

MCDB 4650 Class 2

Developmental control of gene expression



Sign up for Problem Solving Sessions (link from webpage)
Sessions start next week

My office hours, Porter B121 B/C: Fridays 9-10 AM

Some of the topics you say are of interest:

- Stem cells
- Sex determination
- Human genetic disorders
- Applications of developmental biology to health and medicine
- Specialization of cell types
- How gene regulation affects development

Learning goals for class 2

Be able to:

- Evaluate which portions of genomes are critical for complexity of an organism
- Recognize the functions of different portions of the genome.
- Compare the different ways in which gene expression can be regulated, and relate these mechanisms to regulation during development.

Genomic information

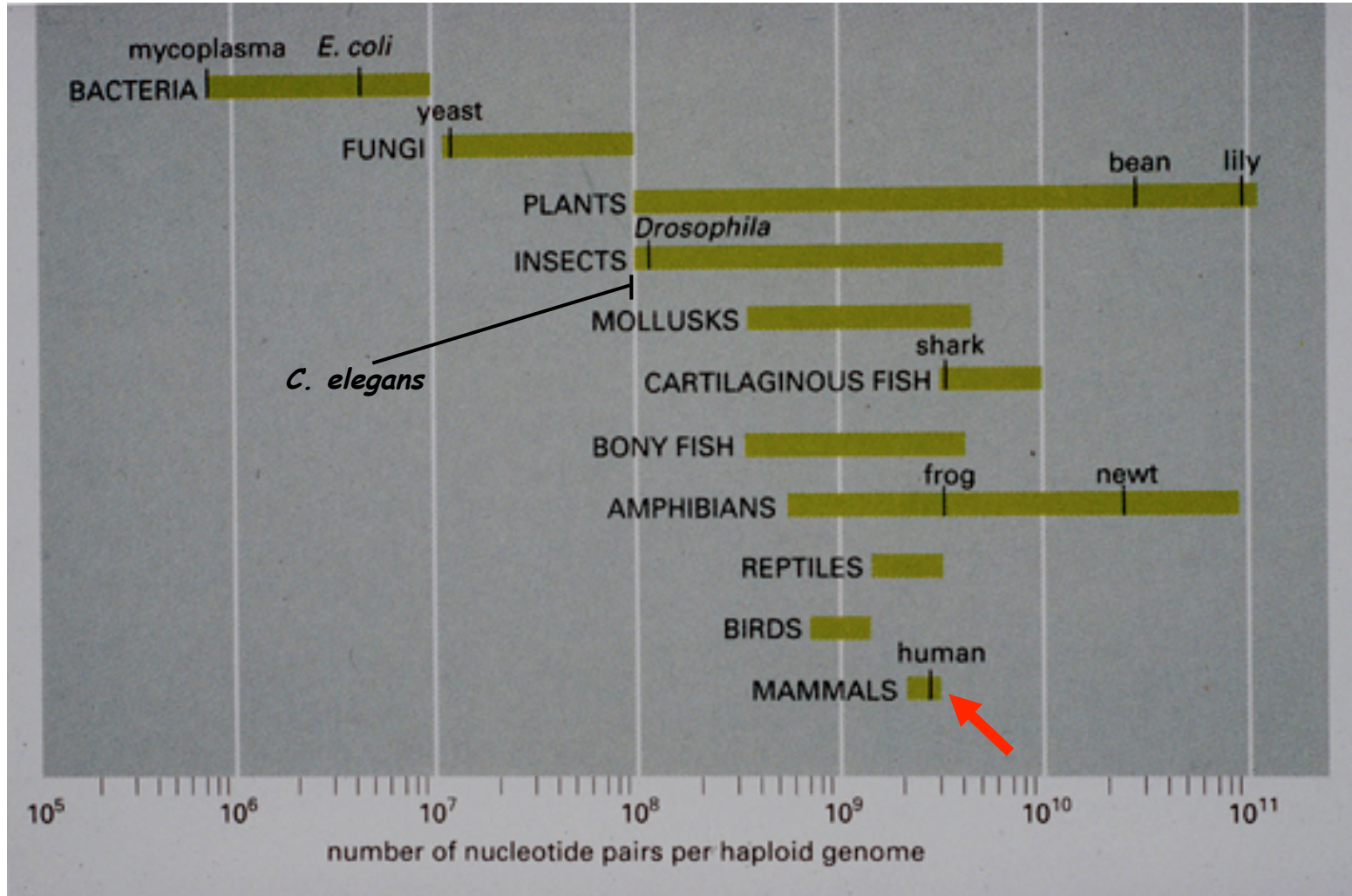
Which of the following is the ideal way to compare the information contained in the respective genomes of two different kinds of organisms?

- a) Compare the complexity (number of DNA nucleotides in non-repetitive sequences) in the two genomes.
- b) Sequence the two genomes, count the number of putative genes based on computer analysis, etc., and compare these numbers.
- c) Count the number of different proteins that are synthesized throughout each animal's life cycle and compare these numbers.
- d) Compare the non-coding regions of the genome

Please vote individually.

Discuss your answers. What is your rationale?

Genome sizes in various phyla



Gene: Genome relationships in five organisms

Organism	Size (Mb)	Estimated gene number	Coding percentage
<i>E. coli</i>	5	~ 4,000	~ 80%
Yeast	20	~ 5,000	~ 50%
Caenorhabditis	97	~ 19,000	~ 20%
Drosophila	180	~ 13,000	~ 8%
Human	3,000	<30,000	~ 1%

In the genomes of more complex organisms, there is a much higher proportion of non-coding DNA than there is in the smaller genomes

What's in the non-coding DNA?

introns

regulatory regions

splicing enhancers and silencers

transcription enhancers and silencers

transposons

untranslated RNAs

micro RNAs

“junk” (unknown function)

Most relevant for us: regulatory regions

Differential gene expression

“Gene expression”: a gene is transcribed into mRNA and ultimately into functional protein or a functional RNA

“Differential”

Different cells (all with the same genome) express:

many shared genes (“house-keeping” for general cellular functions)

only a subset of “cell specific” genes that allow individual cells to take on different fates and different functions

How does it happen?

First, let's review the anatomy of gene regions

- fill out your own sheet of paper, but work in groups around your tables (groups of 3-4 usually work well)
- You may keep your own sheet, but I will ask for volunteers to share their answers (so discuss your ideas with your neighbors)

Work only on question 1

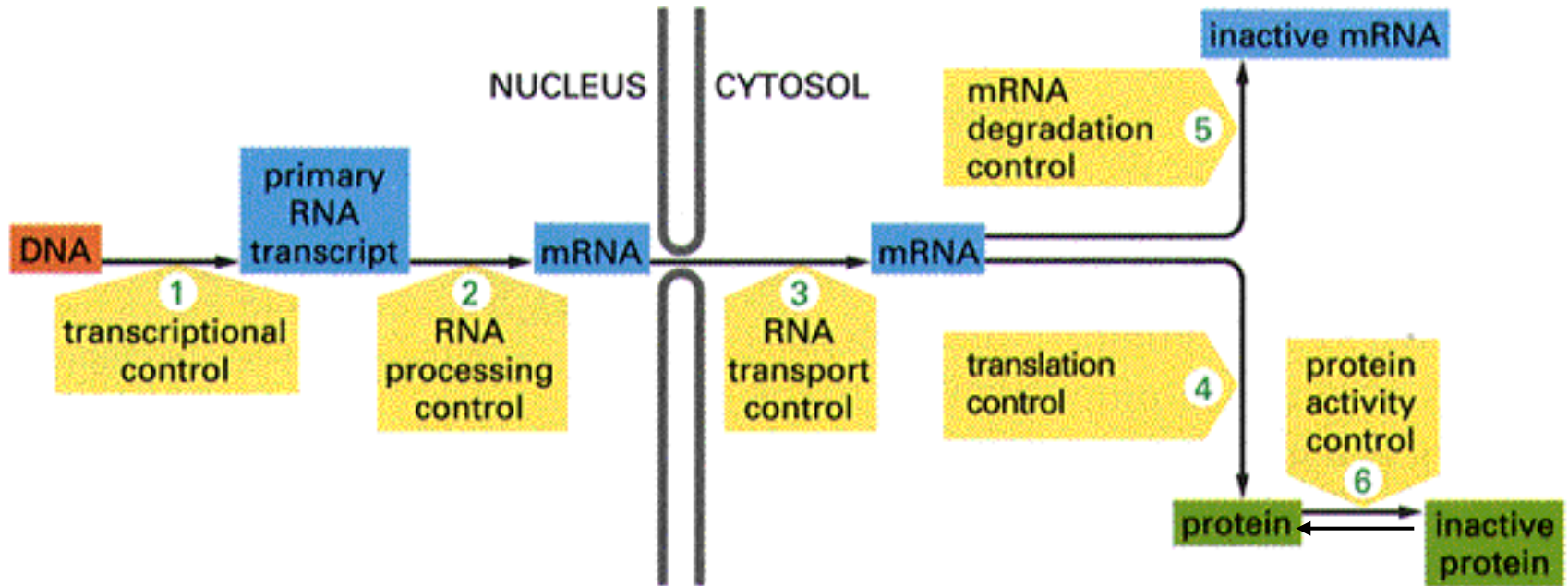
Now let's explore the different ways in which gene expression can be controlled

Question 2 on handout:

Make a concept map

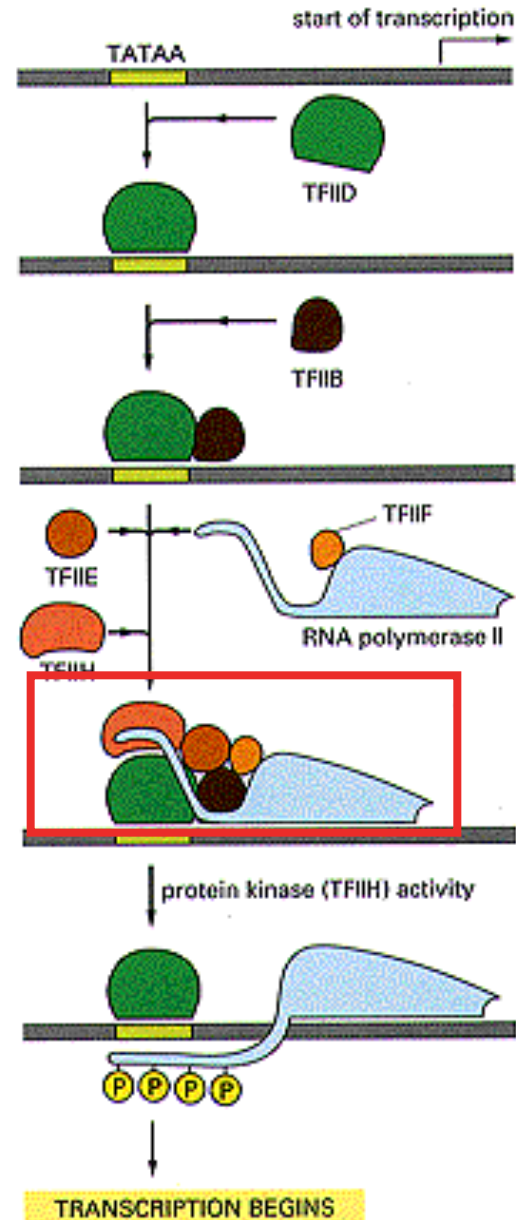
Indicate what kinds of controls are active at each step, and list possible mechanisms of regulation at each of these steps.

Control over RNA and protein expression

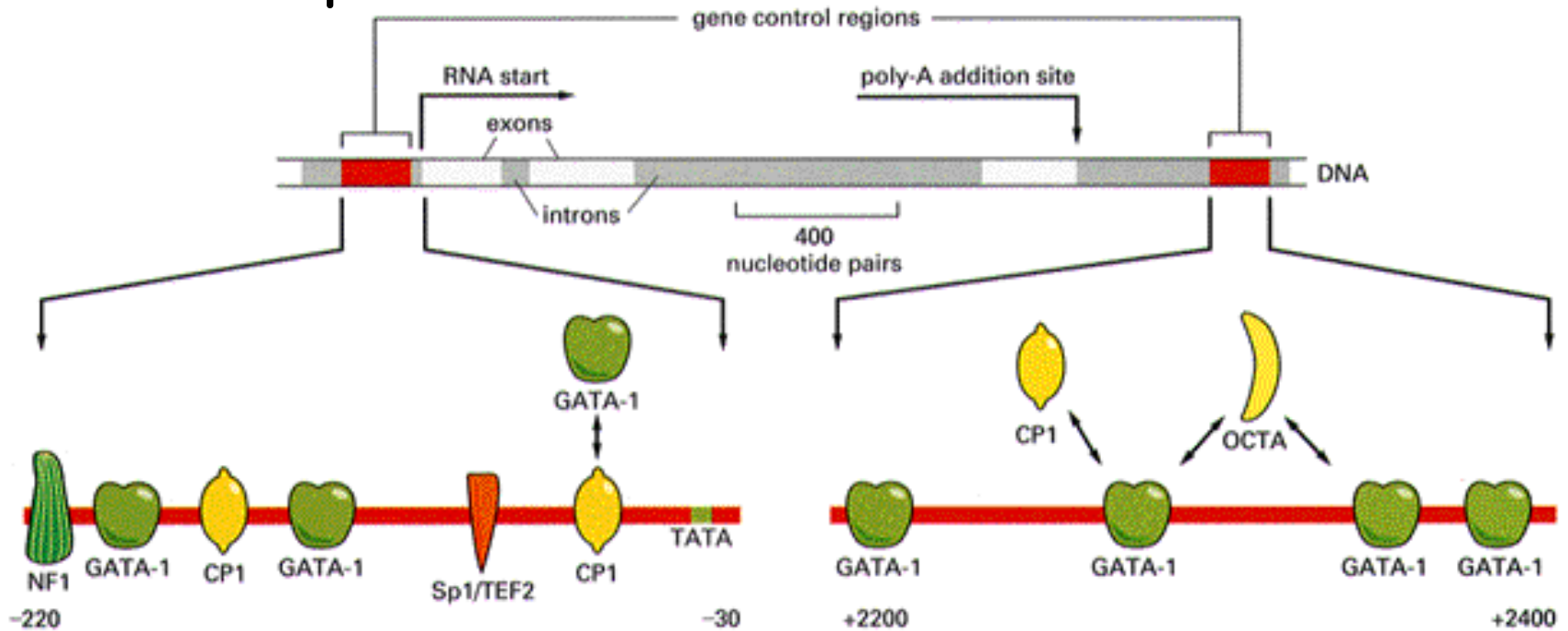


What does transcription initiation require?

- an open or relaxed chromatin configuration
- binding of RNA PolII to the promoter
- activation of PolII by interaction with cell-specific transcription factors

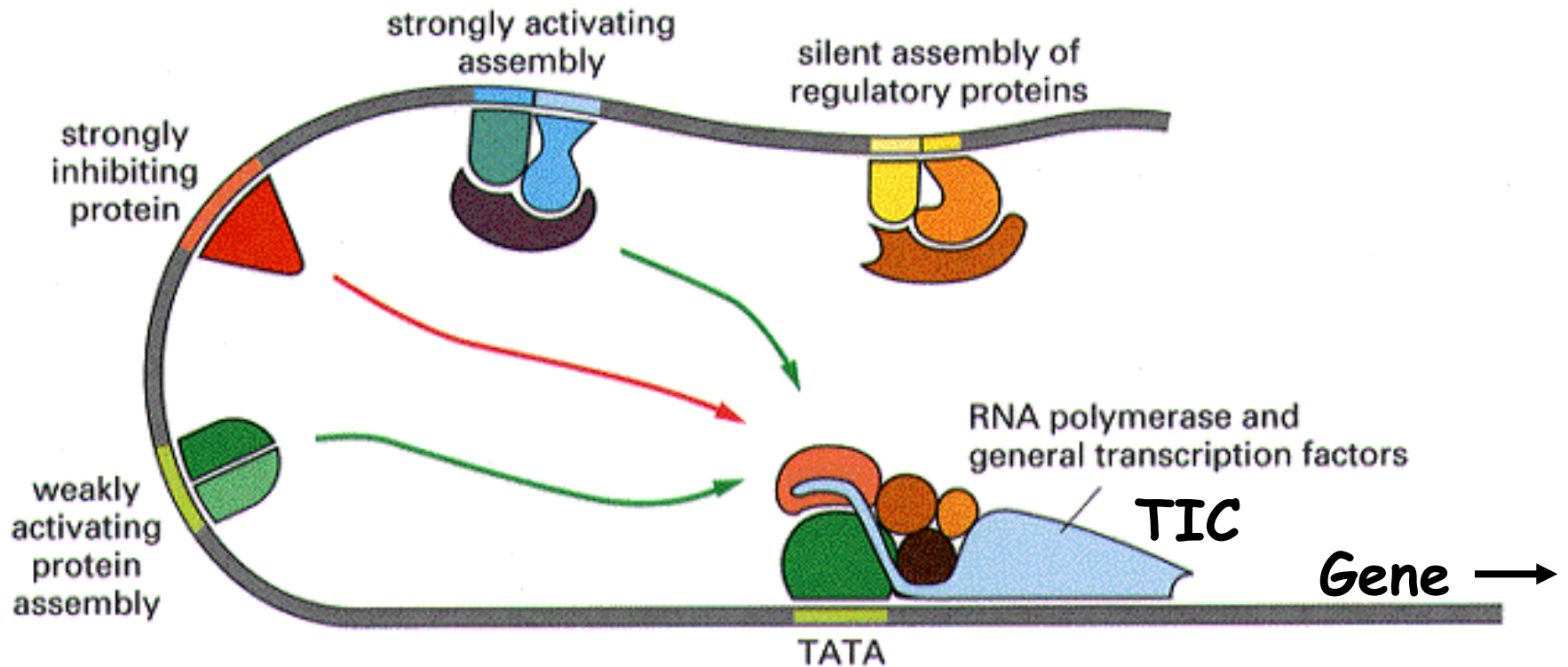


It is the gene-specific **regulatory elements** (enhancers and silencers: the red regions) and the **transcription factors** (the fruits!) that bind to them that are important for **differential control** of this process



Human beta-globin gene

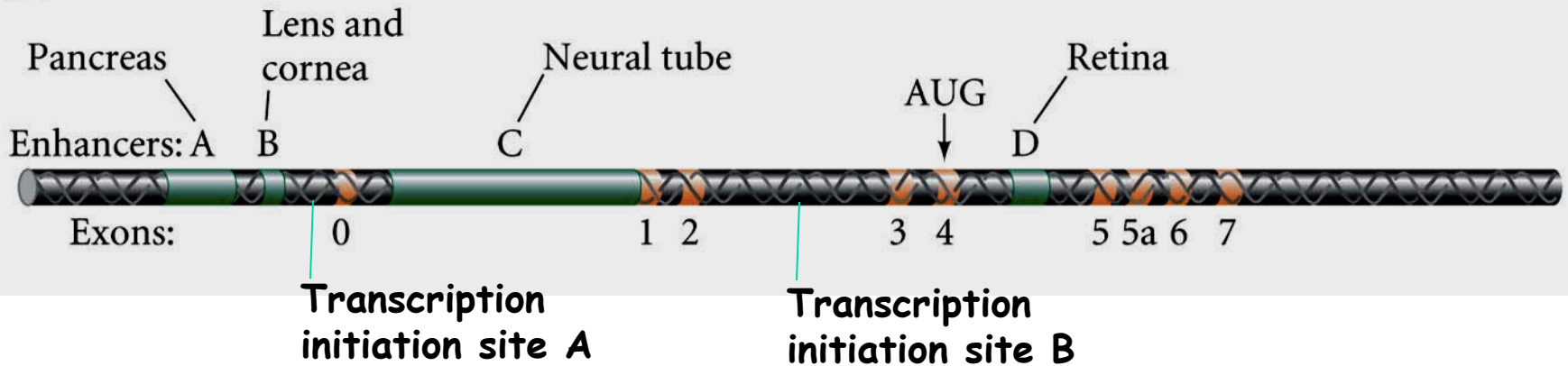
Specific combinations of transcription factors in different cells control the rate of transcription initiation by the TIC



Below is the *pax-6* gene, which encodes a transcription factor expressed in many different places in the embryo

Orange: exons Green: enhancers (REs)

(B)

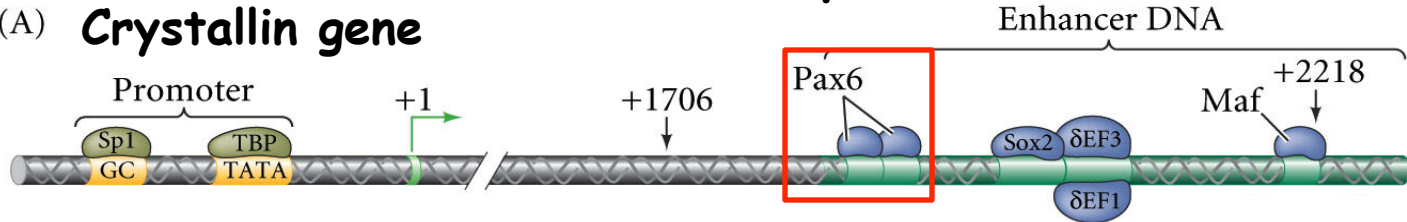


Given this information, what do you expect is true of the RNAs and proteins that will be made from the *pax-6* gene?

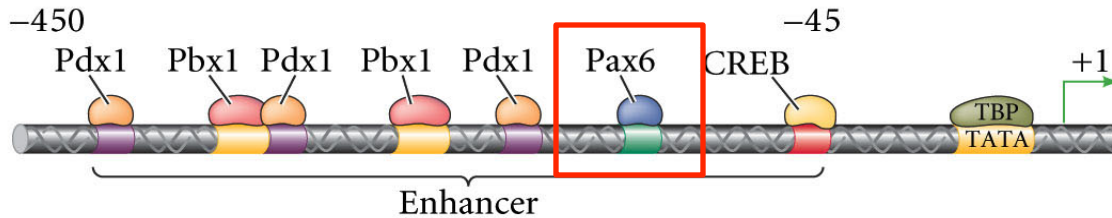
- Two different length RNAs and two different length proteins can be made
- Two different length RNAs are made; the protein is the same length no matter what
- Only transcription site A will be used; one size protein will result
- Only transcription site B will be used, one size protein will result.

Both of the genes shown below have large enhancer regions to which the transcription factor Pax-6 can bind. Crystallin protein is found in the eye; Somatostatin is found in the pancreas.

(A) Crystallin gene



(B) Somatostatin gene

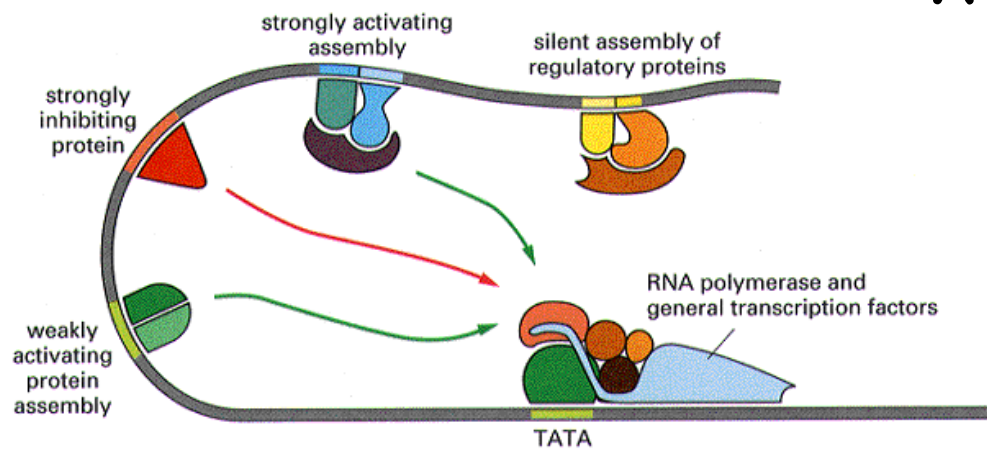


What is the best explanation for why somatostatin protein is found only in the pancreas (not the eye)?

- Pax6 is at a higher concentration in the pancreas.
- Pax6 silences somatostatin expression in the eye.
- Eye cells do not contain the other transcription factors required for somatostatin expression.
- The enhancer regions to which Pax6 binds are different in cells of the pancreas cells vs. cells of the eye.

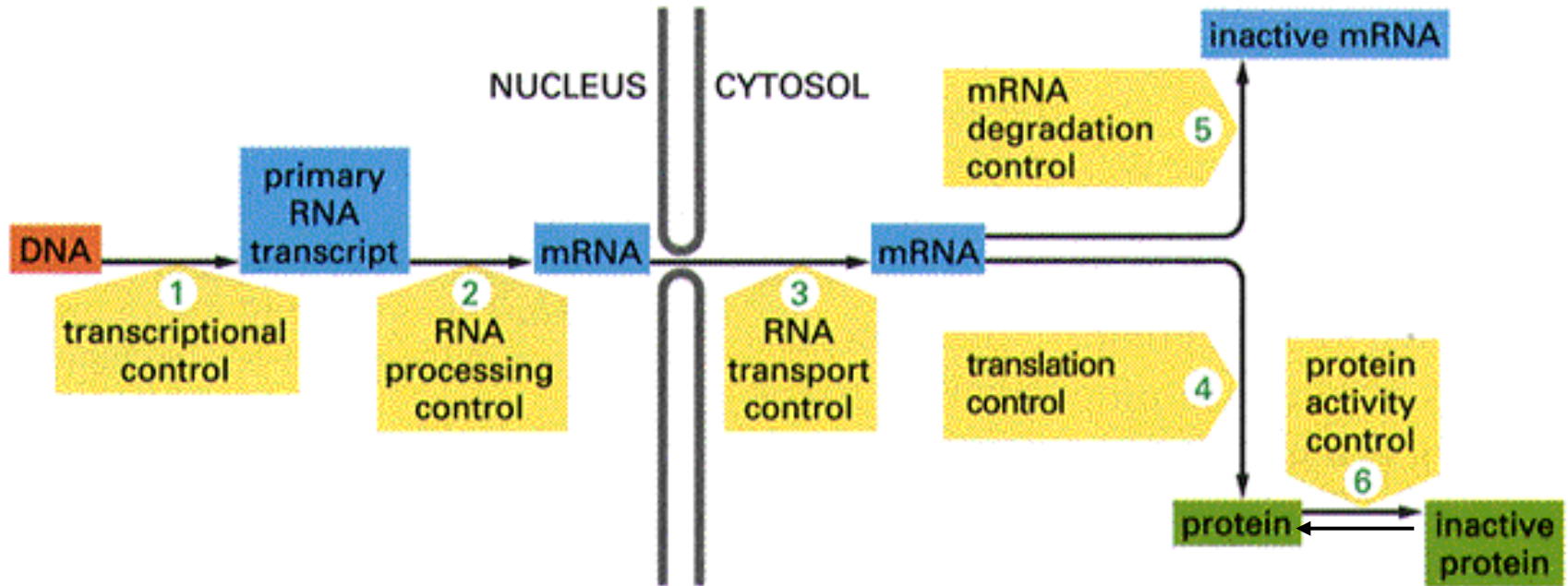
What I have just shown you is the principle of:

Combinatorial control



At any given promoter, it is the combination of interacting transcription factors (activators and repressors) bound to regulatory elements (enhancer or silencer sequences) in the vicinity of the gene itself that controls transcription initiation.

Control over RNA and protein expression

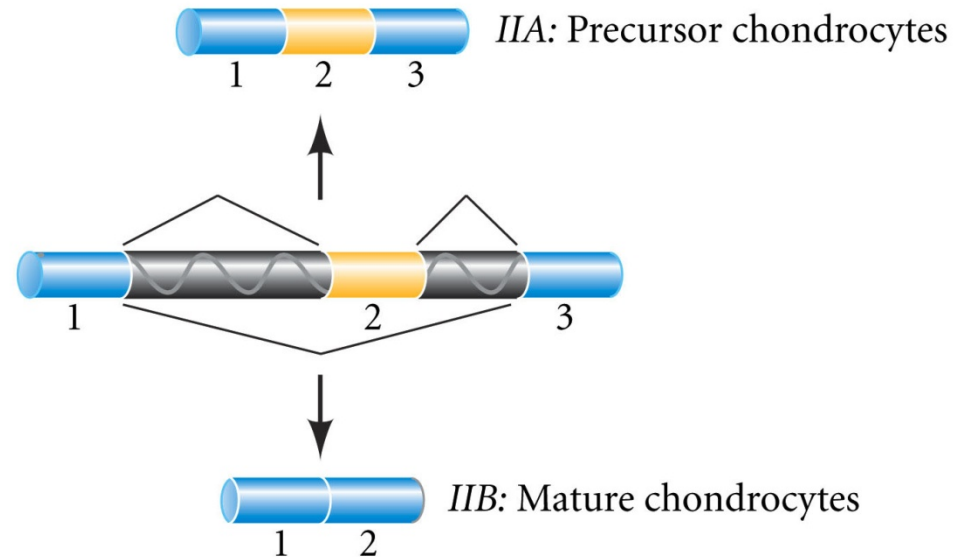


RNA processing control

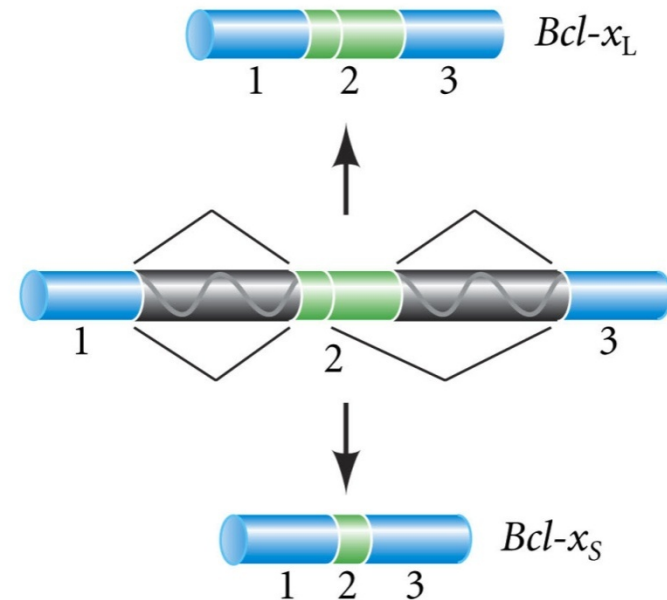
Alternative splicing

Different versions of the same protein

(A) Cassette exon: Type II procollagen

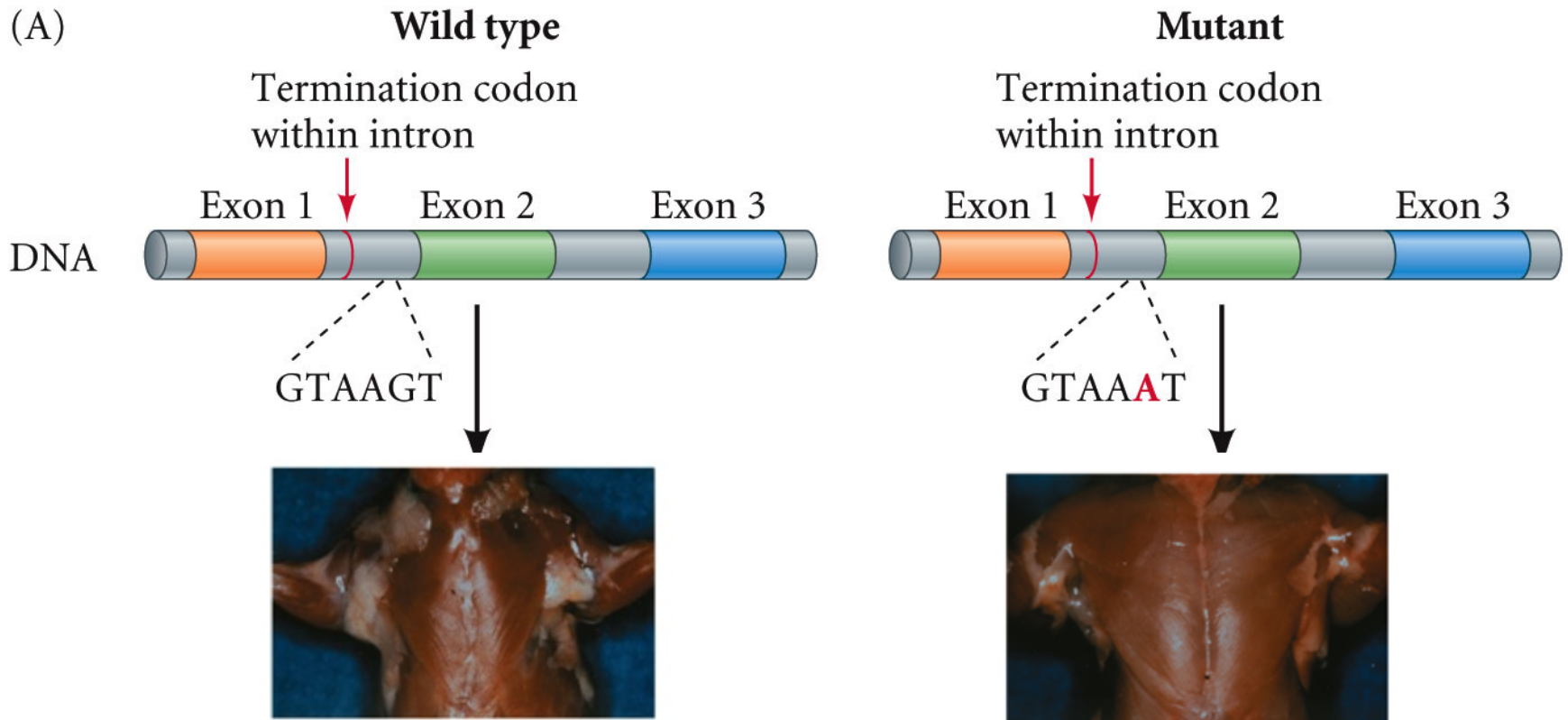


(C) Alternative 5' splice site: *Bcl-x*



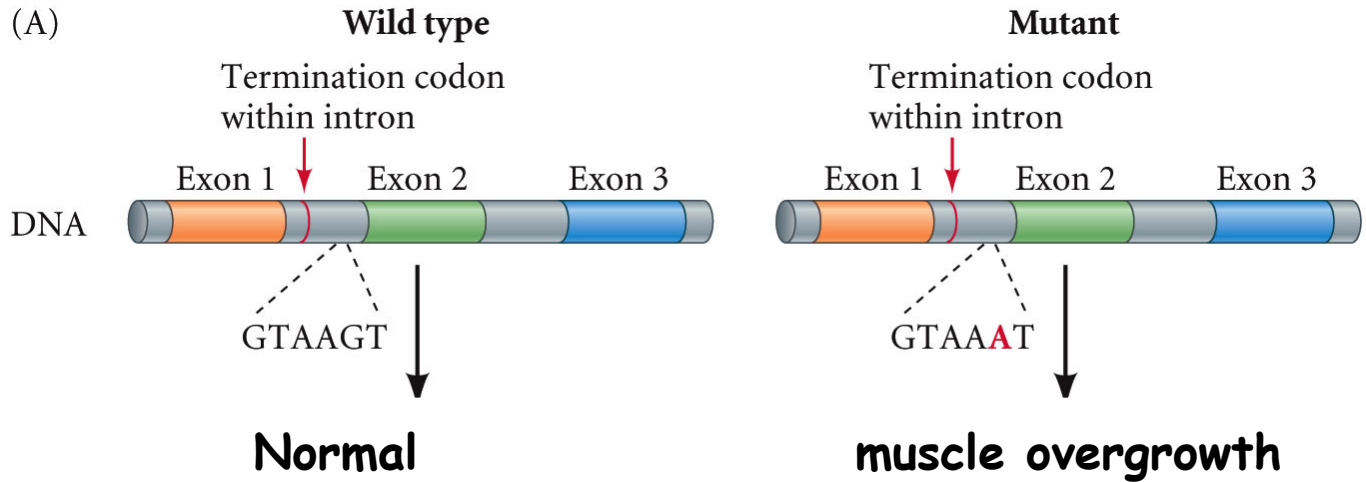
Myostatin: a negative regulator of muscle development

Mice with the mutation (below right) have muscle hypertrophy: **Mighty Mouse!**



The mutation that causes this phenotype is a $G \rightarrow A$ missense, in an intron.

**Myostatin is a negative regulator of muscle growth
Mutation is in an intron, results in a new splice site**



What is the result of this mutation on the mRNA and protein?

- Mature mRNA is truncated at the termination codon; protein is truncated
- Mature mRNA is longer, and includes the termination codon; protein is truncated
- Mature mRNA is the same size, but the protein gets truncated at the site of the mutation