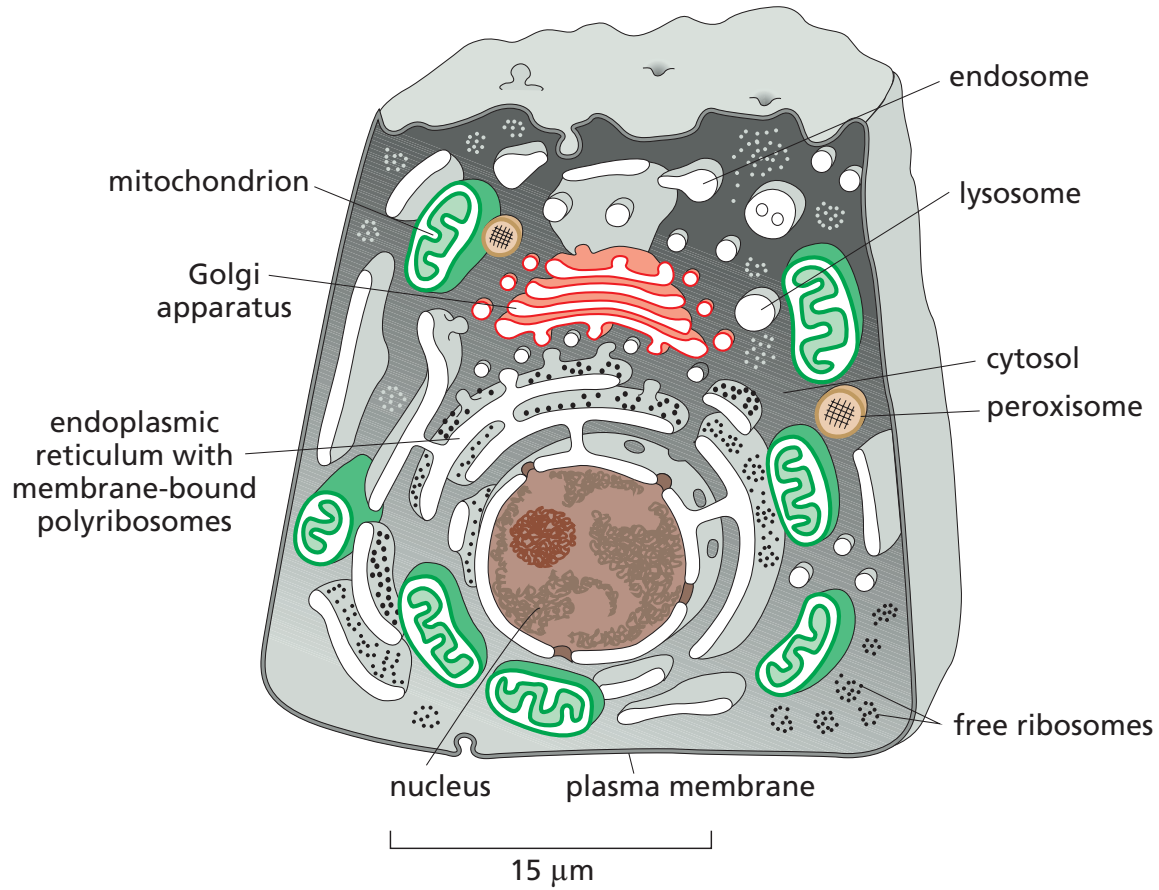
Hence to no introns would be present.

In engulfed organelles, why would there be new introduction of introns in genes that were functioning?

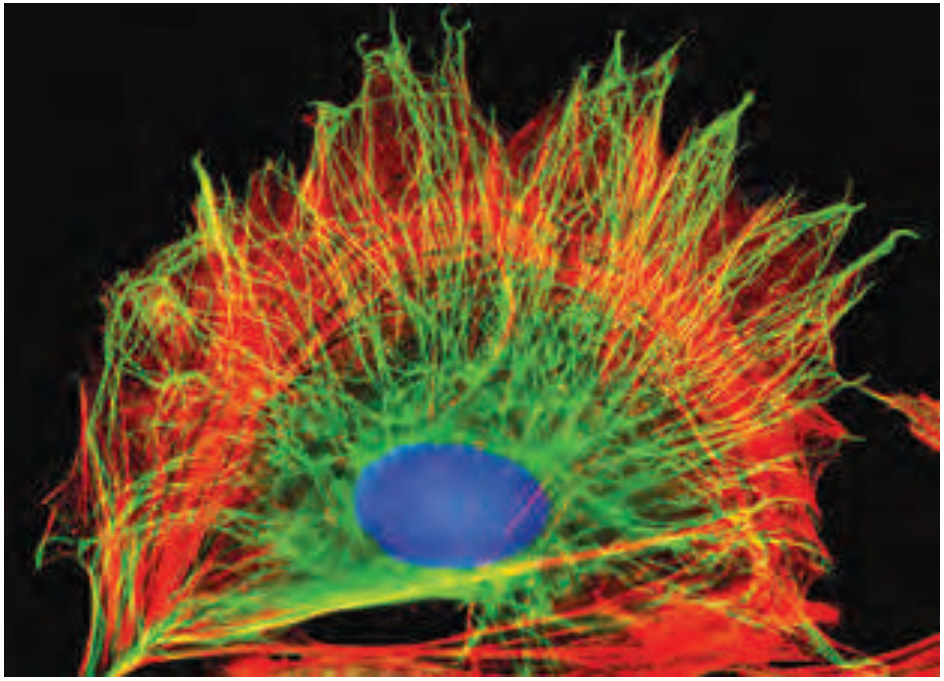
Some of the chapters we've covered are connected. Would it be possible to get an overview of which chapters are linked and how they relate to each other?

It is all connected!

Membranes (structure) (chapter 10)
Membrane transport (chapter 11)
Origin and transmission of mitochondria, and cell death (Chapter 14&18)
Organelles and transport (chapters 12 and 13)
Cellular communication (chapter 15)
Cytoskeleton (chapter 16)
Cellular junctions (chapter 19)

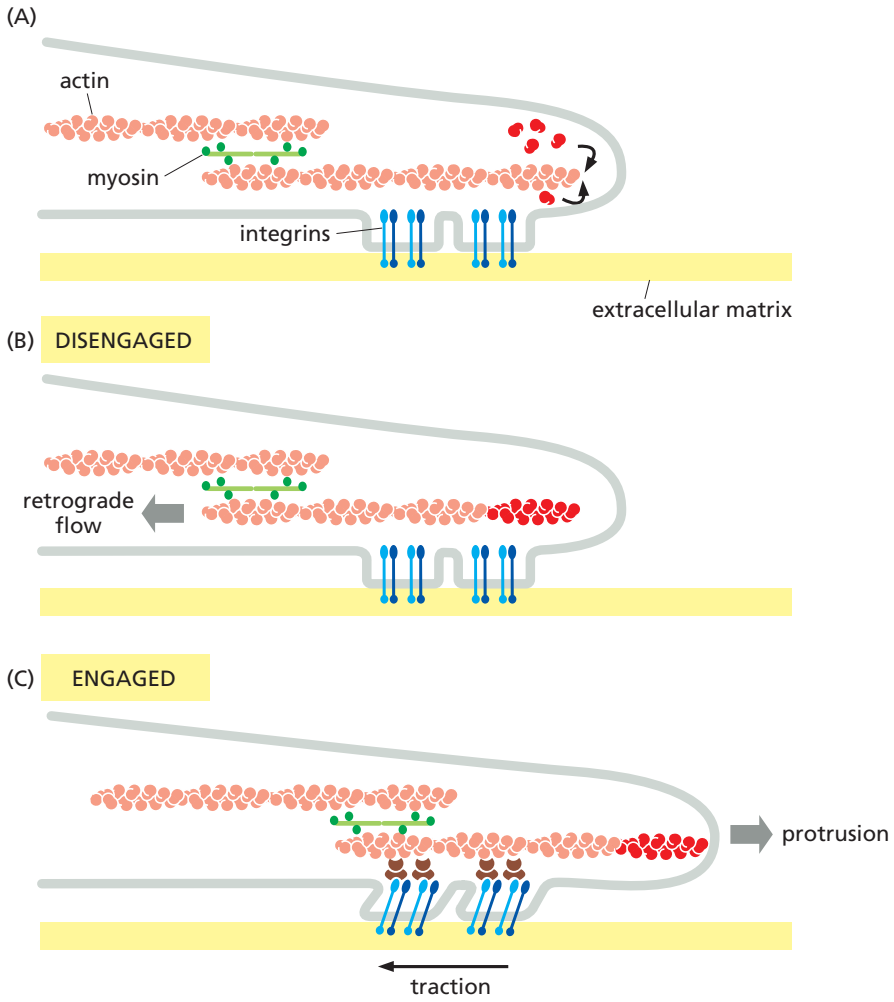


Connection Chapter 16 & 19.Cytoskeleton & ECM

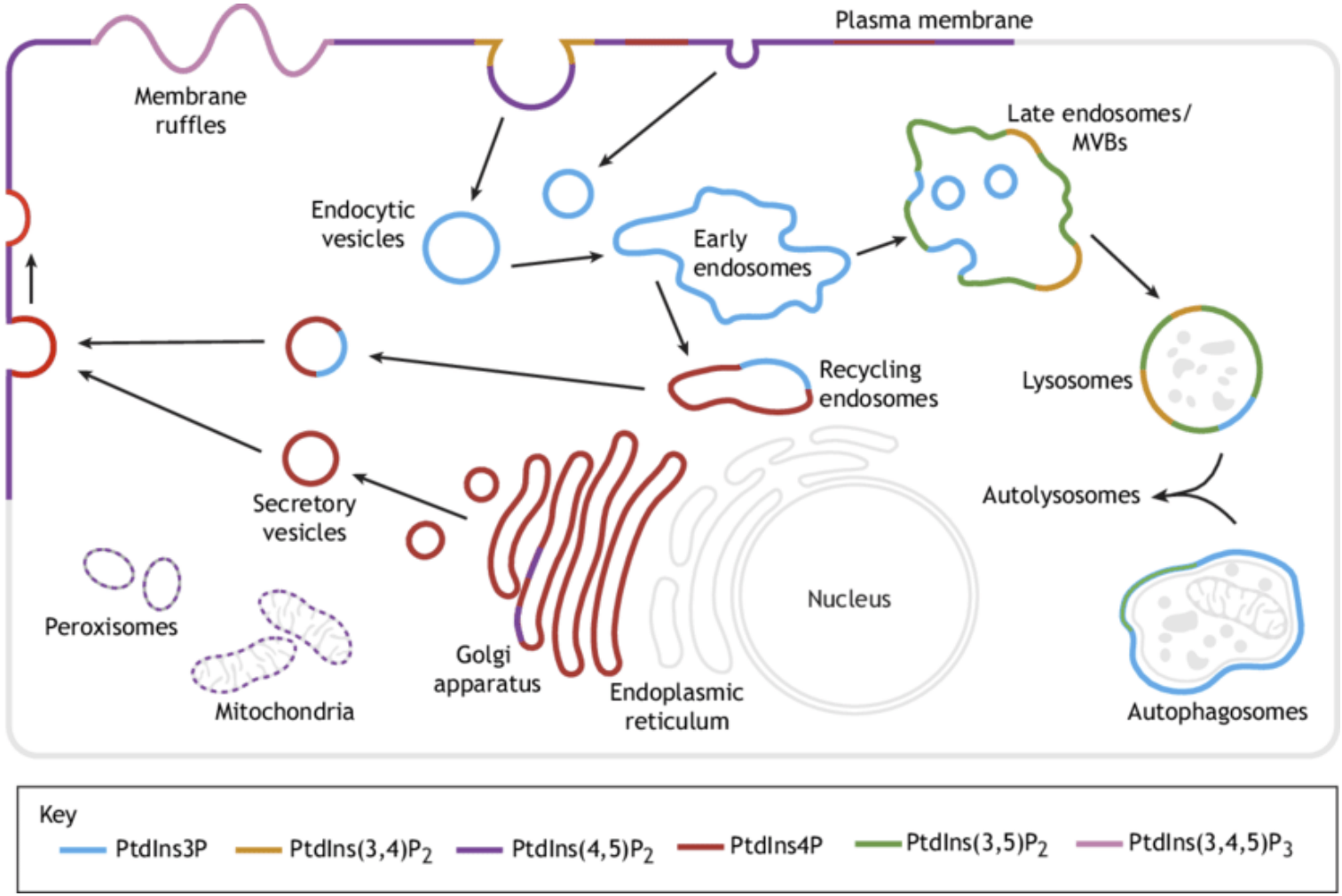


(A)

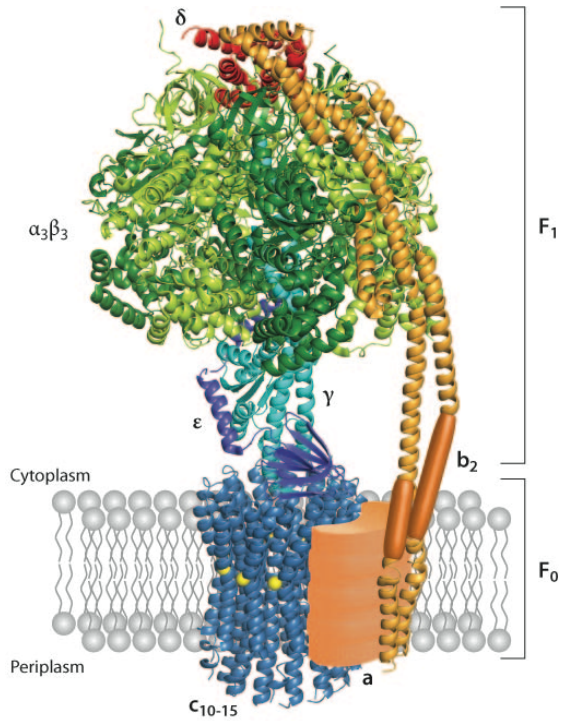
10 μm



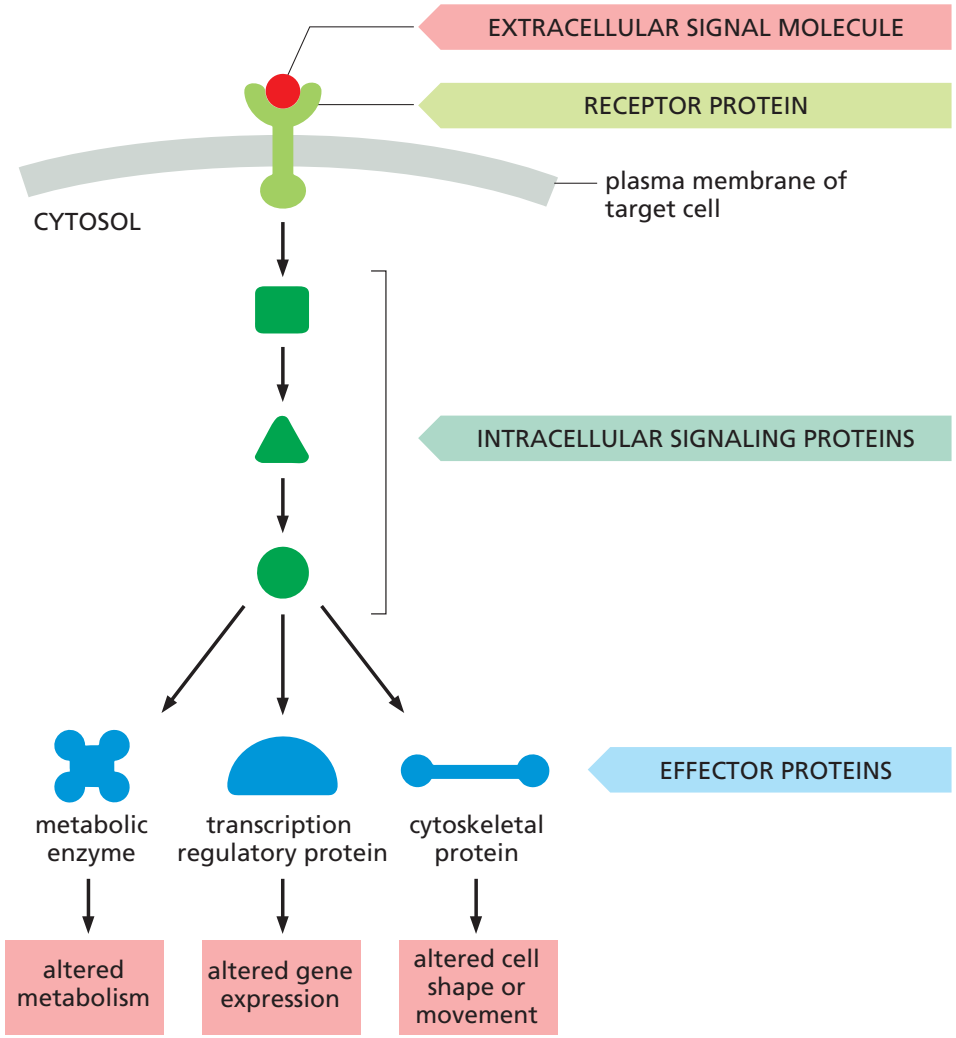
Connection Chapter 10, 12, 13.Lipids & Membrane identity



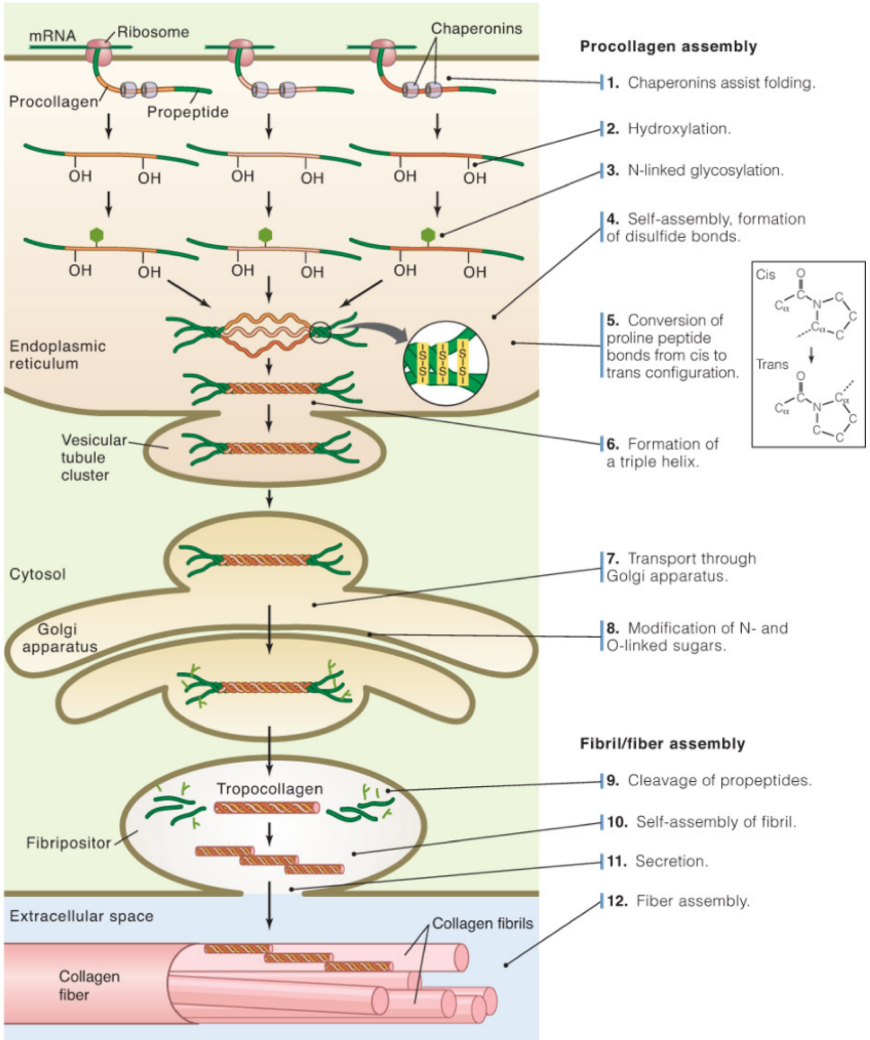
Connection Chapter 10, 11, 15. Membrane spanning proteins & signaling



von Ballmoos C, et al. 2009.
Annu. Rev. Biochem. 78:649–72



Connection Chapter 12, 13, 19. Vesicle transport & ECM



More Q&A (?)