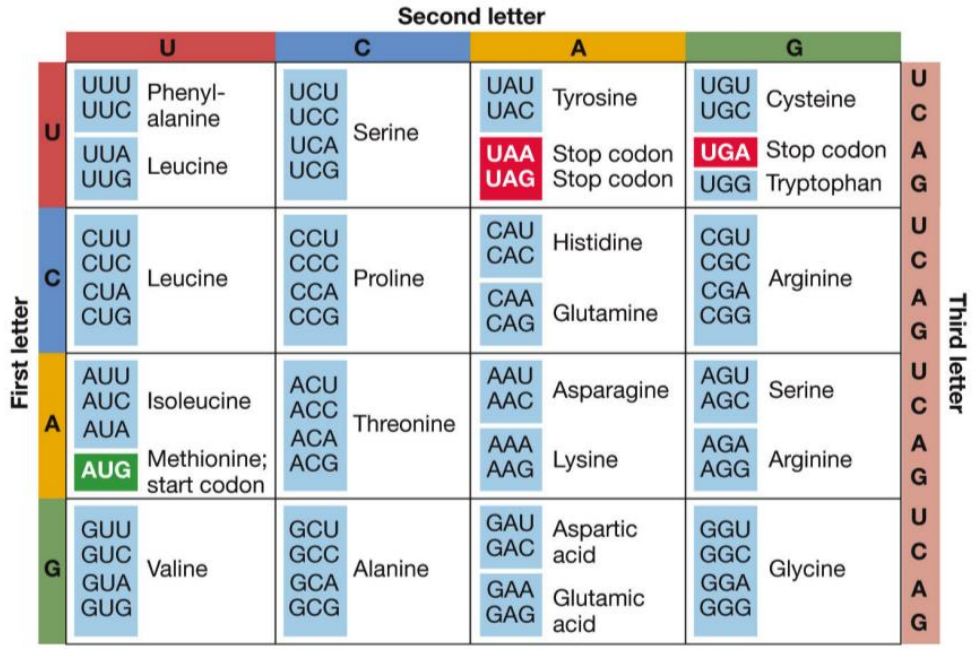
Review: From DNA to Protein

- Consider the following section of a DNA *template strand* as the beginning of transcription:
  - **3’** CTACGGTAAC **5’**

- Given the codon table, what would be the peptide sequence of the *first* three amino acids translated?



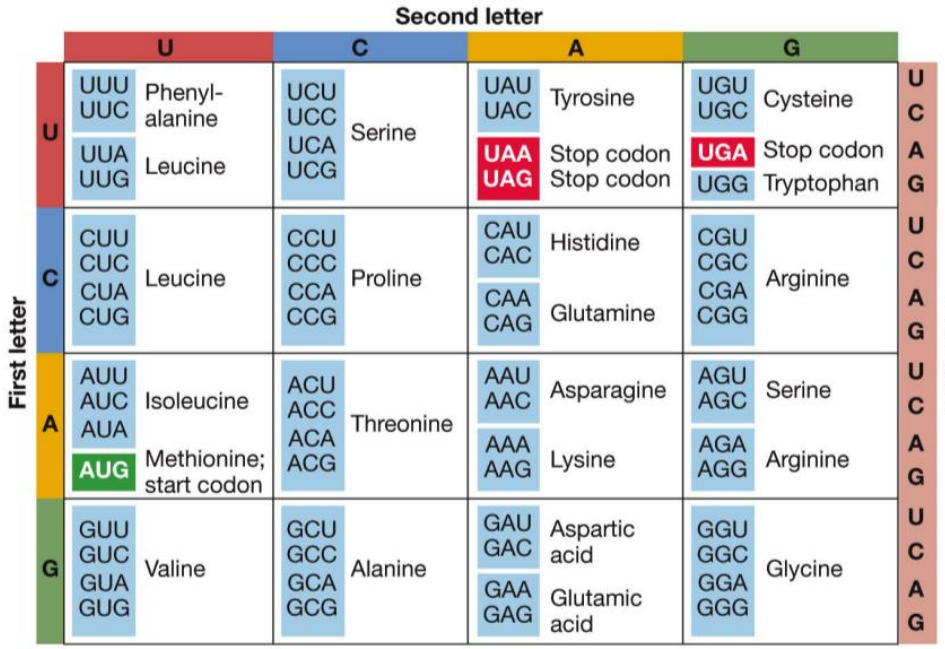
First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phenylalanine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine	UGU, UGC: Cysteine	U C A G
	UUA, UUG: Leucine		UAA, UAG: Stop codon	UGA: Stop codon; UGG: Tryptophan	
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine	CGU, CGC, CGA, CGG: Arginine	U C A G
			CAA, CAG: Glutamine		
A	AUU, AUC, AUA: Isoleucine	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine	AGU, AGC: Serine	U C A G
	AUG: Methionine; start codon		AAA, AAG: Lysine	AGA, AGG: Arginine	
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid	GGU, GGC, GGA, GGG: Glycine	U C A G
			GAA, GAG: Glutamic acid		

Review: From DNA to Protein

- Consider the following section of a DNA *template strand* as the beginning of transcription:
  - **3’** CTACGGTAAC **5’**

  - First, translate the DNA into mRNA:
    - **5’** GAUGCCAUUG **3’**

- Given the codon table, what would be the peptide sequence of the *first* three amino acids translated?
  - Next, note the location of the **start codon** AUG, then separate into codons for translation
    - G **AUG** CCA UUG = Methionine – Proline - Leucine



Chapter 8: Control of Gene Expression

Dr. Matthew Ellis

Chapter 8

BIOL366

Learning Objectives for Chapter 8:

By the end of this module, you should be able to:

- Understand the roles of transcriptional regulators in modulating gene expression (e.g., enhancers and repressors, operons, and master transcription factors)
- Describe the mechanisms of post-transcriptional regulators (e.g., miRNA, lncRNA)
- Detail the role of post-translational modifications as it relates to protein activity and turnover

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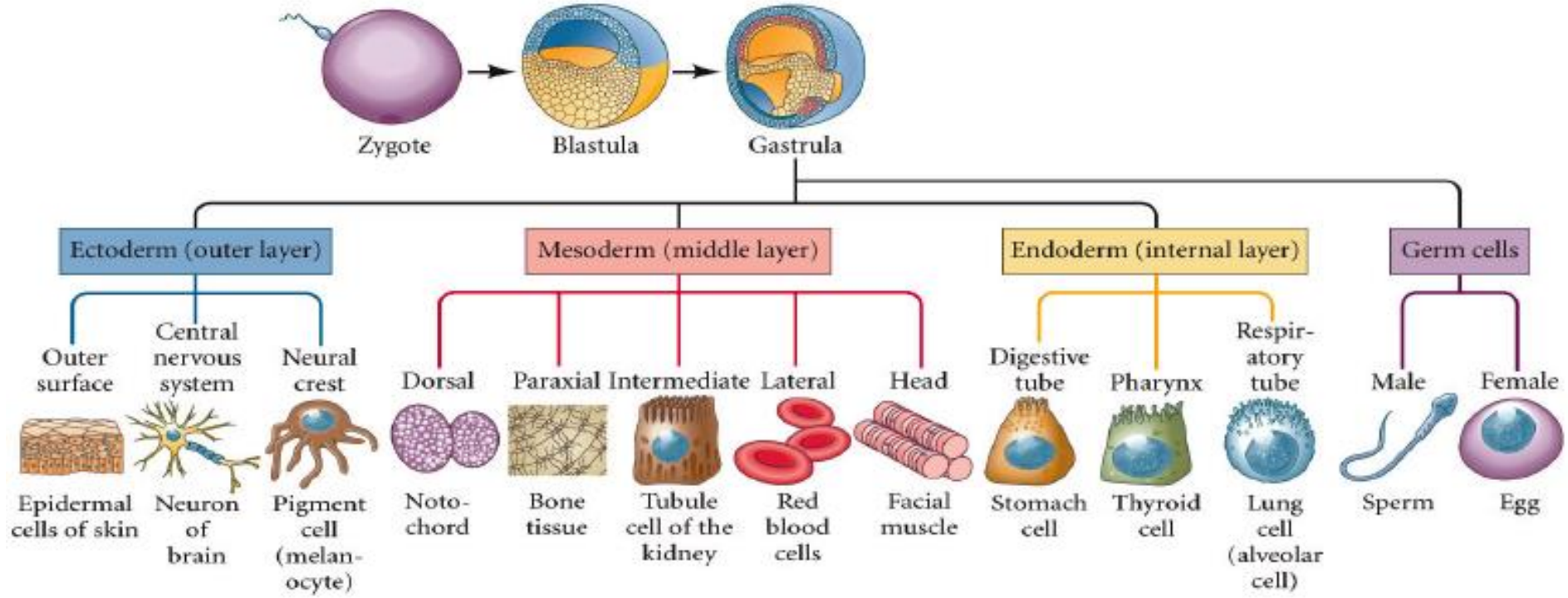
An overview of gene expression



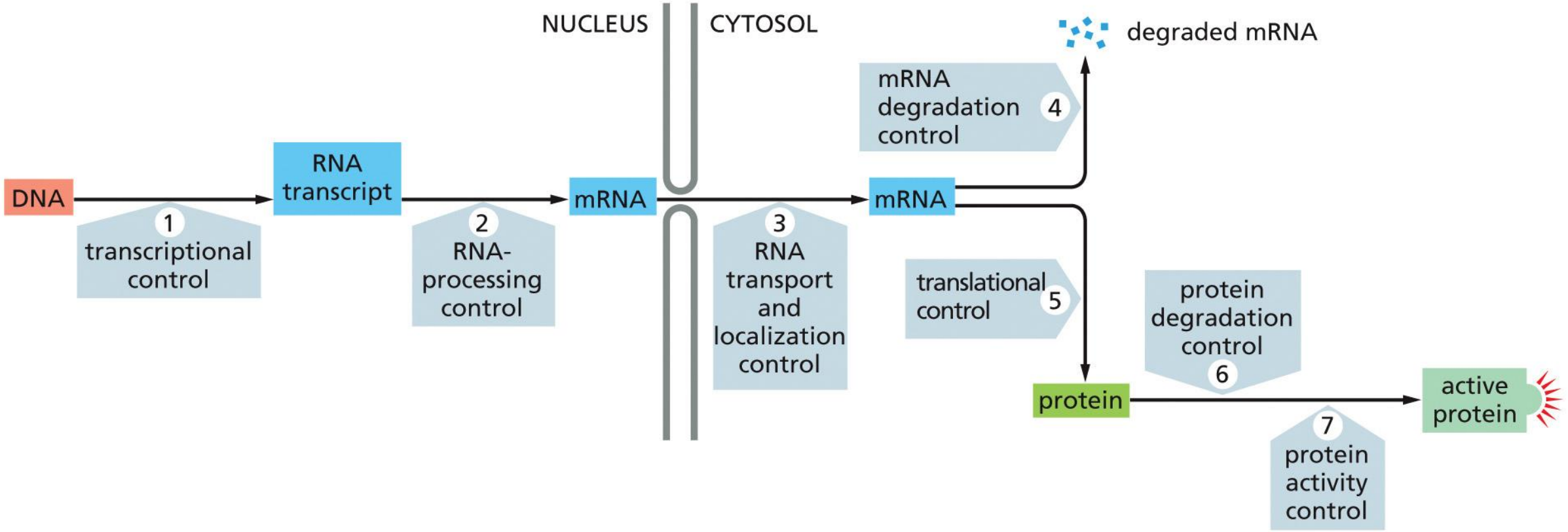
- Recall that the different somatic cell types in a multicellular organism contain the *same DNA*.

- Different cell types produce different sets of proteins, and a cell can change the expression of its genes in response to external signals.

- Gene expression can be regulated at various steps from DNA to RNA to protein.

http://madmnemonics.blogspot.com/2015/06/the-three-primary-germ-layers.html

Eukaryotic gene expression is controlled at many levels



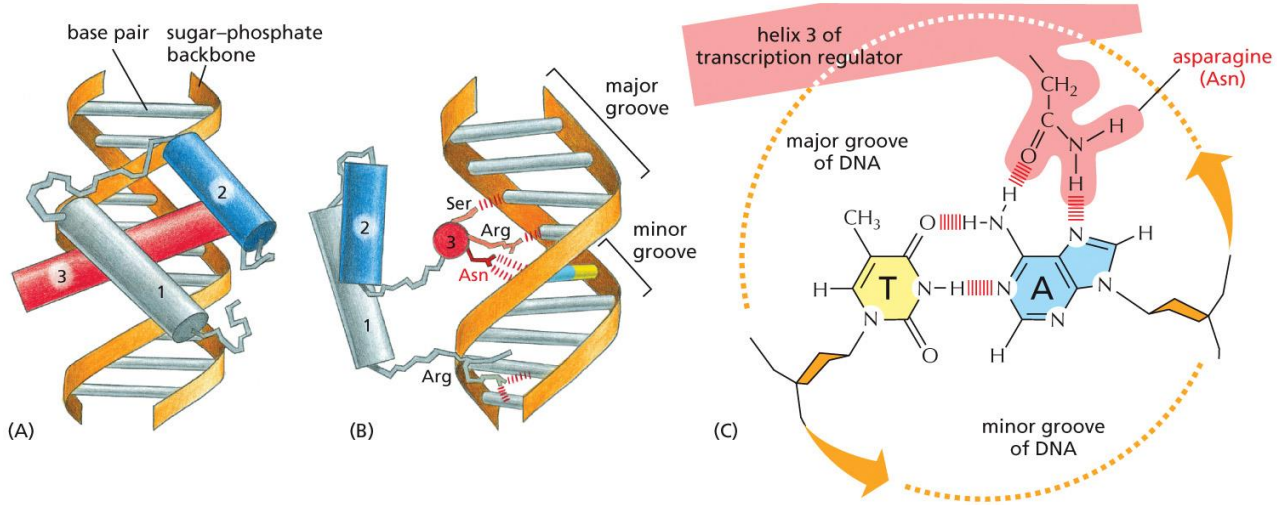
Different DNA sequences in a gene control transcription

- **Promoters** in DNA are bound by RNA polymerase (+ general transcription factors) to synthesize an RNA transcript

- **Regulatory DNA sequences** in DNA are bound by **transcriptional regulators** to switch genes on or off
  - Can be 10-100,000 nucleotides long, and create surface features in the DNA double helix that can be recognized by transcriptional regulators

  - Form hydrogen bonds, ionic bonds, and hydrophobic interactions with multiple bases mostly in major groove

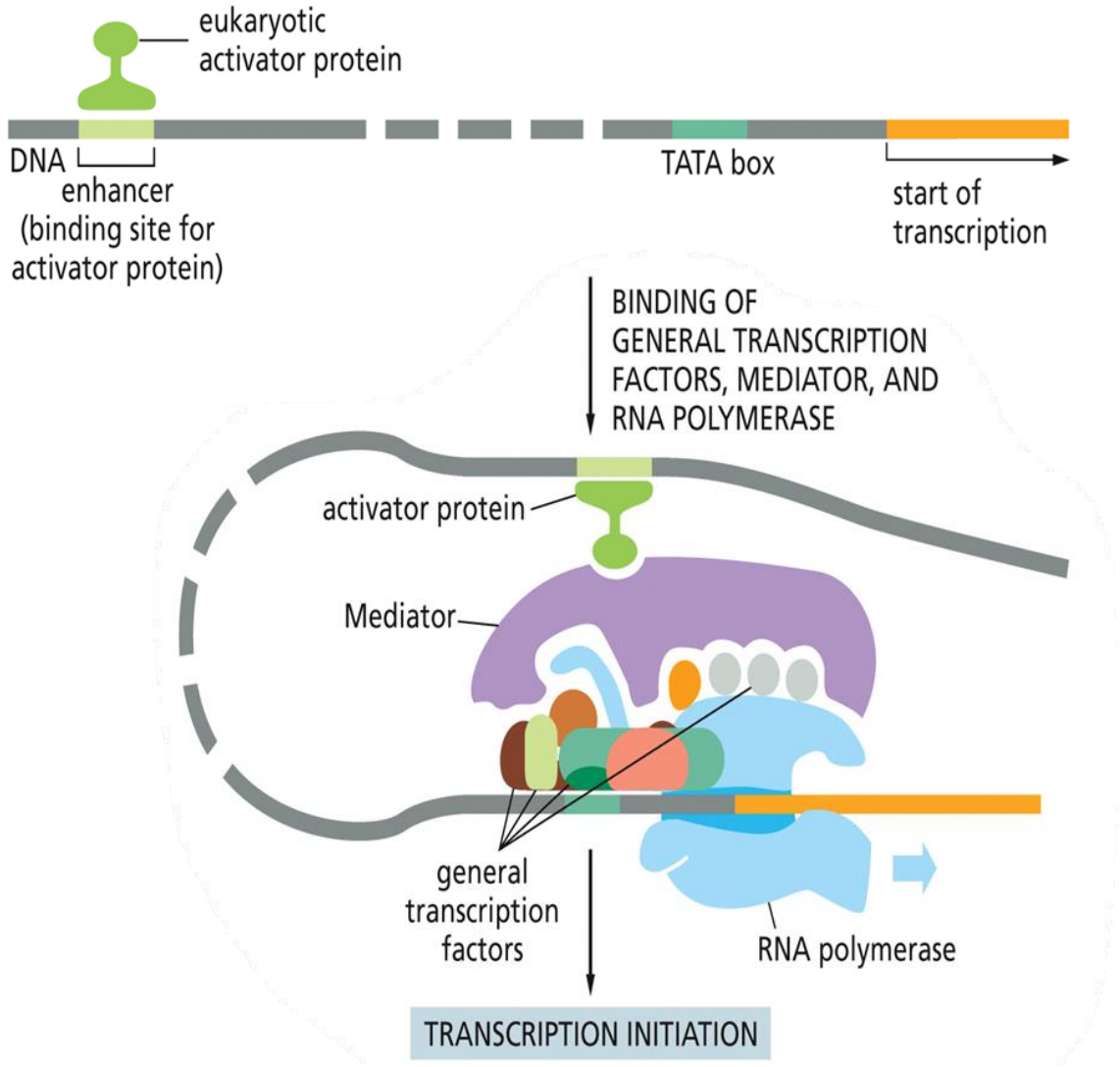
Protein hydrogen bonding with edges of the nucleotide bases



Eukaryotic transcription regulators control gene expression from a distance

- **Activator proteins** bind to **enhancer** (regulatory DNA sequence) and attract RNA polymerase + general transcription factors to the promoter
  - The distance between enhancer and the start of transcription varies (can be tens of thousands of nucleotide pairs away)

- Looping of DNA permits contact between the activator and the **transcription initiation complex** bound to the promoter:
  - By attracting and positioning all necessary factors, transcription can begin
  - **Repressor proteins** do the opposite: decrease transcription by preventing assembly of the mediator complex

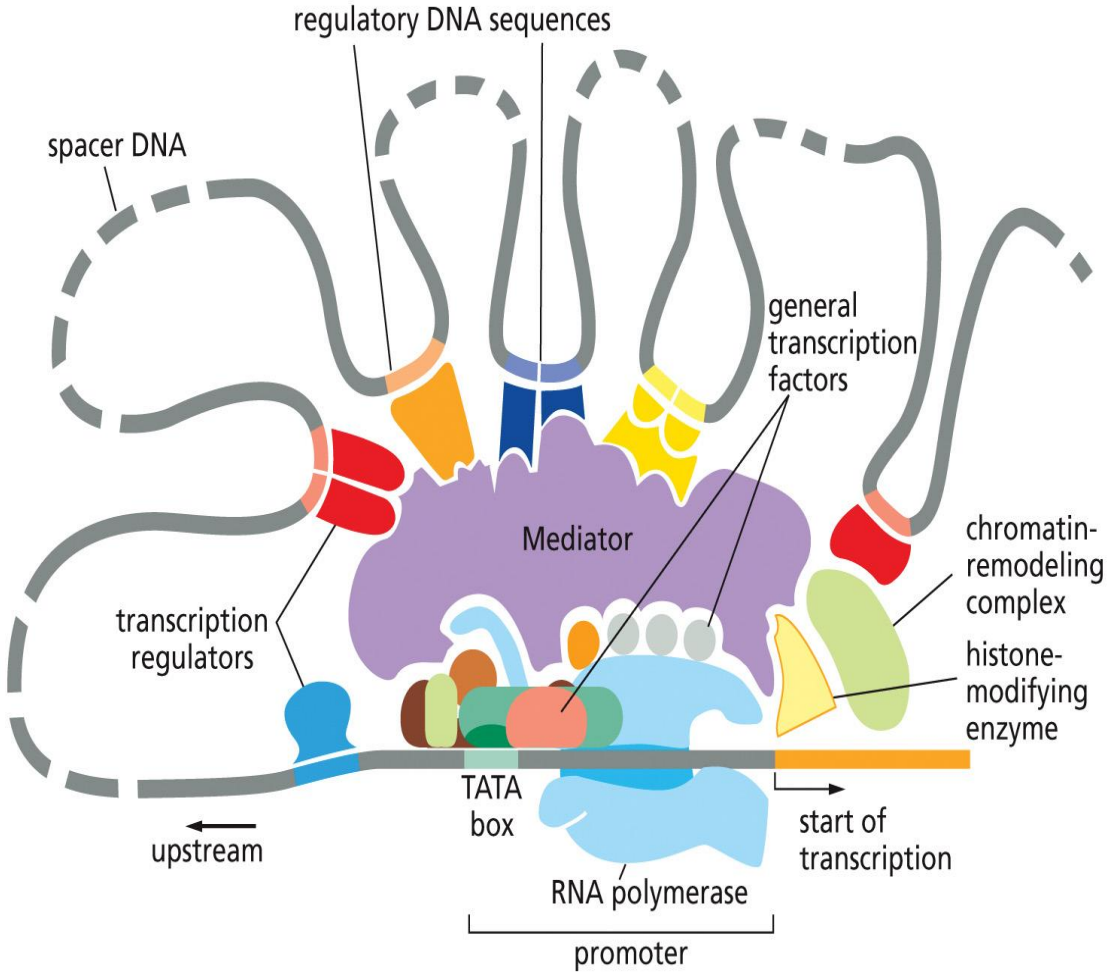


Recall: TATA box- bound by the first general transcription factor TFIID- which contains TATA-binding protein (TBP) subunit (teal unit)

Eukaryotic genes are controlled by combinations of transcription regulators

- Eukaryotic genes are under **combinatorial control**: groups of transcription regulators work together to control the expression

- While the general transcription factors that assemble at the promoter are the same for all genes transcribed by RNA polymerase, the transcription regulators and the locations of their DNA binding sites relative to the promoters are different for different genes

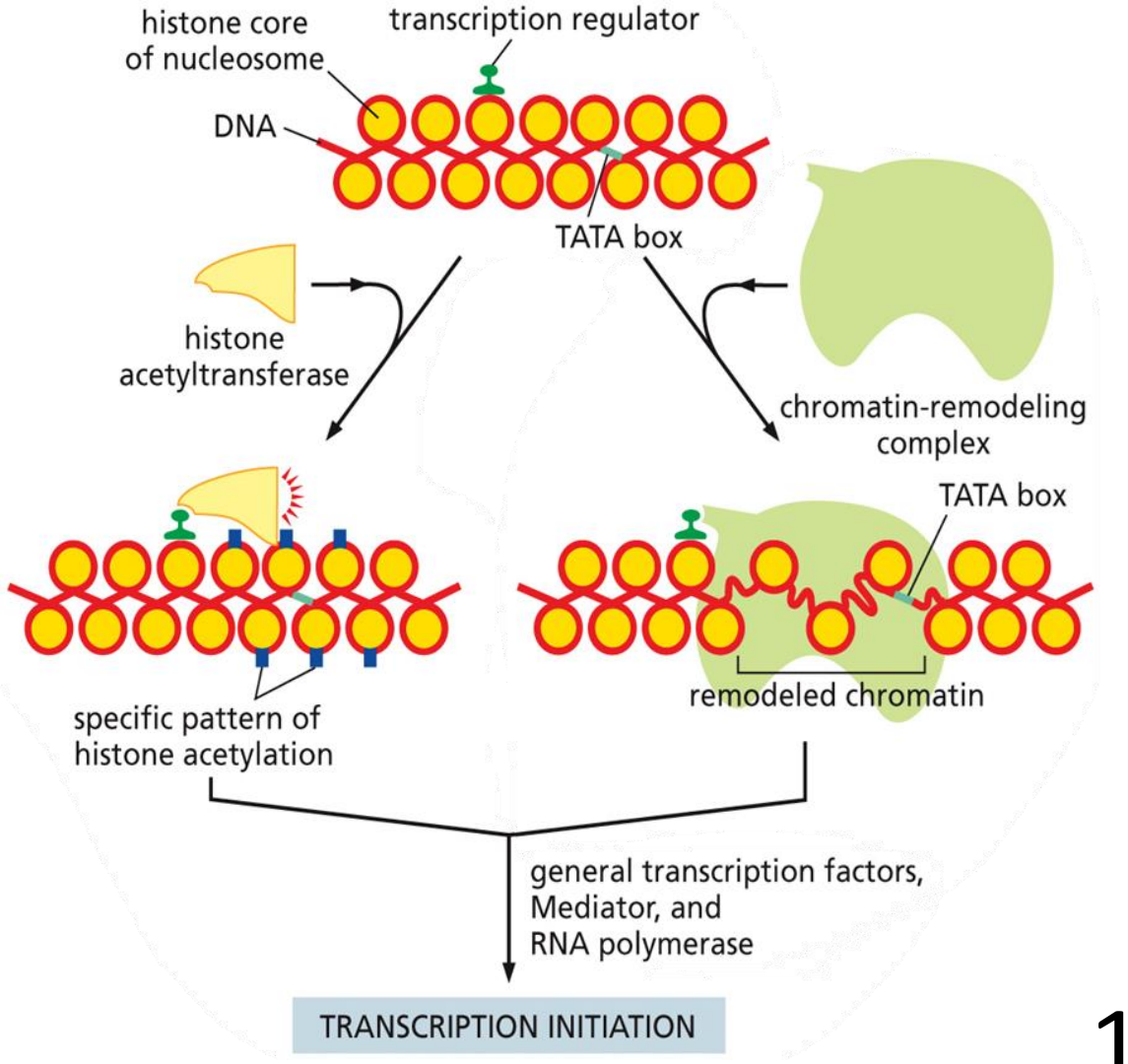


Eukaryotic transcription regulators help initiate transcription by recruiting chromatin-modifying proteins

- Histone acetyltransferase add **acetyl** groups to specific histones
  - Serve as binding sites for proteins such as general transcription factors that stimulate transcription initiation

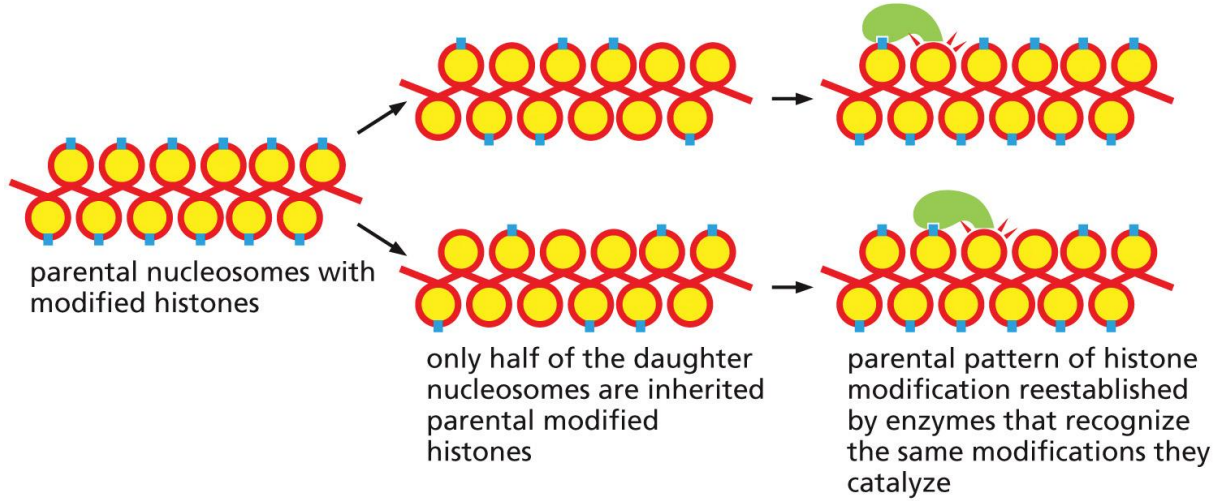
- Chromatin-remodeling complexes help make the DNA packaged in nucleosomes more accessible to proteins that mediate transcription initiation
    - Ex. Increased exposure of the TATA box

- Repressor proteins recruit enzymes to reduce transcription initiation
  - Histone deacetylase remove acetyl groups
  - Histone methyltransferase adds methyl groups

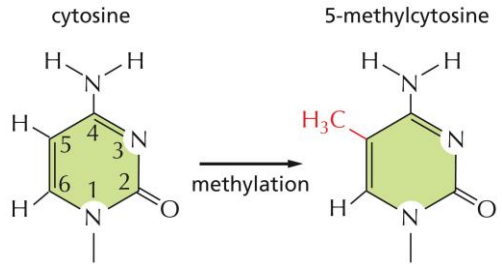


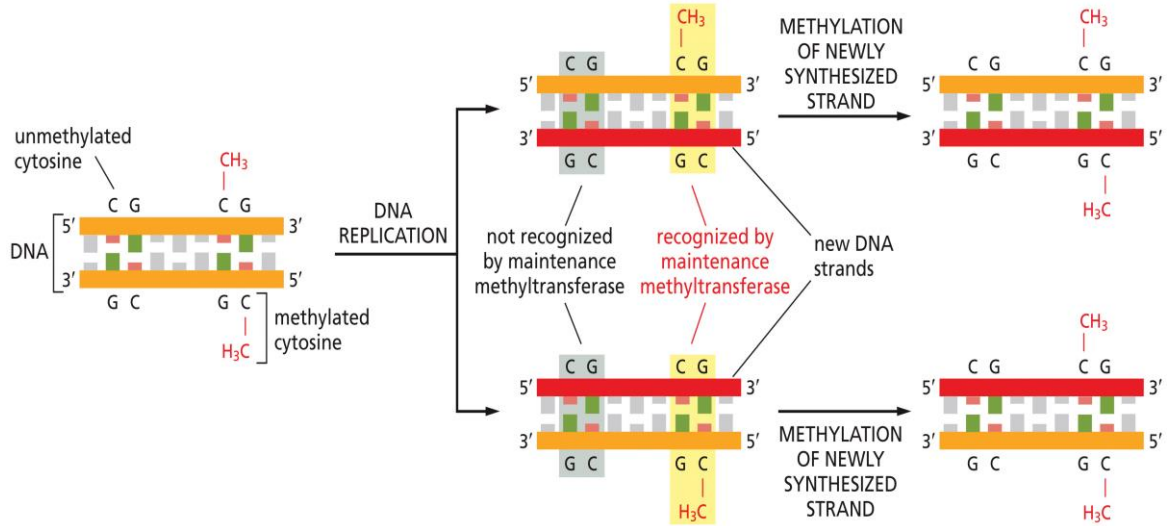
Epigenetic memory: Histone modifications and DNA methylation

- Histone modifications:
  - When a chromosome is replicated, each daughter chromosome will inherit half of the parental histones
  - Enzymes that create these modifications can bind and catalyze the same modification on the new histones, allowing inheritance of the parental chromatin structure



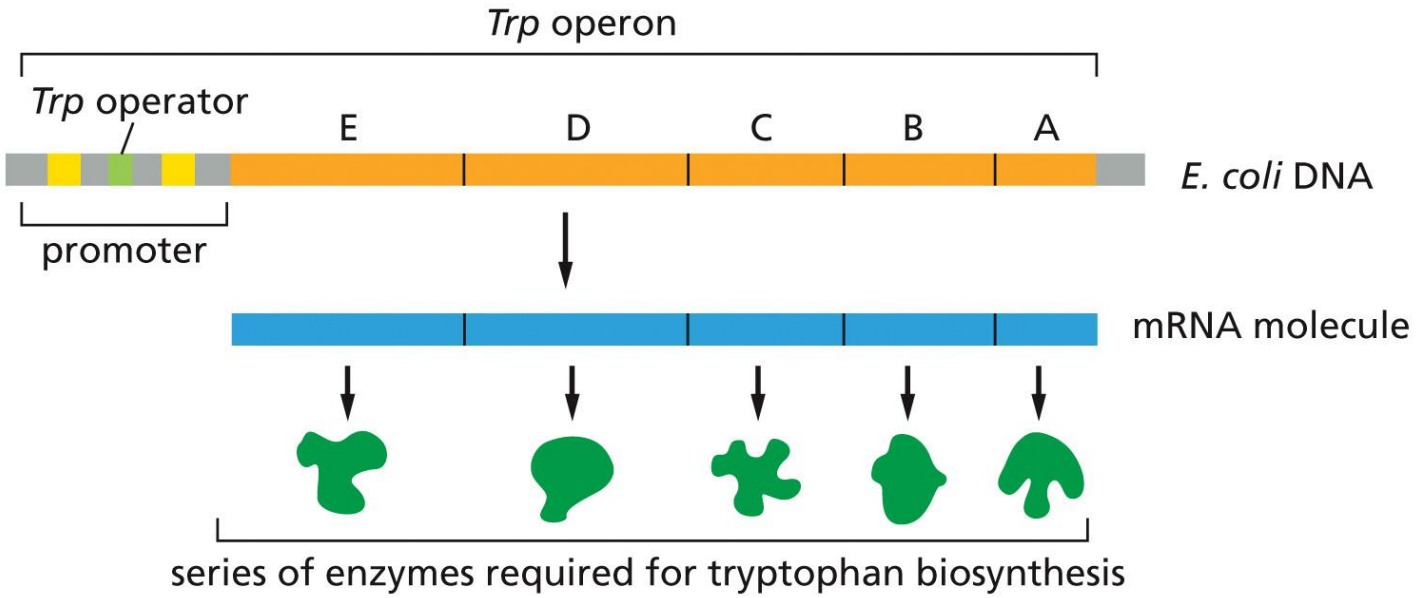
- DNA methylation-addition of methyl groups to certain cytosine bases in DNA:
  - Blocks access of transcription factors and recruits proteins that inhibit transcription
  - Can be inherited when a cell divides using a special methyltransferase





A cluster of bacterial genes can be transcribed from a single promoter

- Recall: Bacteria have **operons** - clusters of genes transcribed together from a single promoter to produce one mRNA molecule that encodes multiple proteins
  - Ex. each of the 5 genes here encode a different enzyme needed for tryptophan synthesis
  - Operon is controlled by the Trp operator
    - Green = transcriptional regulator (operator) binding site
    - Yellow = RNA polymerase binding site



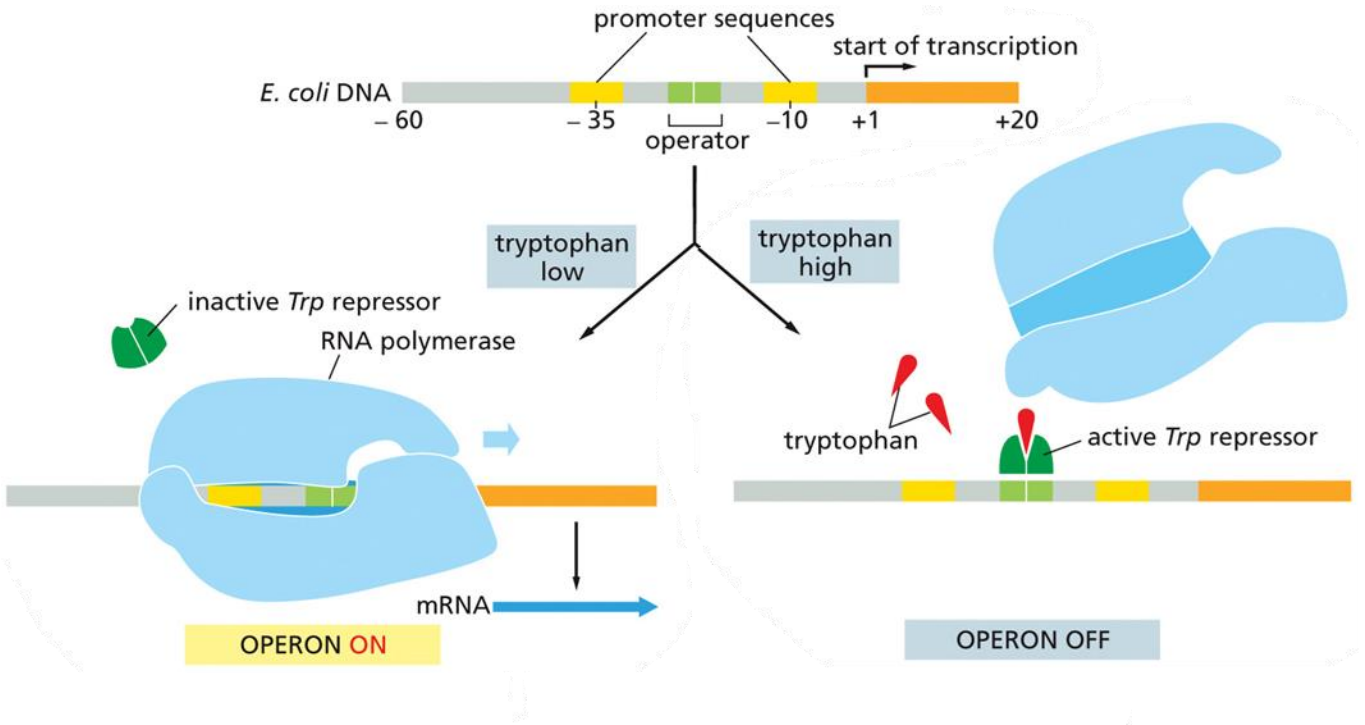
Genes can be switched off by repressor proteins

Ex. with Trp operon in bacteria:

- If tryptophan concentration inside a bacterium is low, RNA polymerase binds to the promoter and transcribes the 5 genes of the tryptophan operon

- If tryptophan concentration is high, the repressor protein binds tryptophan and becomes active
  - Repressor binds DNA (operator) and blocks binding of RNA polymerase

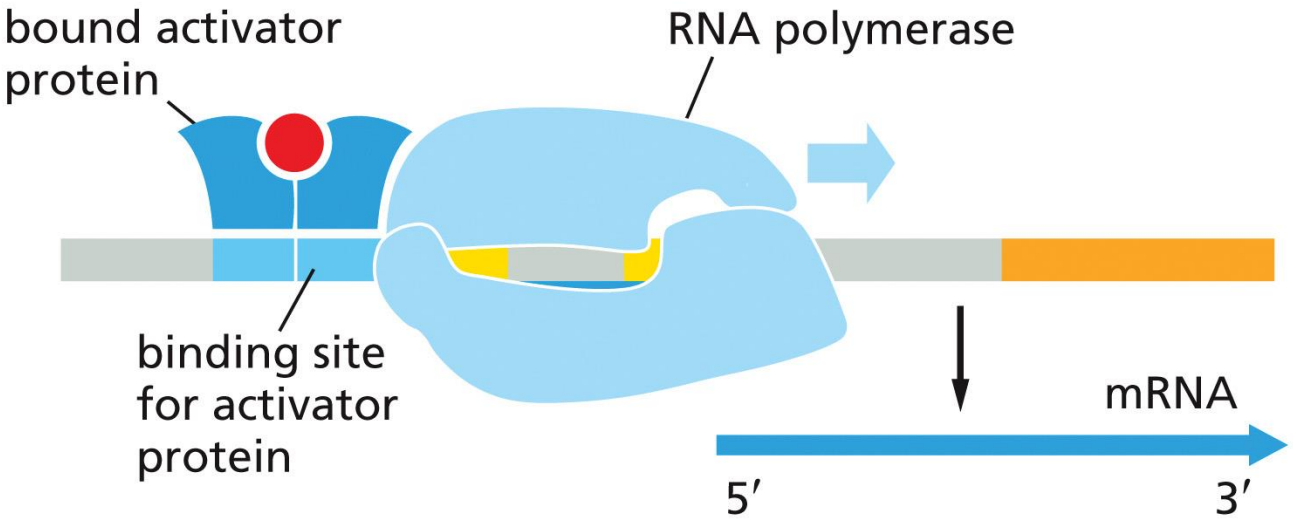
- When the concentration of tryptophan drops, the repressor releases the DNA, allowing RNA polymerase to again transcribe the operon (*feedback inhibition*)



*See video 8.4 for lac operon example

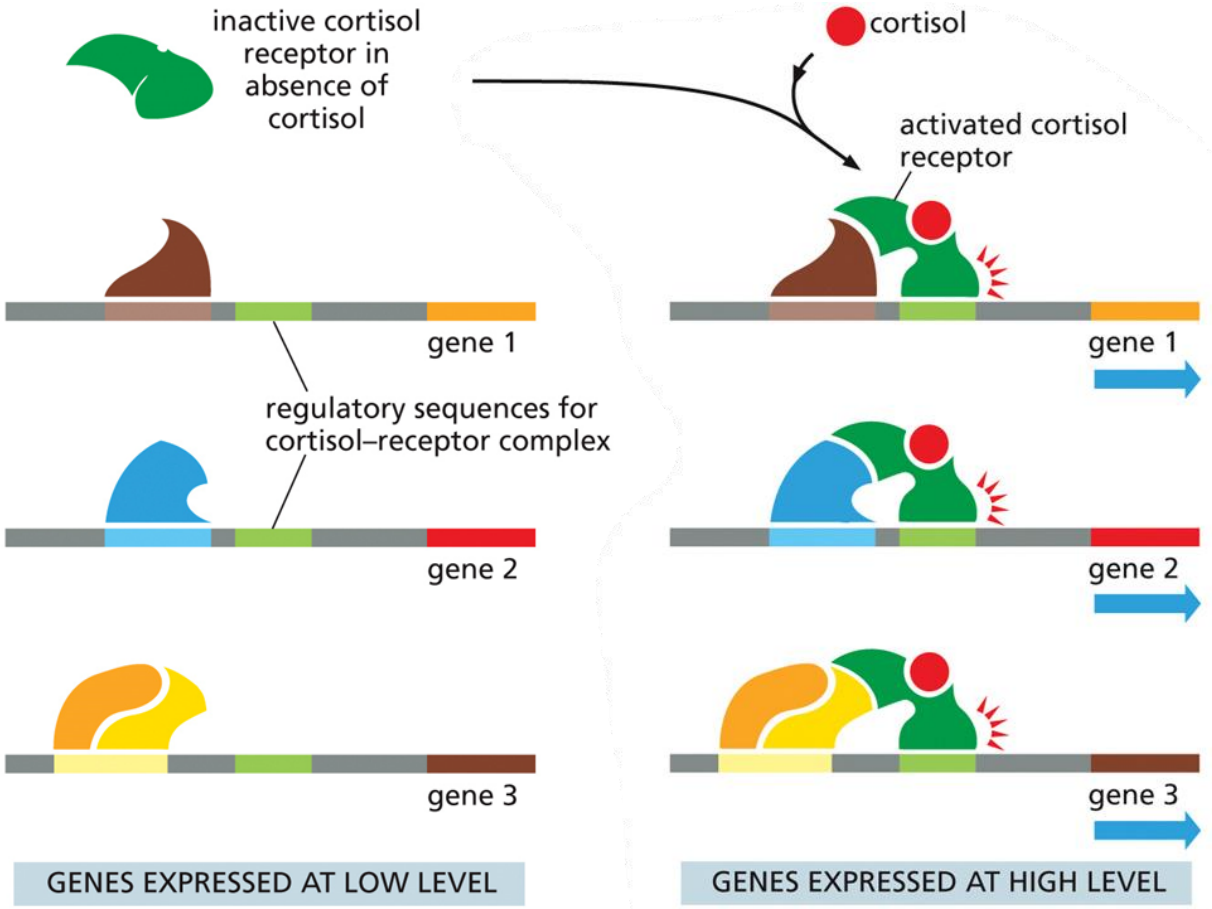
Genes can be switched on by activator proteins

- Some promoters are inefficient at binding and positioning RNA polymerase
- Activator proteins bind to a regulatory sequence on the DNA and to interact with the RNA polymerase to help it initiate transcription
    - Like the trp repressor, binding of the activator to DNA is often controlled by the interaction of a metabolite or other small molecule (*red* circle) with the activator protein



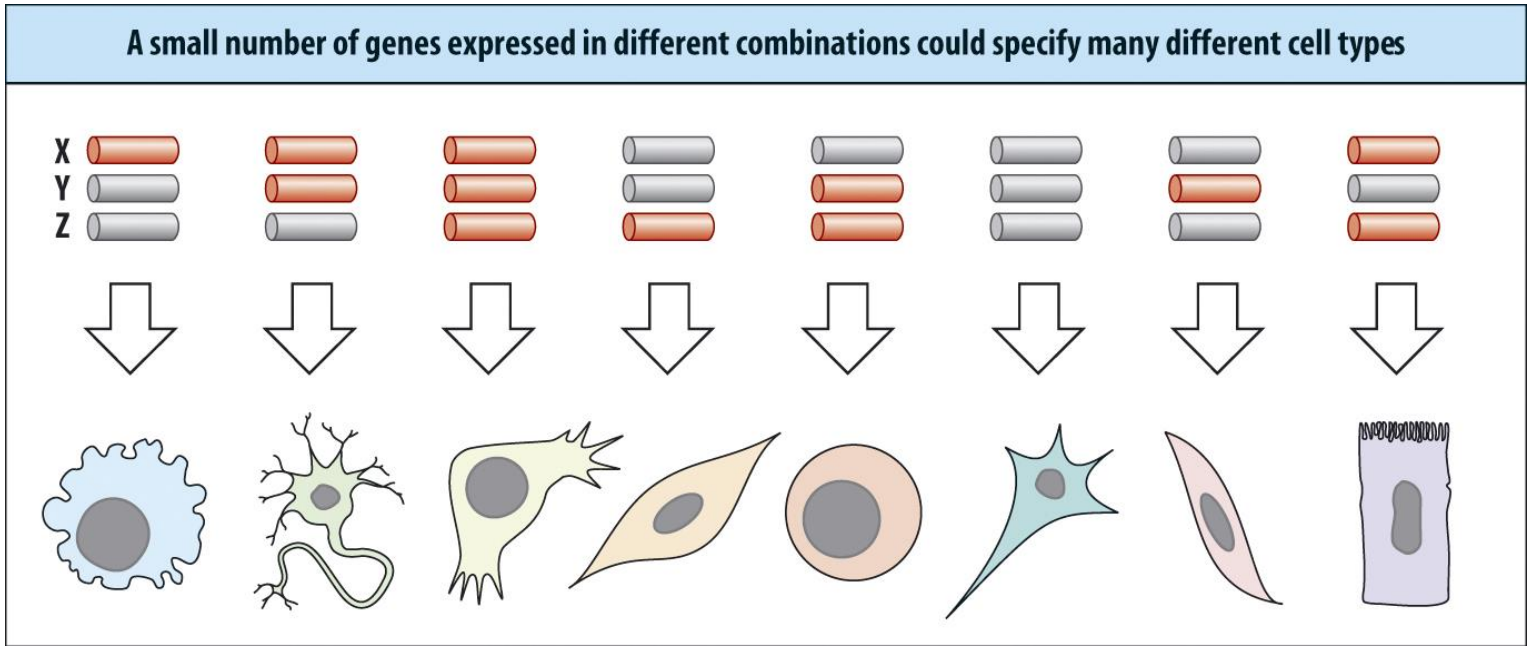
In eukaryotes, a single transcription factor can coordinate the transcription of many different genes

- Whereas bacteria use operons to initiate transcription of multiple genes at once, eukaryotes do this by using specific transcription regulators that combine with other gene-specific activators to initiate transcription
  - Same regulator can bind at different initiation complexes

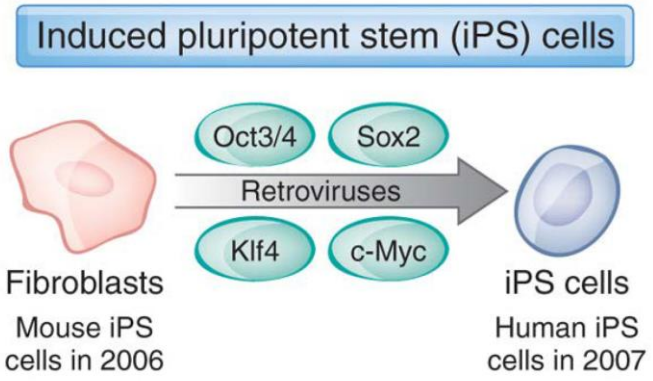


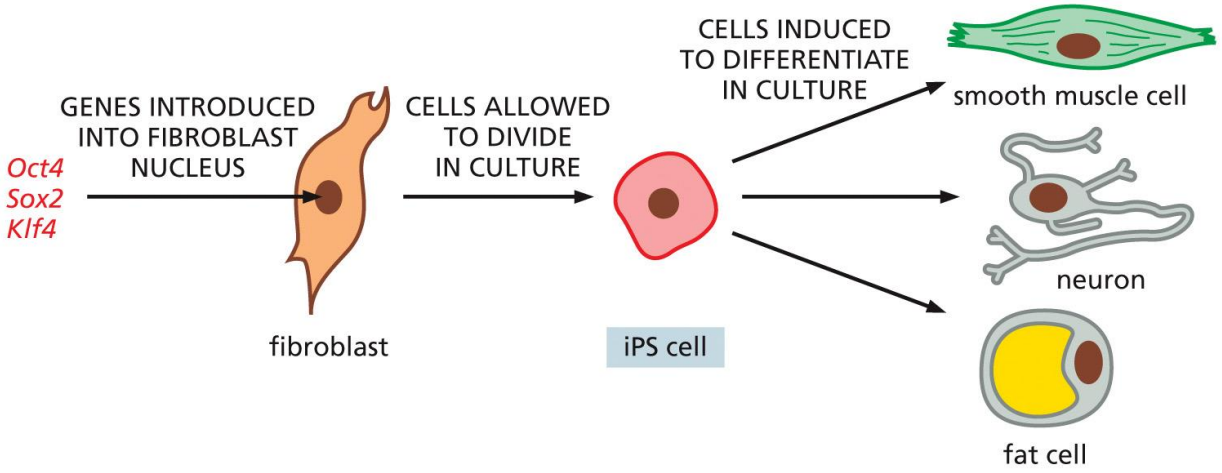
A combination of a few transcription regulators can generate many cell types during development

- Different combinations of just 3 transcription regulators can generate multiple cell types with different sets of genes turned on (hundreds or thousands)



Four transcription regulators form the regulatory network that specifies an embryonic stem cell





- Shinya Yamanaka discovered four **master regulators** that can reverse the developmental clock to induce differentiated adult specialized cells back into an embryonic like state
  - These iPSCs are therefore returned to a stage of development prior to the specialization and gene regulatory patterns that define unique cell types

- This technology can be leveraged to study specific individuals' specialized cells and their physiology without the need to isolate those specific cells
  - Such as studying heart rhythm and mechanics without needing to biopsy the heart

Squarecap Q#1-3

Learning Objectives for Chapter 8:

By the end of this module, you should be able to:

- Understand the roles of transcriptional regulators in modulating gene expression (e.g., enhancers and repressors, operons, and master transcription factors)
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- Detail the role of post-translational modifications as it relates to protein activity and turnover

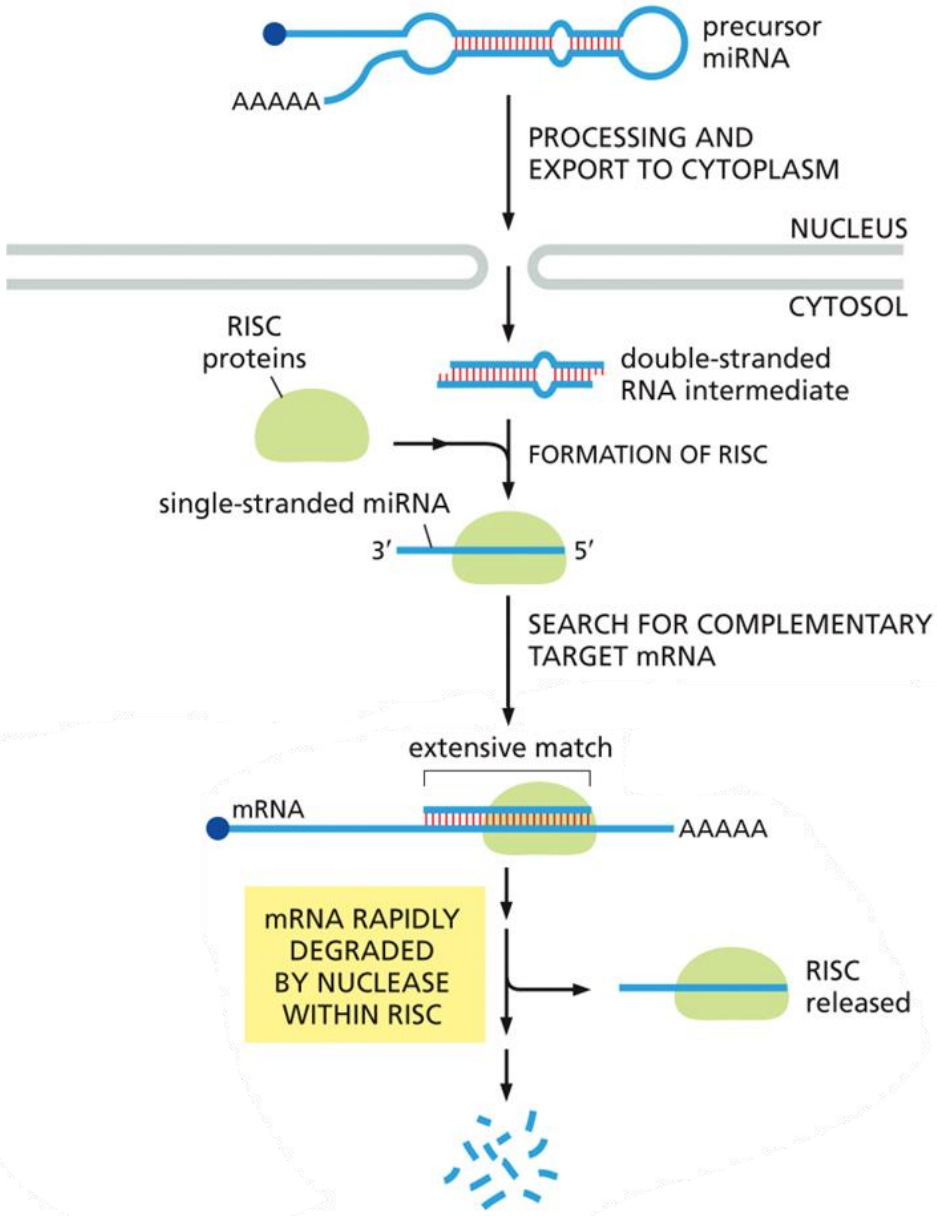
Post-transcriptional control is mediated via non-coding RNAs

- Regulatory RNAs control the expression of thousands of genes after transcription has taken place
  - **microRNAs** are endogenous molecules that direct the destruction of target mRNAs
  - **long noncoding RNAs**, serving as scaffolds, may also regulate mammalian gene activity
  - Bacteria use **small noncoding RNAs** to protect themselves from infection

MicroRNAs direct the destruction of target mRNAs

- A **microRNA (miRNA)** is a ~22 RNA nucleotide sequence that base pairs to specific mRNAs targeting them for destruction
  - A precursor miRNA transcript (~64 nucleotides with 5’ cap and poly A tail) with a hairpin structure is processed at several steps to form a mature, single-stranded miRNA

- miRNA assembles with a set of proteins into a complex called **RNA-induced silencing complex (RISC)**
  - Searches for mRNAs that have a nucleotide sequence complementary to the miRNA
    - Leads to rapid degradation of mRNA by RISC nucleases

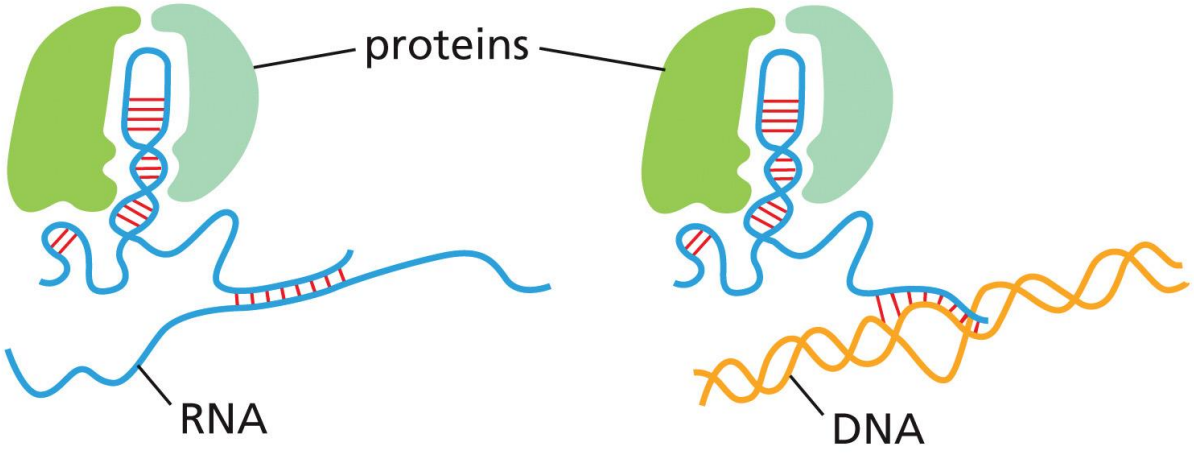


Thousands of long noncoding RNAs may also regulate eukaryote gene activity

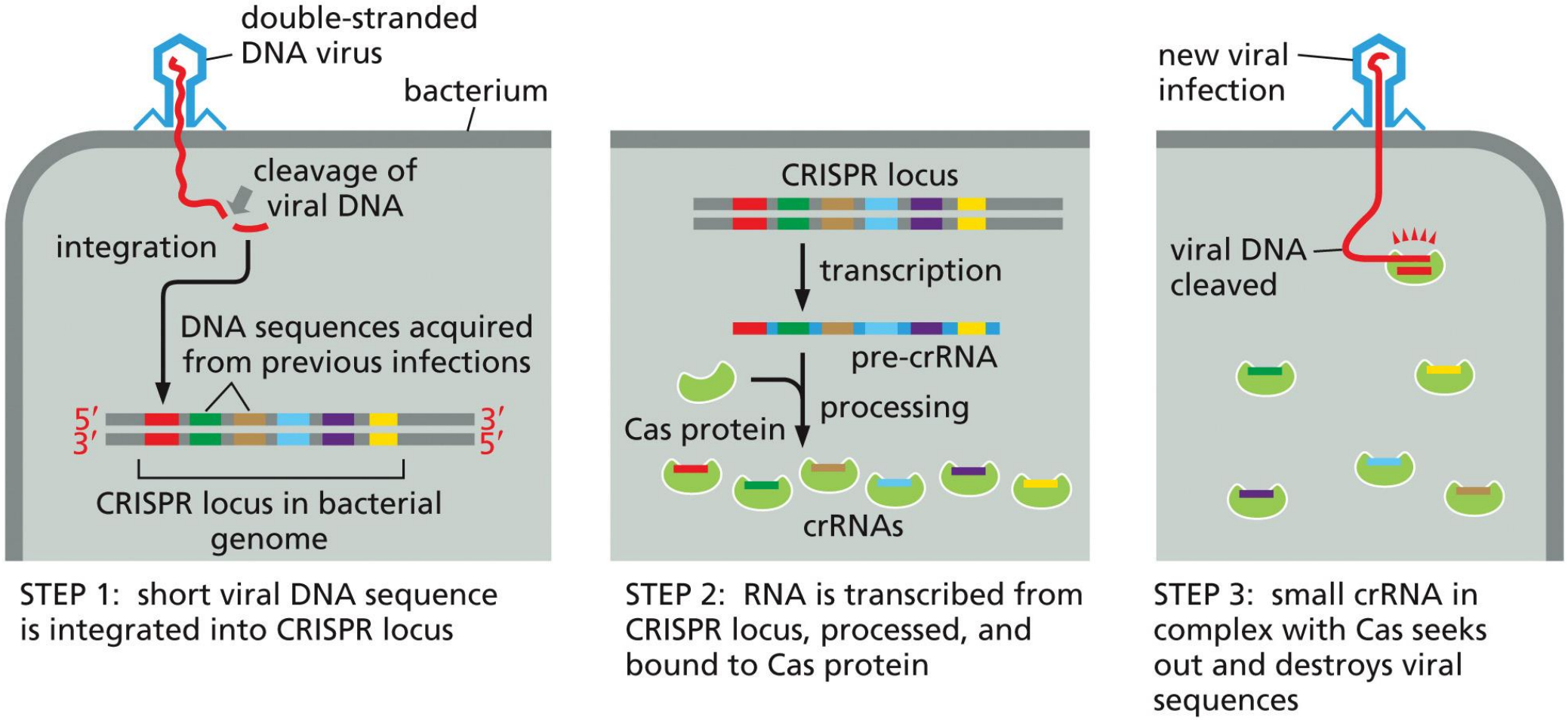
- **Long noncoding RNAs (lncRNA)** (>200 nucleotides) can fold into three-dimensional structures that can be recognized by specific proteins

- By engaging in complementary base-pairing these long noncoding RNAs can localize proteins to specific sequences in RNA or DNA molecules

- Serve as **scaffolds,** bringing together proteins that function together
  - Ex. Xist is a lncRNA that assists in the complete inactivation of the second X chromosome in XX individuals



Bacteria use small noncoding RNAs to protect themselves from viruses (similar to adaptive immunity in humans)



STEP 1: short viral DNA sequence is integrated into CRISPR locus

STEP 2: RNA is transcribed from CRISPR locus, processed, and bound to Cas protein

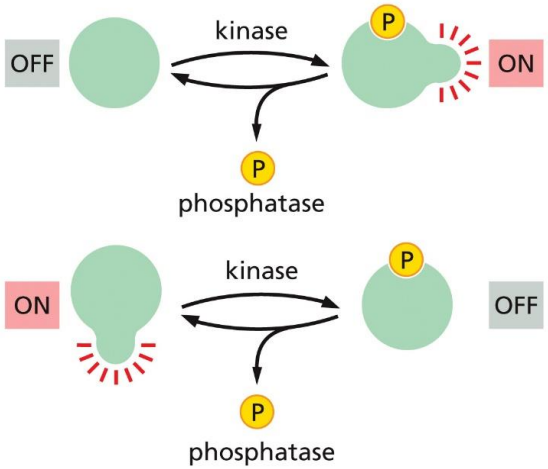
STEP 3: small crRNA in complex with Cas seeks out and destroys viral sequences

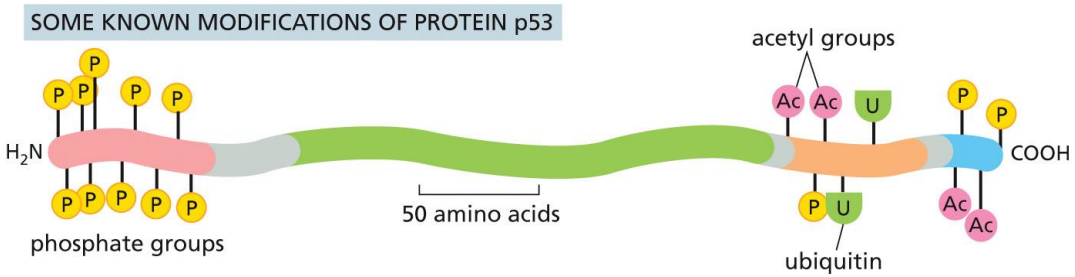
This is the basis for the CRISPR/Cas9 gene editing approach

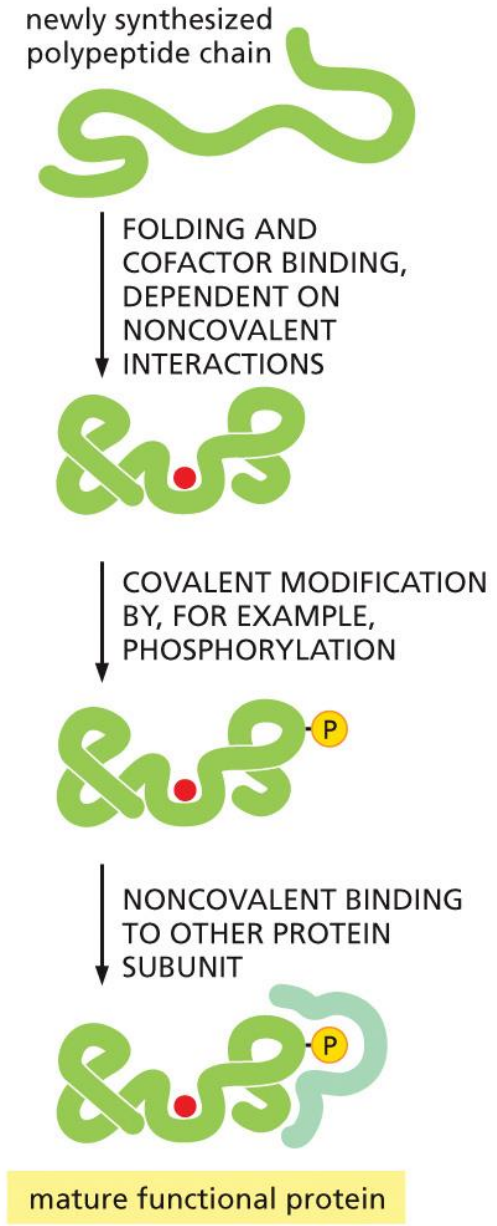
Post-translational modifications also affect protein activity

- Covalent addition of functional groups can influence activity/localization/interactions with other molecules

Recall: Kinases and phosphatases

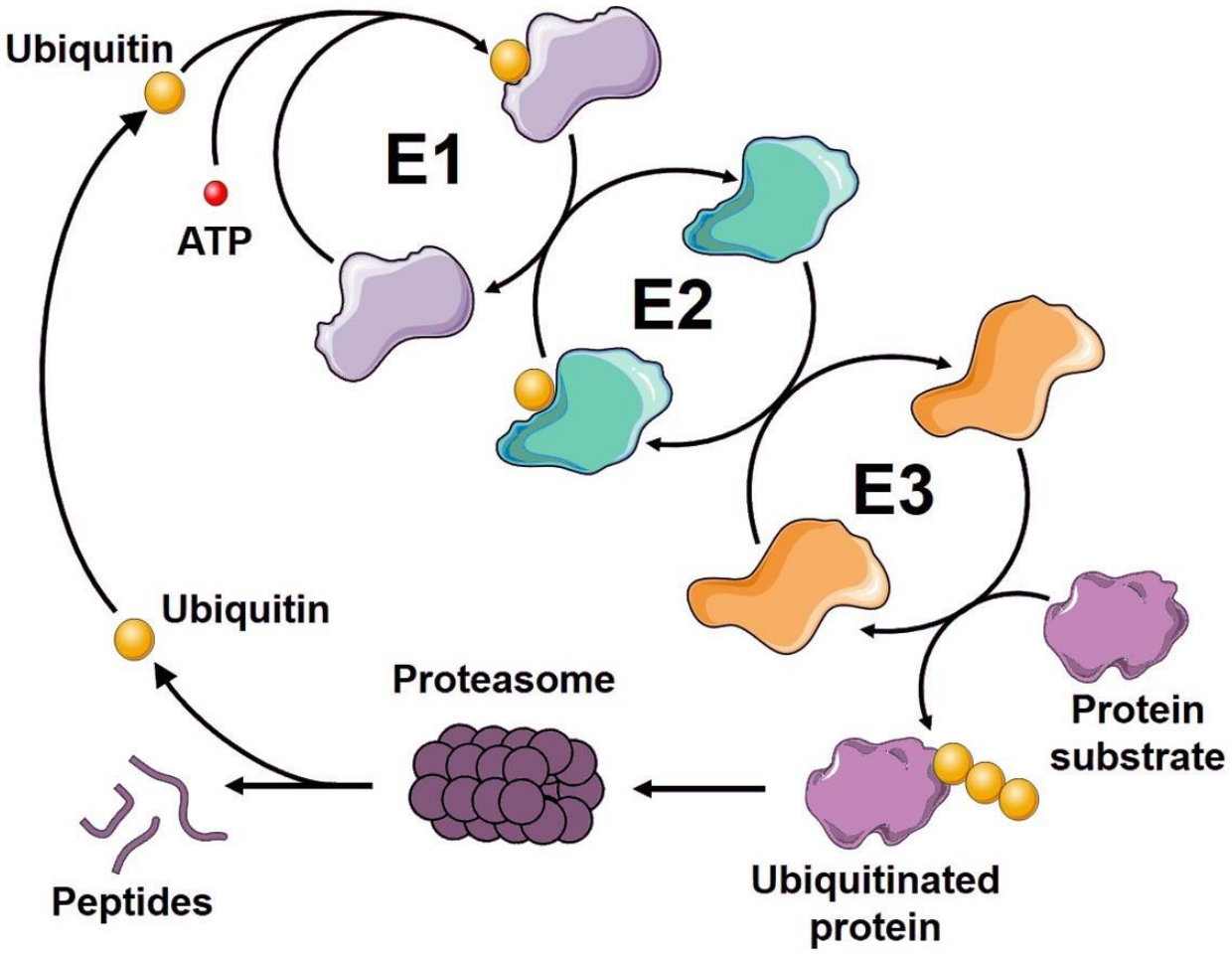






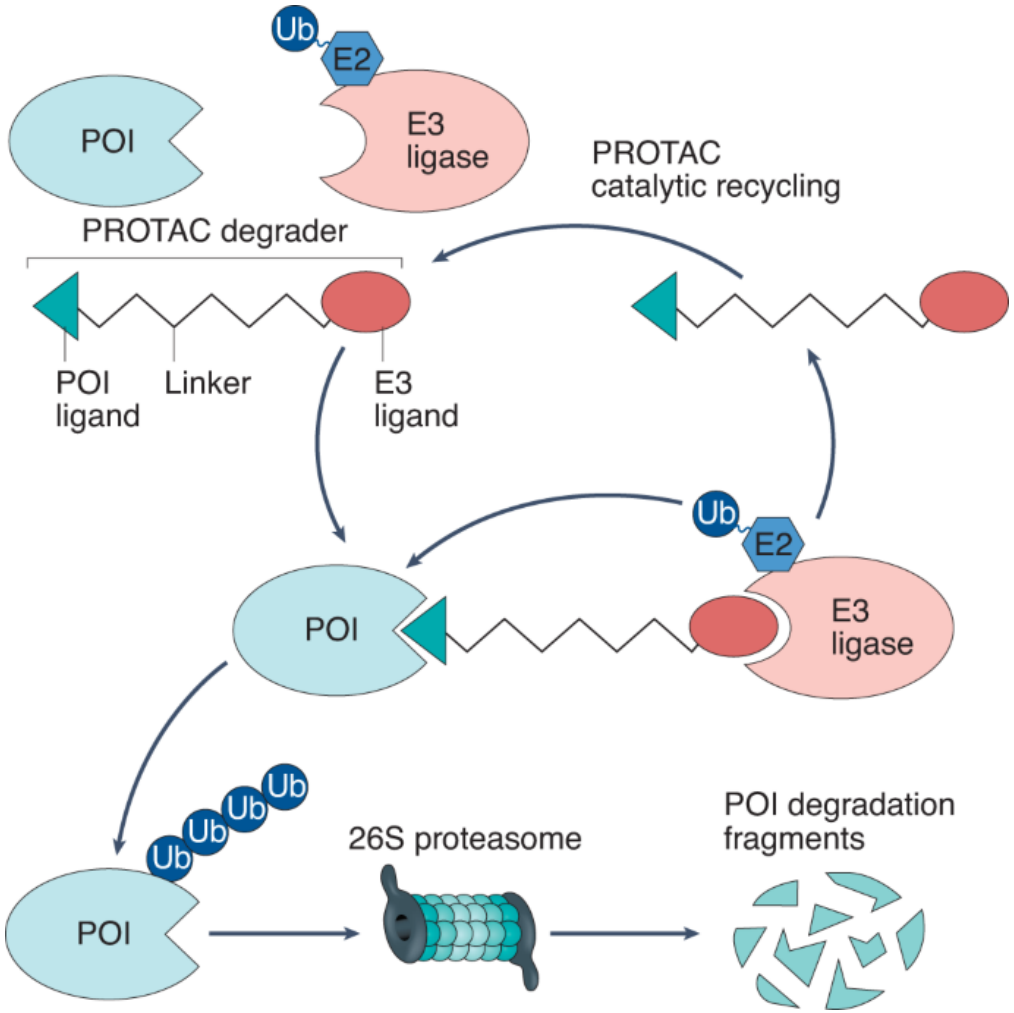
Targeted Protein Degradation: The Ubiquitin Proteasome System

- Protein turnover can be regulated by **ubiquitination**
- A series of enzymes lead to the addition of ubiquitin groups to a protein of interest
- A poly-ubiquitin chain is recognized by the **proteasome** which degrades proteins to be recycled for their components



https://lifesensors.com/ubiquitinproteasomesystem/

Proteolysis-Targeting Chimeras (PROTACs) are a tool that leverages the ubiquitin pathway to degrade specific proteins of interest (POIs)



- This technique allows researchers to regulate protein expression in cells to study cell biology and develop therapeutics

https://www.nature.com/articles/s41587-021-01173-2

Squarecap Q#4-5

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Reflection/Feedback

https://forms.gle/vbdZXee89Dh9diY78