Chapter 18 Regulation of Gene Expression

Differential gene expression

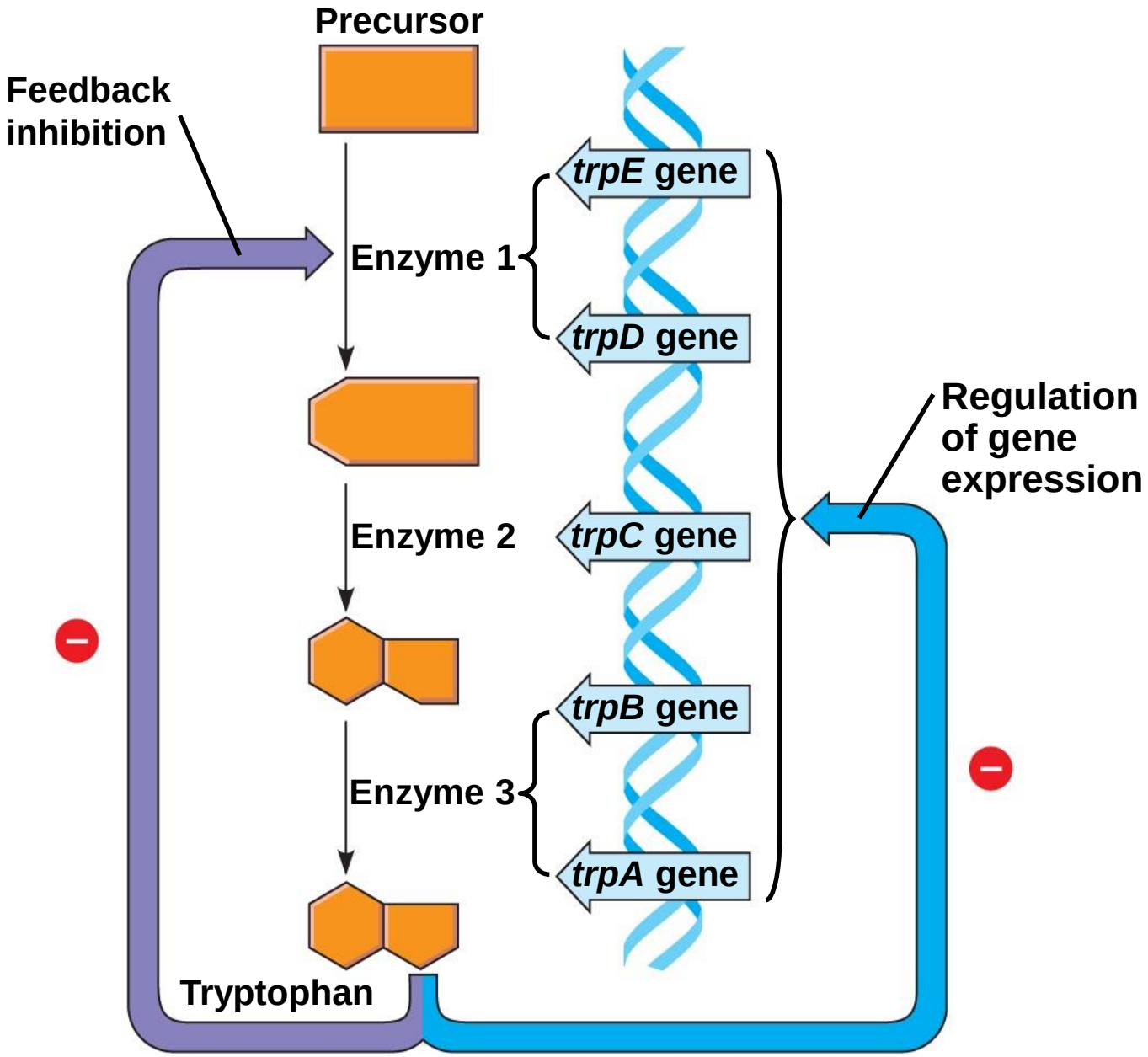
- Every somatic cell in an individual organism contains the same genetic information and replicated from the same original fertilized zygote.
- Why then, do we have differences such as tissues and organs that develop?
- At different points in time and in different parts of the body, gene expression varies. The amount of transcription and translation occurring for each is regulated and controlled.
- In bacterial cells, regulation of gene expression occurs as well, but it is controlled differently.

Concept 18.1
Bacteria often respond to environmental change by regulating transcription

- Bacteria have operons which can coordinately control groups of functionally related genes
- Gene expression can be controlled through the binding or unbinding of proteins to the operator
- The operator acts as a switch that can turn gene expression on or off for the operon

Bacterial regulation

- The processes of transcription and translation use energy and resources. Expressing unnecessary genes results in wasted energy and resources.
- For example, if a nutrient is freely available from the environment, there is no need for the bacteria to synthesize it. The energy and resources can then be diverted to another more useful task.
- Regulation of metabolism can occur in two ways:
  - Control of enzymatic activity through feedback inhibition
  - Control of gene expression through repression



(a) Regulation of enzyme activity

(b) Regulation of enzyme production

Operons

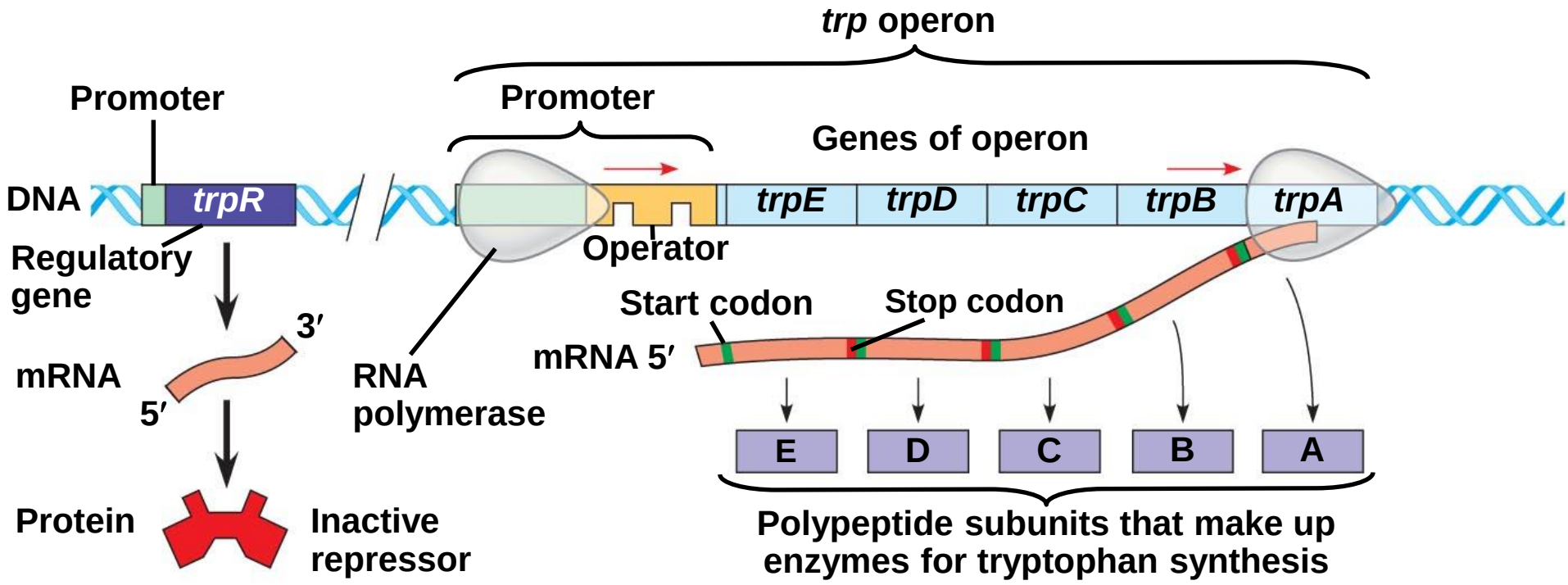
- The synthesis of certain molecules consists of a multistep pathway that requires specific enzymes at each step.
- An **operon** is a stretch of DNA that includes an operator, a promoter, and a group of functionally related genes.
- The **operator** acts as an on-off switch for the operon, controlling transcription of the group of genes.
- This means the genes are under **coordinate control**, as they are all controlled together.

The *trp* operon

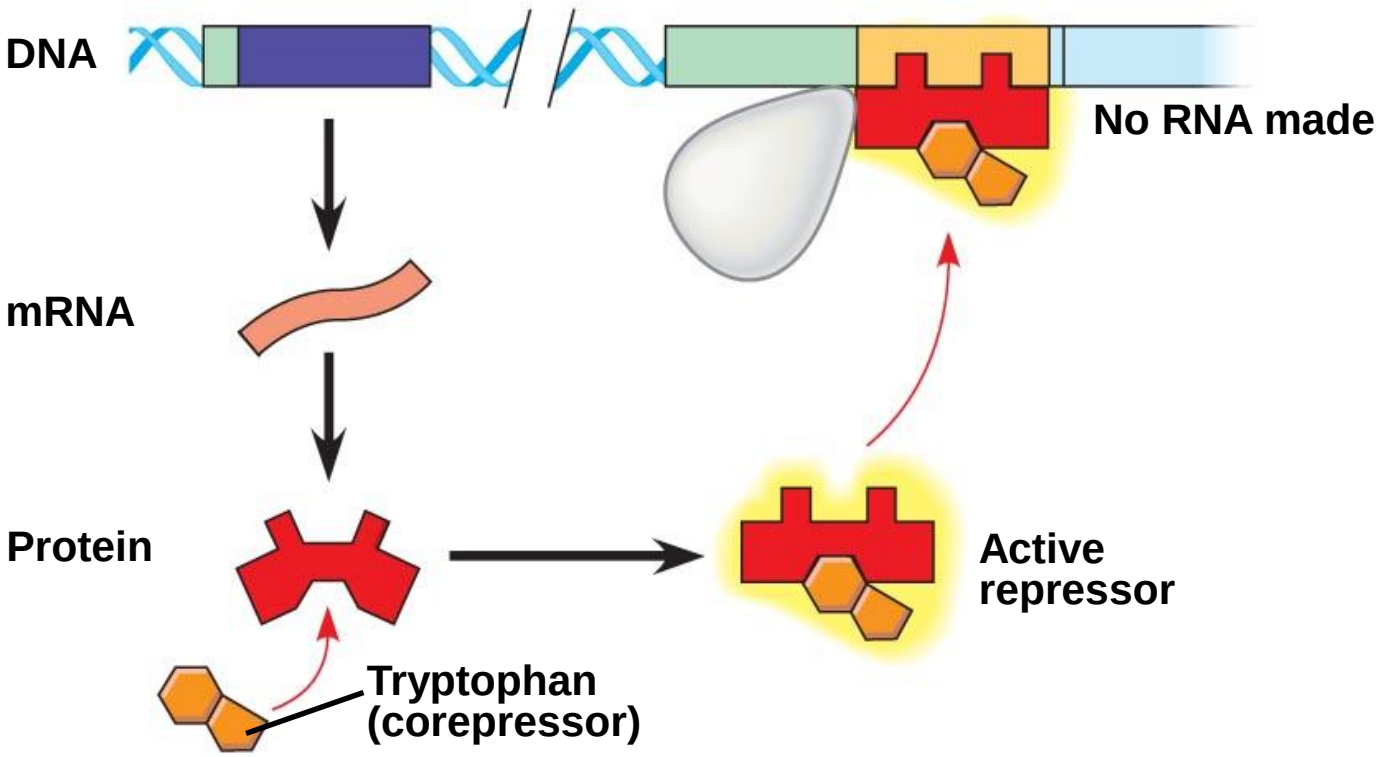
- *E. coli* synthesizes tryptophan through a series of reactions catalyzed by different enzymes. The subunits for these enzymes are under the control of the same operator and are part of the ***trp* operon**.
- The operon produces one long transcript that codes for all five subunits. During translation, five separate polypeptides are produced.
- Production of these enzymes can be turned off by a protein known as the ***trp* repressor**. If the repressor binds to the operator, it prevents the attachment of the RNA polymerase and therefore prevents transcription.

The *trp* repressor

- The *trp* repressor is produced from a regulatory gene and is continuously expressed in low amounts.
- However, it is an **allosteric protein**. This means that there are two conformations of the protein: active and inactive.
- In its inactive form, it cannot bind to the operator.
- To become active, it must be bound to a tryptophan molecule which acts as a **corepressor**.
- Therefore, when tryptophan is abundant, it activates the *trp* repressor, which in turn can now bind to the operator to repress synthesis of enzymes that catalyze tryptophan synthesis.



(a) Tryptophan absent, repressor inactive, operon on



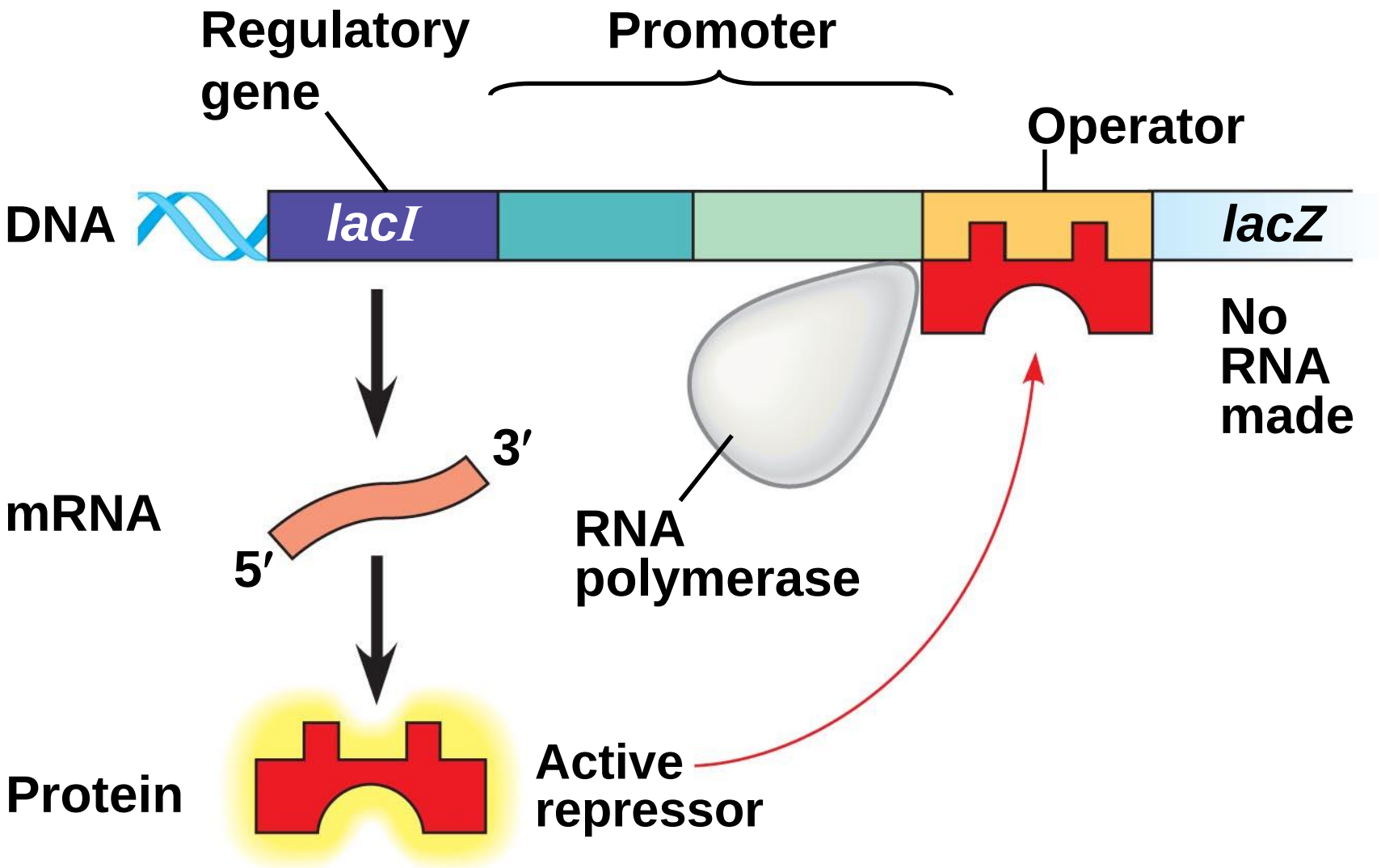
(b) Tryptophan present, repressor active, operon off

The *lac* operon

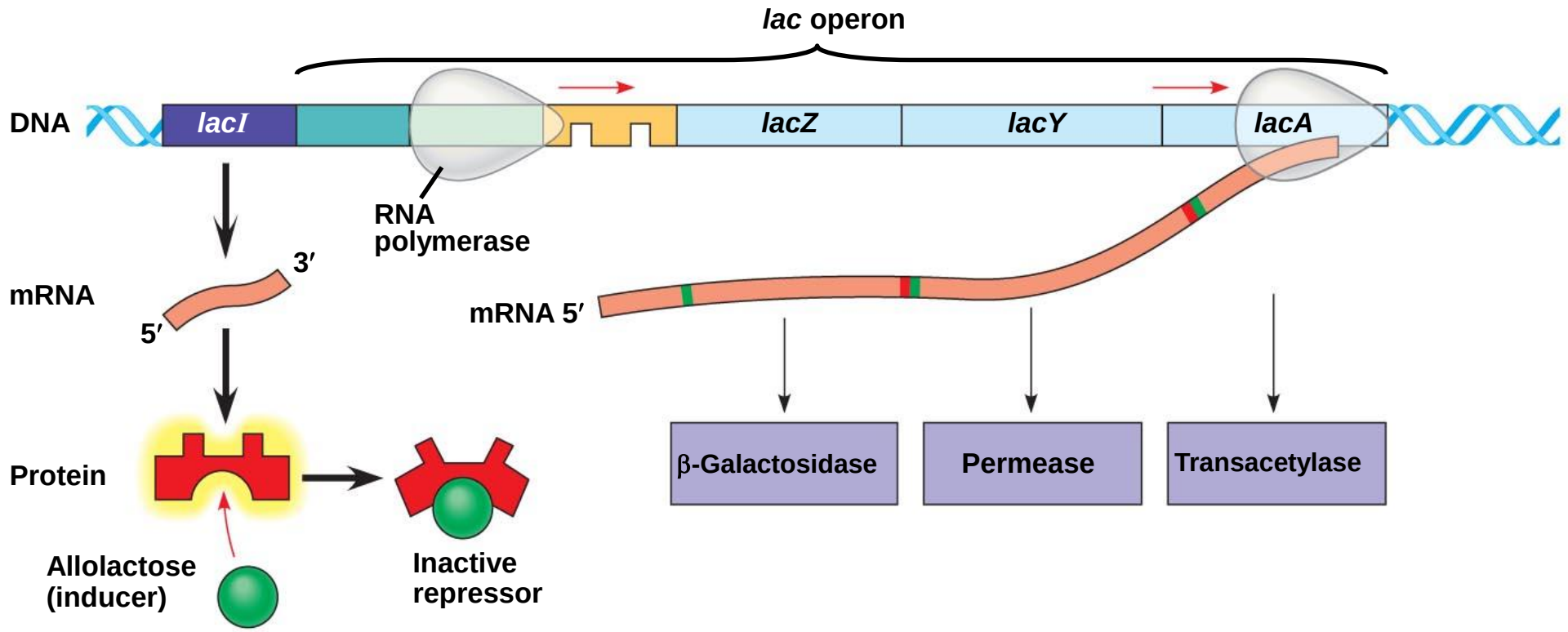
- Through the enzyme β-galactosidase, *E. coli* are able to breakdown the disaccharide lactose into its component monosaccharides.
- In environments with little lactose, very little β-galactosidase is present. However, if lactose increases, so does the amount of β-galactosidase.
- The ***lac* operon** is responsible for controlling the expression of β-galactosidase, along with two other genes involved in lactose regulation.

The *lac* repressor

- The *lac* repressor is an allosteric protein that can bind to the *lac* operator
- In its regular state, it is active and can bind to the *lac* operon. For it to be inactivated, it has to be bound to an **inducer** which inactivates the repressor but induces the operon.
- For the *lac* operon, the inducer is allolactose. Allolactose is an isomer of lactose that is formed in the presence of lactose.
- Hence, more lactose means more allolactose which induces the operon by repressing the *lac* repressor.



(a) Lactose absent, repressor active, operon off



(b) Lactose present, repressor inactive, operon on

Inducible and Repressible Enzymes

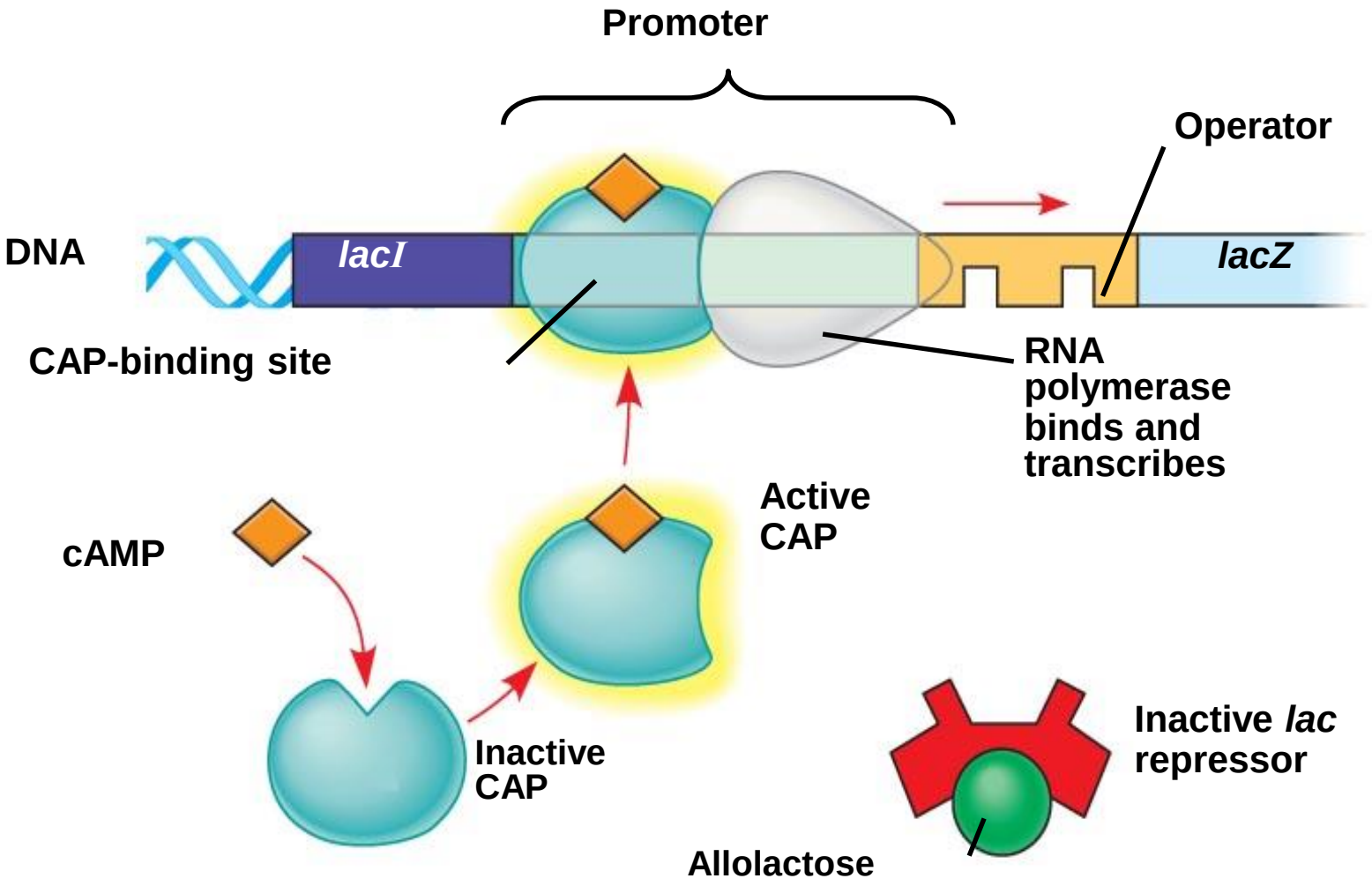
- **Inducible enzymes** – enzymes whose synthesis can be induced by the presence of a substance.
  - Usually part of **catabolic pathways** which break down a nutrient.
  - E.g. *lac* operon induced by presence of allolactose.
- **Repressible enzymes** – enzymes whose synthesis can be repressed by the presence of a substance.
  - Usually part of **anabolic pathways** which synthesize essential products.
  - E.g. *trp* operon repressed by presence of tryptophan.

Repressible and Inducible Operons

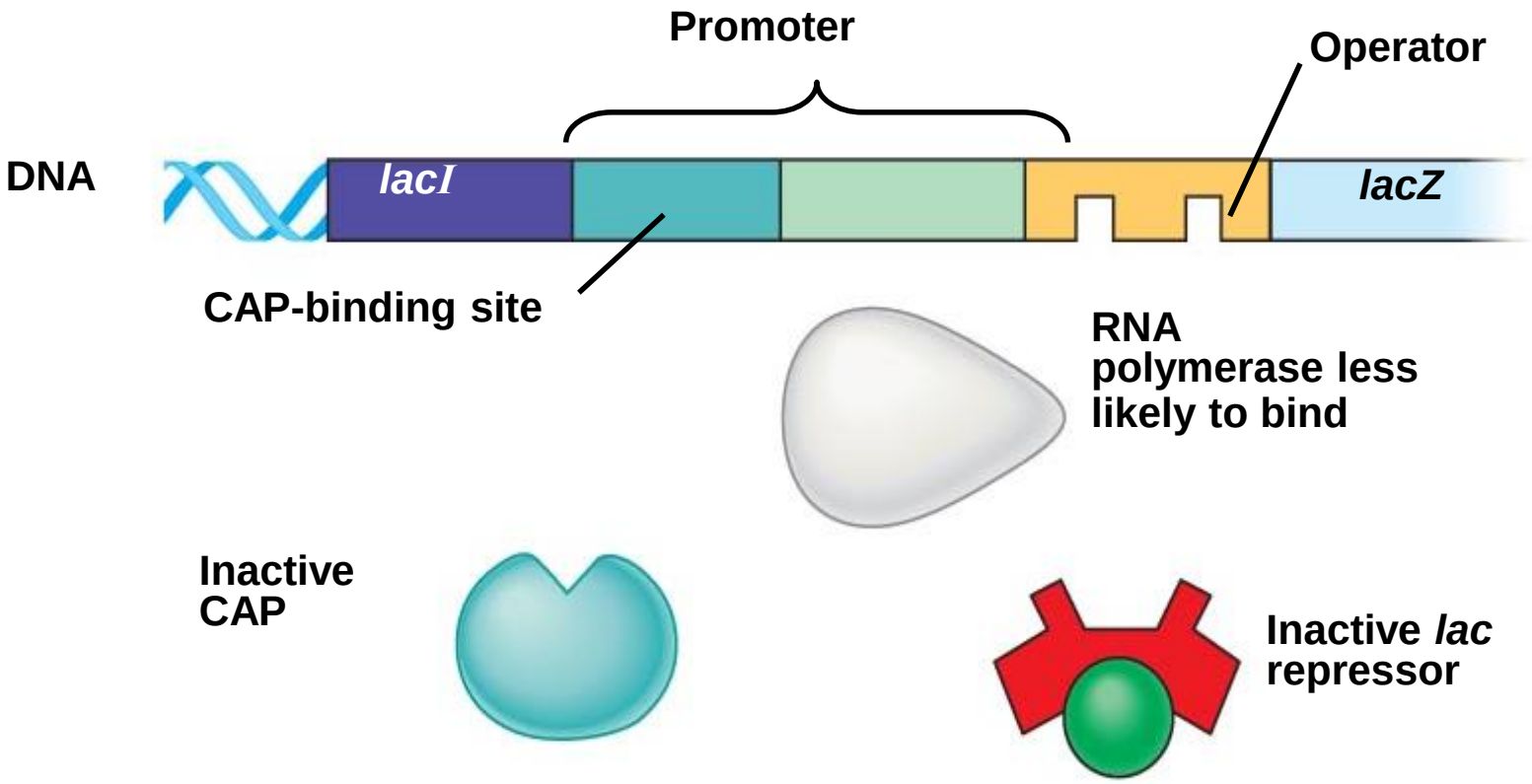
- **Repressible operon** – usually on but transcription can be turned off (repressed)
  - E.g. *trp* operon
- **Inducible operon** – usually off but transcription can be turned on (inducible)
  - E.g. *lac* operon
- These are both examples of **negative gene regulation** because the operons can be switched off by the active form of a repressor protein.

Positive Gene Regulation

- The *lac* operon can also provide an example of **positive gene regulation**.
- When glucose is lacking, lactose can be broken down as an energy source.
- Cyclic AMP (cAMP) accumulates when glucose is low and can bind to catabolite activator protein (CAP) which activates it.
- An **activator** is a protein that can bind to DNA and stimulate transcription (increasing rate of transcription).
- Once active, it binds upstream of the *lac* promoter and increases affinity of RNA polymerase for the promoter, thereby increasing transcription.



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



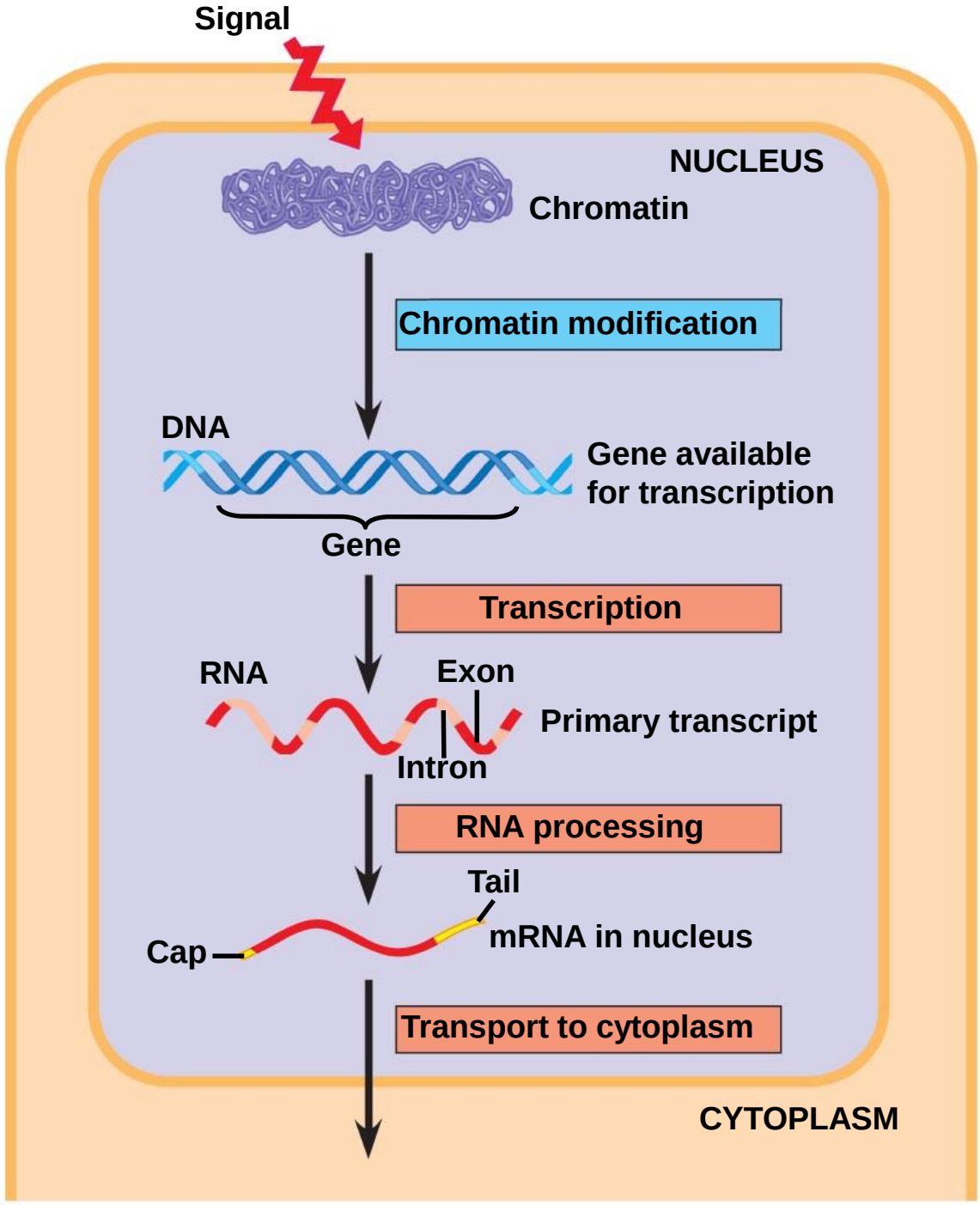
(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

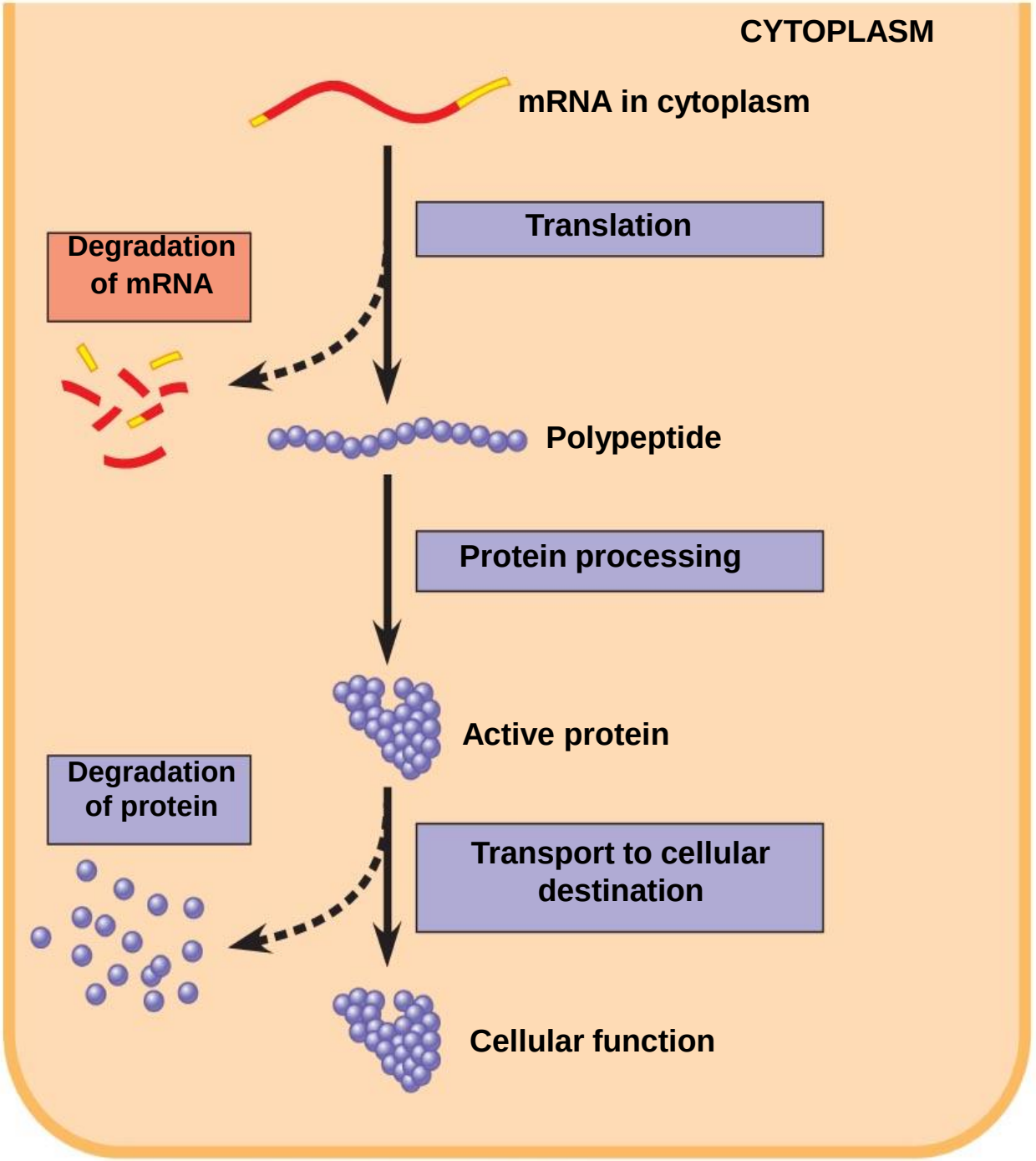
Concept 18.2
Eukaryotic gene expression is regulated at any stage

- Different cell types in the same organism will express a different combination of genes
- There are many steps to gene expression in eukaryotic cells
- Control of gene expression in eukaryotic cells can be controlled at many of these steps

Differential Gene Expression

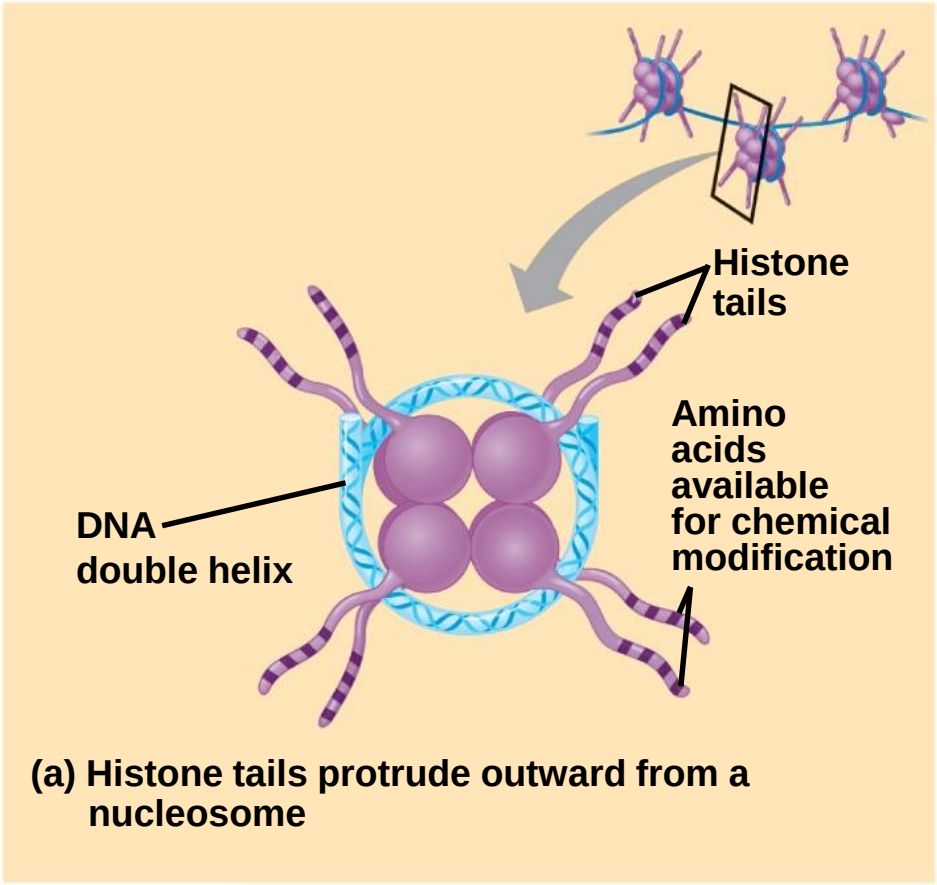
- **Differential gene expression** is the expression of different genes by cells with the same genome.
- In each cell, only a fraction of the genes are expressed and the subset of genes expressed depends on the cell type.
- Control of gene expression can occur at different stages of the process



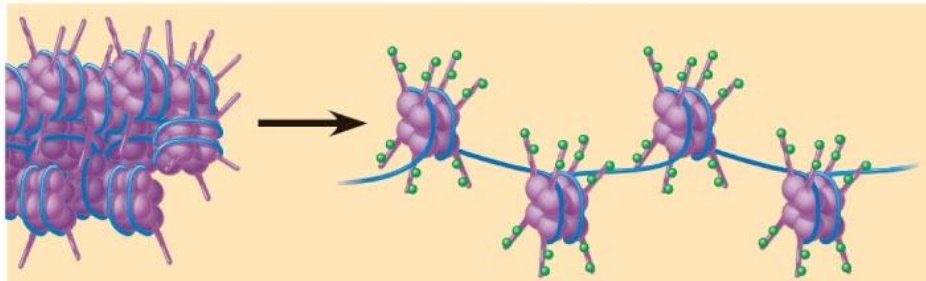


Regulation of Chromatin Structure

- **Heterochromatin**
  - Highly condensed areas of chromatin where genes are not usually expressed
- Histone Modifications
  - **Histone acetylation** (addition of acetyl groups) to histone tails
    - Prevents folding of chromatin into more compact structure
    - Easier access for transcription
  - Other modifications such as methylation and phosphorylation can also occur.
  - The histone code hypothesis proposes that specific combinations of modifications influence transcription



(a) Histone tails protrude outward from a nucleosome



(b) Acetylation of histone tails promotes loose chromatin structure that permits transcription

Regulation of Chromatin Structure

- **DNA Methylation**
  - Methylation (addition of a methyl group) to nitrogen bases in DNA.
  - Genes are usually more heavily methylated in cells in which they are not expressed.
  - Methylated DNA can promote histone deacetylation
  - Demethylation of DNA can switch on expression of certain genes.
  - Methylation patterns are passed on to replicated daughter cells.
  - Also a factor in genomic imprinting.

Regulation of Chromatin Structure

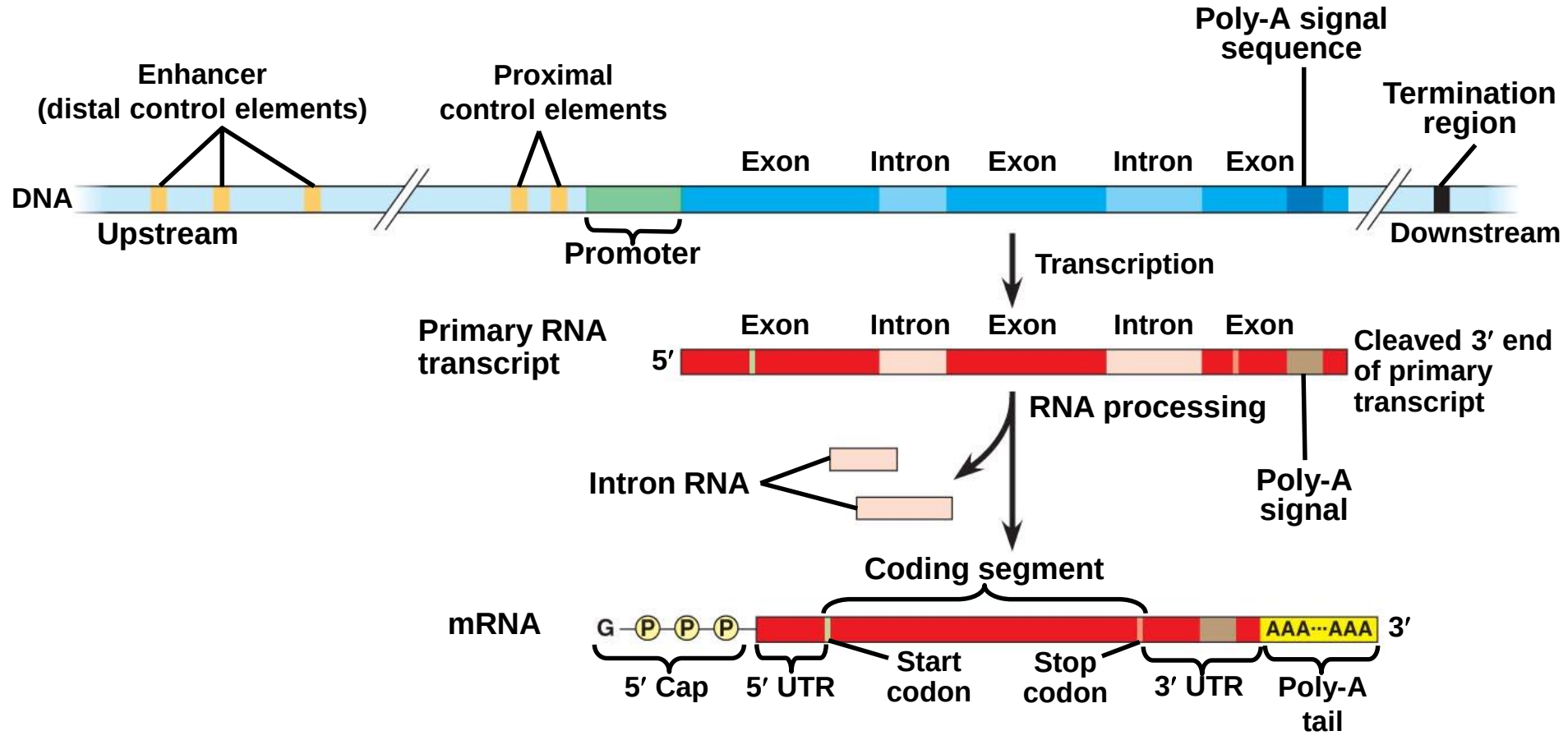
- **Epigenetic Inheritance**
  - Modifications to chromatin structure do not alter DNA sequences.
  - However, these modification can be passed on to future generations.
  - This is known as epigenetic inheritance

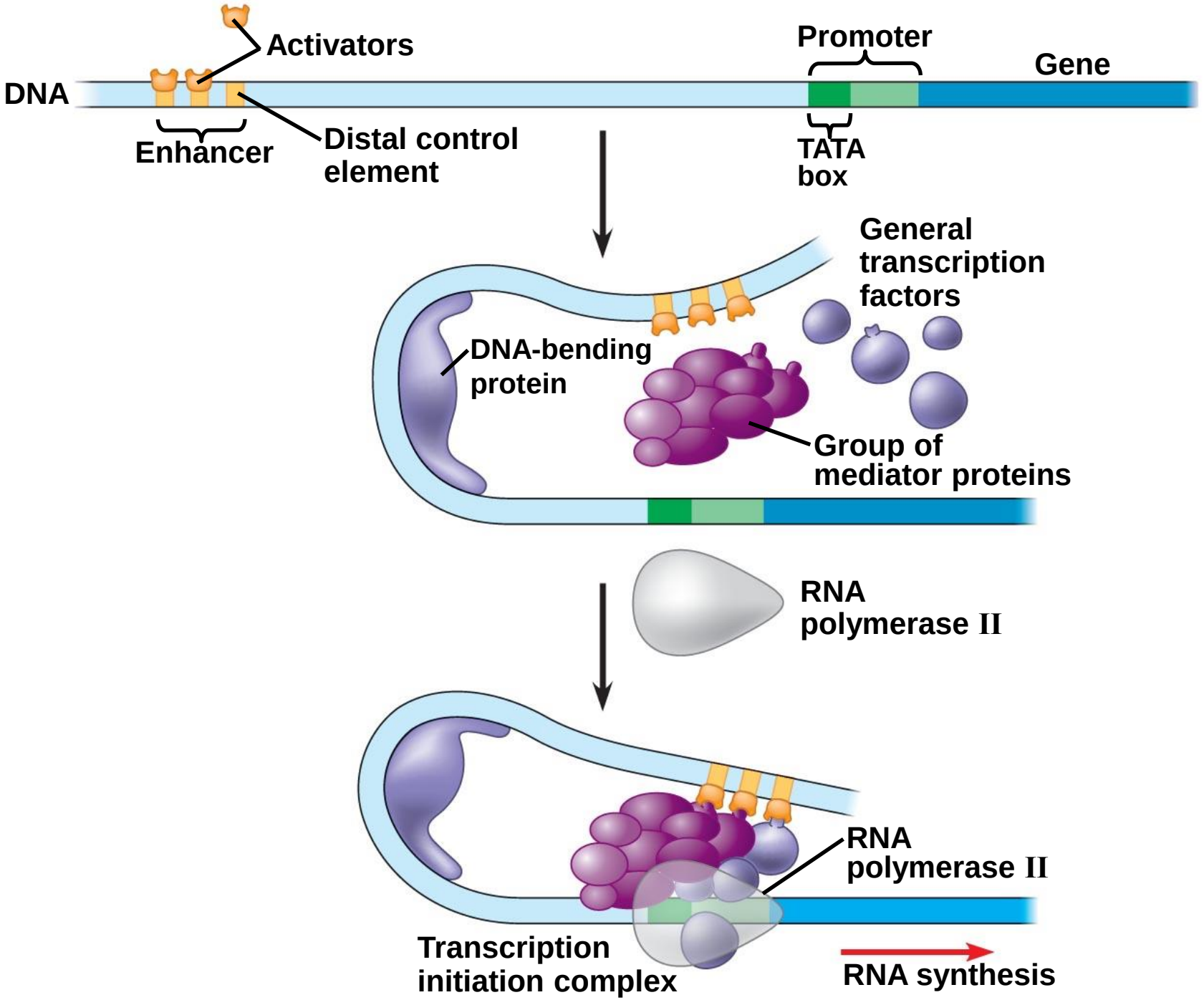
Regulation of Transcription Initiation

- Recall: Transcription is initiated in a eukaryotic cell through assembly of the transcription initiation complex at the promoter sequence that is upstream of the gene.
- **Control elements**, which are segments of noncoding DNA, help to regulate transcription through binding of proteins.
- **General transcription factors** are essential for the transcription of all protein-coding genes. However, general transcription factors initiate a low rate of transcription. For increased transcription, **specific transcription factors** are needed.

Specific Transcription Factors

- **Proximal control elements** are sequences located close to the promoter
- **Distal control elements**, groups of which are called enhancers, are sequences located more distantly upstream or downstream of a gene, or within an intron.
- **Activator proteins** can bind to the enhancer elements. A **DNA-bending protein** can bend the DNA to bring the activator proteins into contact with mediator proteins which interact with protein at the promoter.



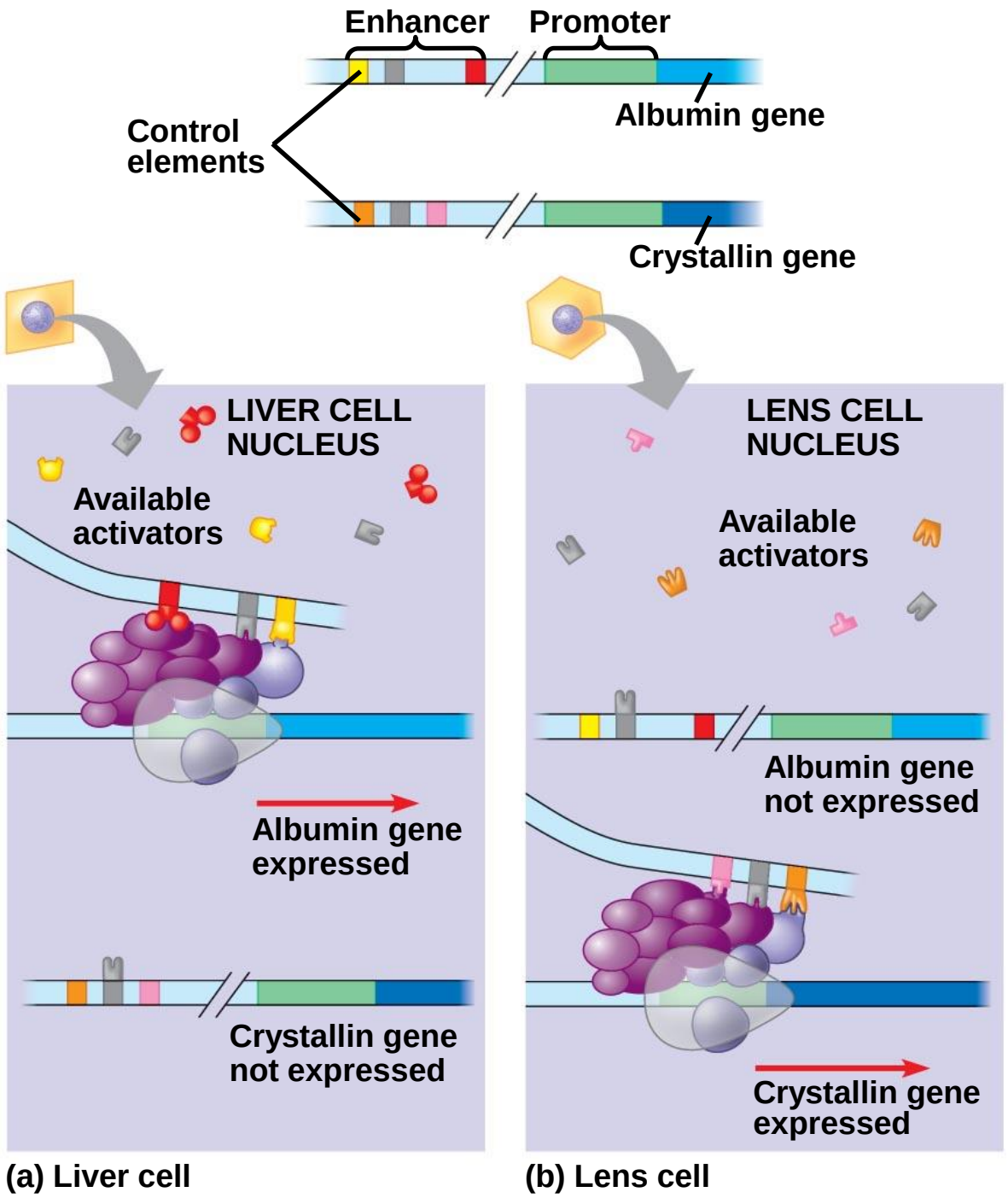


Specific Transcription Factors

- Specific transcription factors can also function as repressors that inhibit gene expression.
- **Repressors** can bind directly to control elements which can in turn block activator binding.
- Along with directly binding to DNA, other activators and repressors can indirectly influence gene expression by altering chromatin structure through recruitment of proteins to acetylate or deacetylate histones

Combinatorial Control of Gene Activation

- Though there are many genes that must be regulated, only a few sequences appear in control elements.
- Each enhancer is composed of around ten control elements which can each bind one or two specific transcription factors.
- A particular combination of control elements can activate transcription only when the appropriate activator proteins are present.



(a) Liver cell

(b) Lens cell

Coordinately Controlled Genes in Eukaryotes

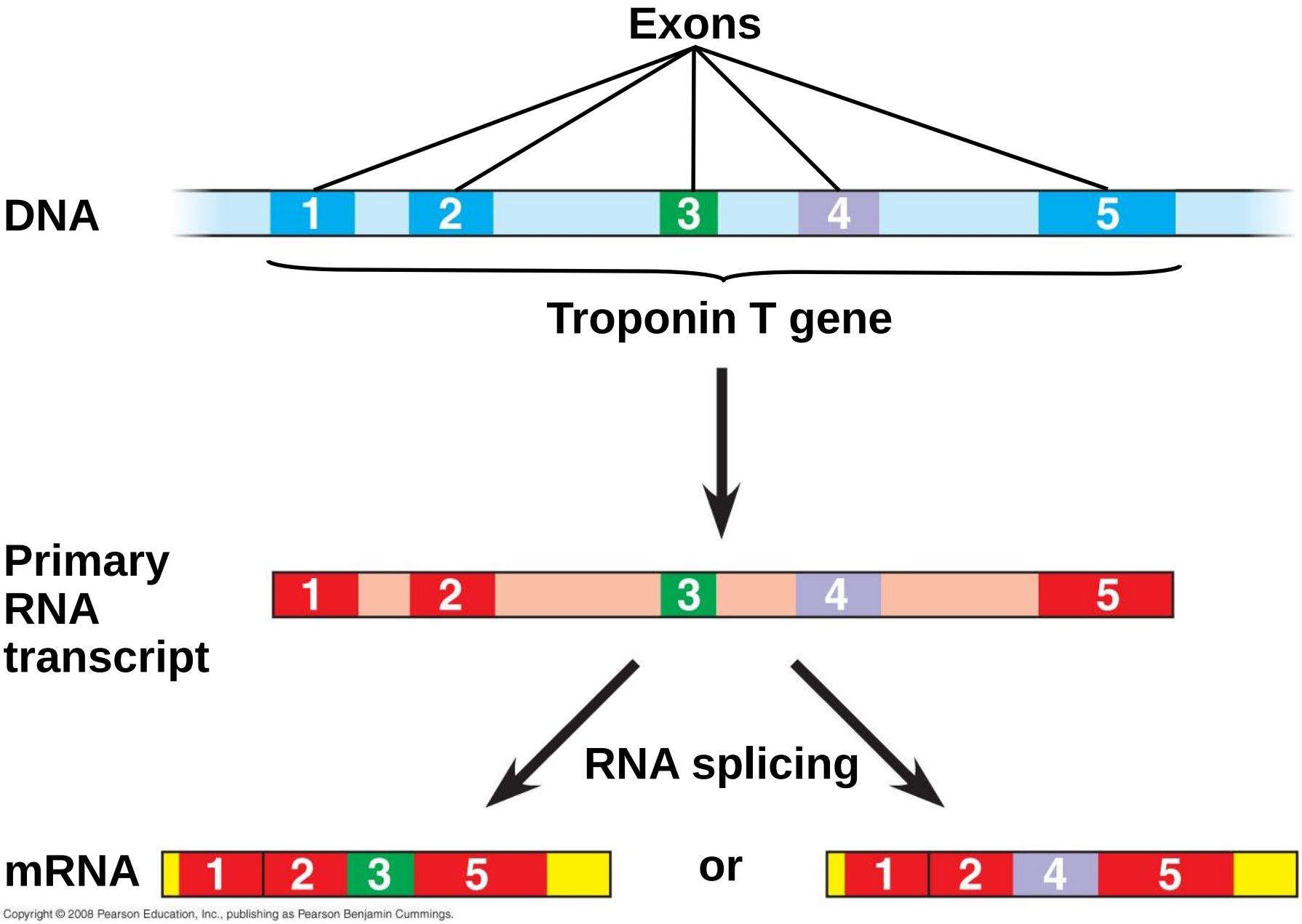
- Unlike functionally related genes in bacteria, functionally related genes in eukaryotic cells are usually each controlled by their own promoter.
- To coordinate expression, changes in chromatin structure occurs that renders the group of genes available or unavailable for transcription.
- The association of a specific combination of control elements can also coordinate expression of a widely dispersed group of genes.
- For example, molecules binding to receptors on the cell surface and trigger a signal transduction pathway that leads to activation of transcription activators or repressors based on a certain combination of control elements.

Mechanisms of Post-Transcriptional Regulation

- Production of a transcript does not equate with gene expression. Gene expression is measured in the amount of functional product.
- Therefore, post-transcription, there are still mechanisms that can control gene expression through regulation of RNA processing and translation.

Regulation of RNA Processing

- In **alternative RNA splicing**, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns
- For example, the tropoin T gene produces one primary transcript. However, this primary transcript can produce two possible proteins, based on alternative RNA splicing.



mRNA Degradation

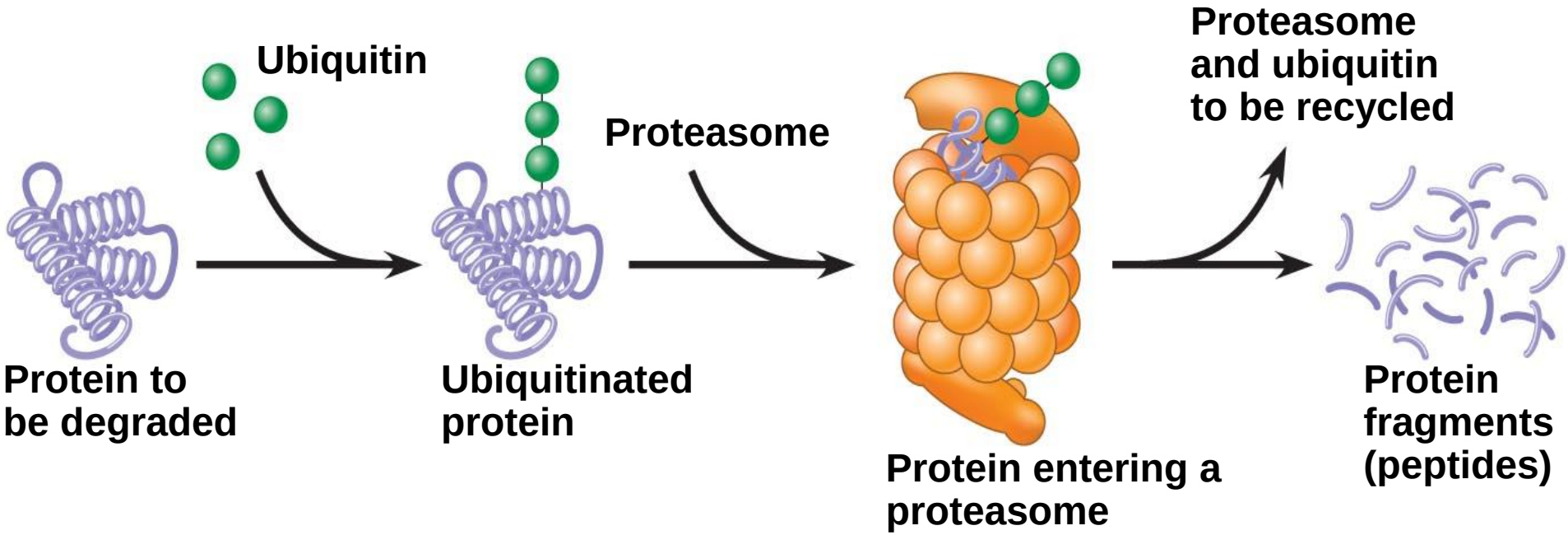
- Eukaryotic mRNA is more long-lived than bacterial mRNA.
- Research shows that removal of the poly-A tail can result in the beginning of enzymatic mRNA breakdown.
- Sequences that are found in the untranslated region (UTR) of the 3' end of the mRNA can affect the life-span of that mRNA.

Regulation of Initiation of Translation

- The initiation of translation of selected mRNAs can be blocked by regulatory proteins that bind to sequences or structures of the mRNA
- Alternatively, translation of all mRNAs in a cell may be regulated simultaneously
- For example, translation initiation factors are simultaneously activated in an egg following fertilization

Protein Processing and Degradation

- After translation, various types of protein processing can occur.
- **Cleavage** of the initial polypeptide can activate many proteins.
- As well, the addition of chemical groups (such as phosphate groups by phosphorylation) can be used to regulate activity of certain regulatory proteins.
- **Ubiquitination** (addition of the protein ubiquitin) can often signal a protein for degradation.
- **Proteasomes** are giant protein complexes that bind protein molecules and degrade them once they have been tagged with ubiquitin.



Concept 18.3
Noncoding RNAs play multiple roles in controlling gene expression

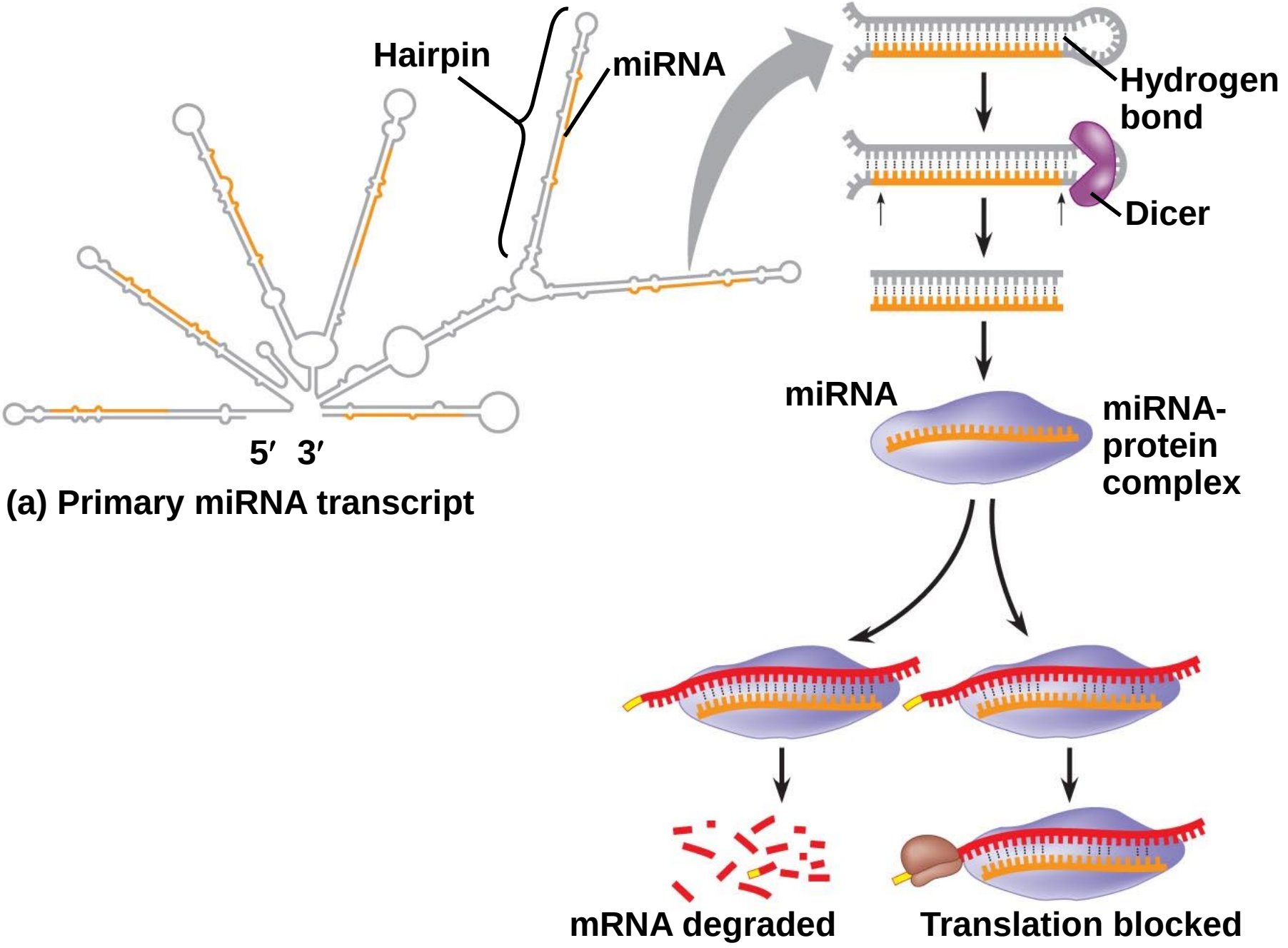
- microRNAs (miRNAs) and small interfering RNAs (siRNAs) are small noncoding RNAs
- They are able to bind to complementary sequences of mRNA to inhibit gene expression through RNA interference (RNAi)
- These small RNAs also play a role in heterochromatin formation and transcription

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- Some non-coding regions of DNA can produce small RNA molecules known as **noncoding RNAs**.
- **MicroRNAs** (**miRNAs**) are small single-stranded RNA molecules that can bind to mRNA
- They are formed from longer mRNA molecules that fold to form double-stranded hair pin structures that are held together by hydrogen bonds.
- Once it is trimmed by the dicer enzyme, one of the strands is degraded and the other strand forms the miRNA.
- These miRNAs can then bind to target mRNAs of a complementary sequence and either degrade them or block translation.

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- When researchers injected double stranded RNA into a cell, it would turn off the gene expression of genes with that sequence.
- The phenomenon of inhibition of gene expression by RNA molecules is called **RNA interference** (**RNAi**)
- RNAi is caused by **small interfering RNAs (siRNAs)**
- siRNAs and miRNAs are similar but form from different RNA precursors



(a) Primary miRNA transcript

(b) Generation and function of miRNAs

Chromatin Remodeling and Silencing of Transcription

- As well as interfering with mRNAs, siRNAs can play a role in heterochromatin formation
- Small RNAs may also block transcription of specific genes
- This means that these small non-coding RNAs are involved with regulating multiple steps of gene expression.
- Many of the miRNAs that have been characterized thus far have been shown to play a role in embryonic development.

Concept 18.4
A program of differential gene expression leads to the different cell types in a multicellular organism

- During embryonic development, a fertilized egg gives rise to many different cell types
- Cytoplasmic determinants and regulation of gene expression can control differentiation of cells

Biological organization and heirarchy

- A zygote and the organism is becomes are very different. The genetic information is the same but differential gene expression leads to formation of different cells.
- Cell differentiation leads to the formation of many cell types.
- These specialized cells are organized into tissues, which are organized into organs.
- Multiple organs together can form organ systems, which work together to form the whole organism.

A Genetic Program for Embryonic Development

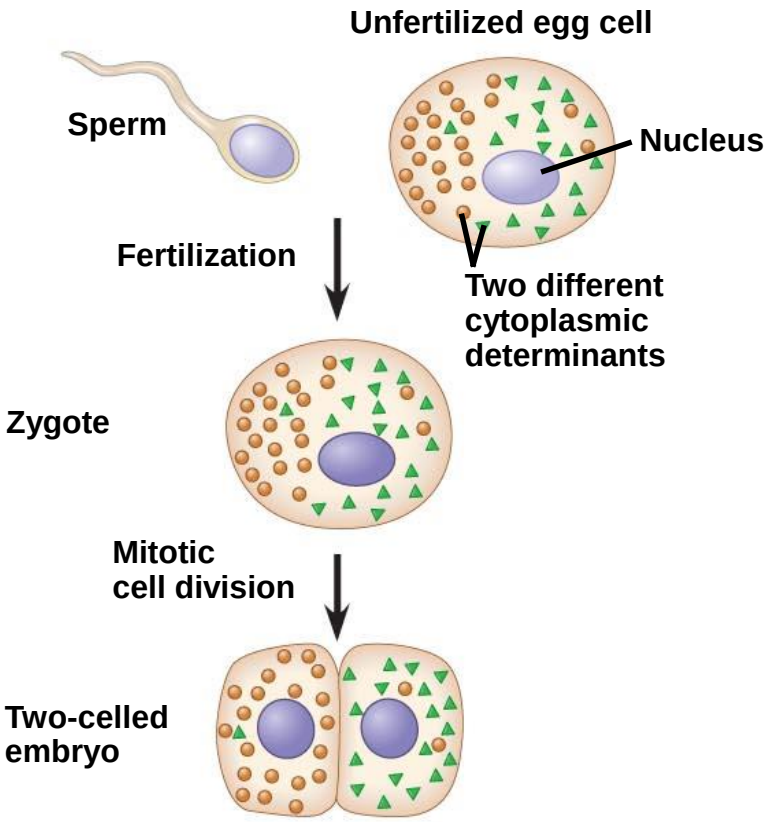
- The transformation from zygote to an adult organisms results from **cell division**, cell differentiation, and morphogenesis.
- **Cell differentiation** is the process by which cells become specialized in structure and function.
- The physical processes that give an organism its shape constitute **morphogenesis**
- Differential gene expression results from genes being regulated differently in each cell type
- Materials in the egg can set up gene regulation that is carried out as cells divide

(a) Fertilized eggs of a frog

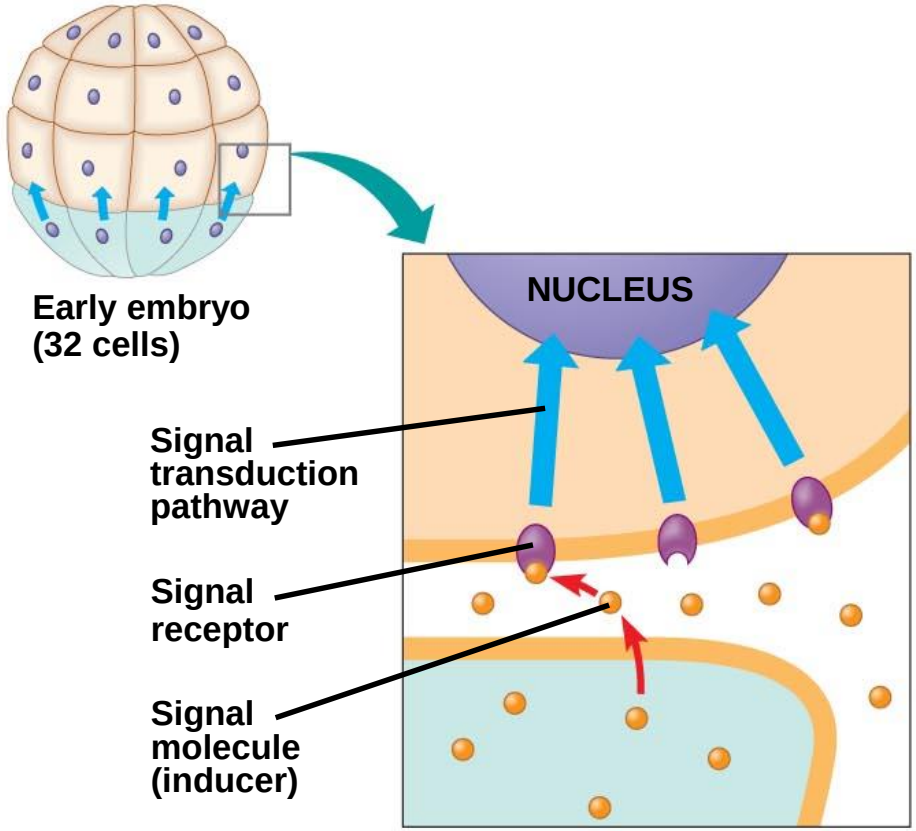
(b) Newly hatched tadpole

Cytoplasmic Determinants and Inductive Signals

- An egg's cytoplasm contains RNA, proteins, and other substances that are distributed unevenly in the unfertilized egg
- **Cytoplasmic determinants** are maternal substances in the egg that influence early development
- As the zygote divides by mitosis, cells contain different cytoplasmic determinants, which lead to different gene expression
- Along with this, environmental factors can also influence cell differentiation through signaling molecules in a process called induction.



(a) Cytoplasmic determinants in the egg



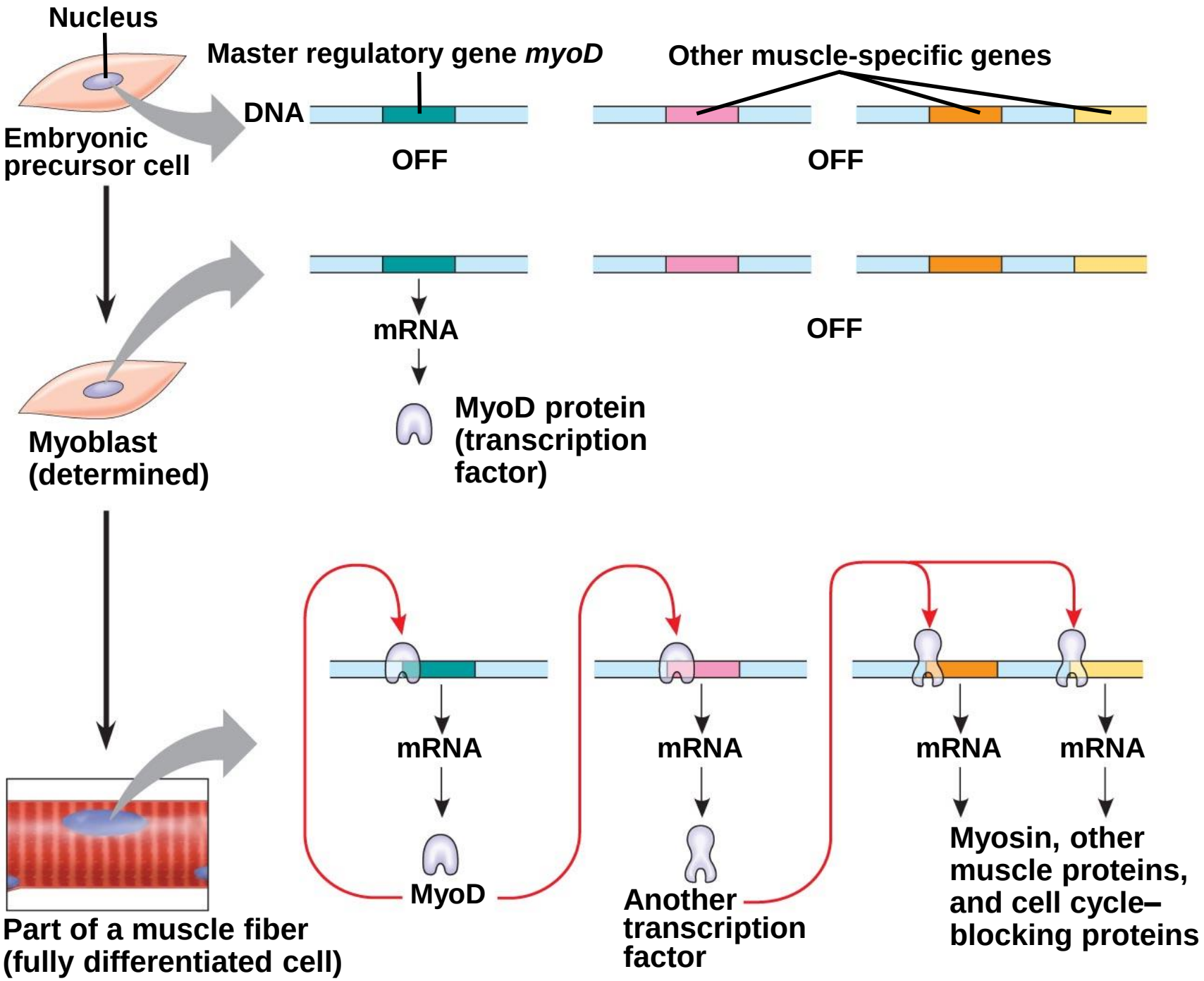
(b) Induction by nearby cells

Sequential regulation of gene expression during cellular differentiation

- **Determination** refers to the events that lead to the observable differentiation of a cell.
- Once determination has occurred, it is irreversible.
- With observations of molecular changes, it can be seen that determination is directed through gene expression of tissue-specific proteins.
- Sets of genes are sequentially expressed as cells arise from division of precursor cells.

Sequential regulation of gene expression during cellular differentiation

- For example, the precursor cell for muscle cells can be has the potential to develop into a number of cell types.
- Researchers examined the effect of isolating different genes to be expressed and identified master regulatory genes which are responsible for determination.
- The master regulatory gene *myoD* encodes for the MyoD protein which is a transcription factor that can stimulate expression when bound to control elements.

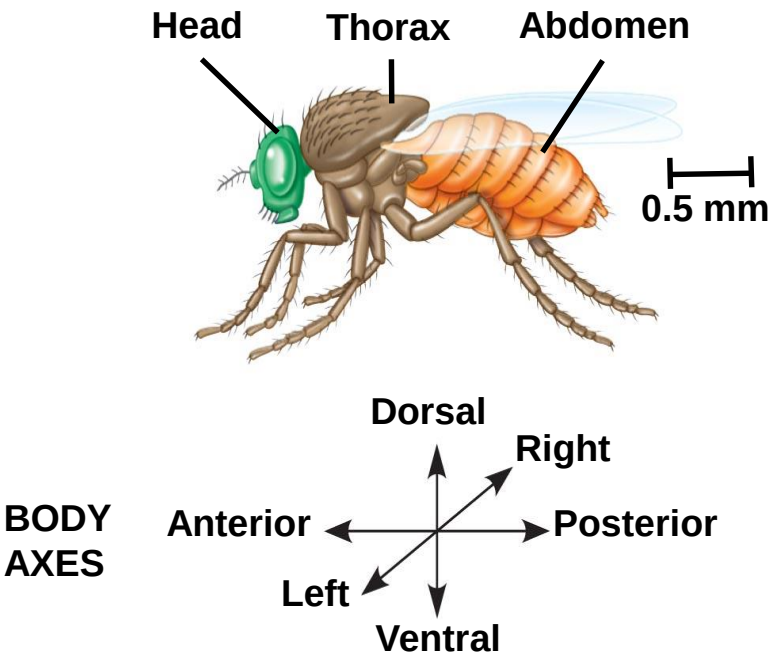


Pattern Formation: Setting Up the Body Plan

- **Pattern formation** is the development of a spatial organization of tissues and organs in characteristic places.
- In animals, pattern formation begins with the establishment of the major axes (anterior-posterior, right-left, dorsal-ventral).
- **Positional information** are molecular cues that control pattern formation and are provided by cytoplasmic determinants and inductive signals.
- The genetics of pattern formation has been studied in *Drosophila melanogaster*.
- Combining anatomical, genetic, and biochemical approaches, researchers have discovered developmental principles common to many other species, including humans.

The Life Cycle of *Drosophila*

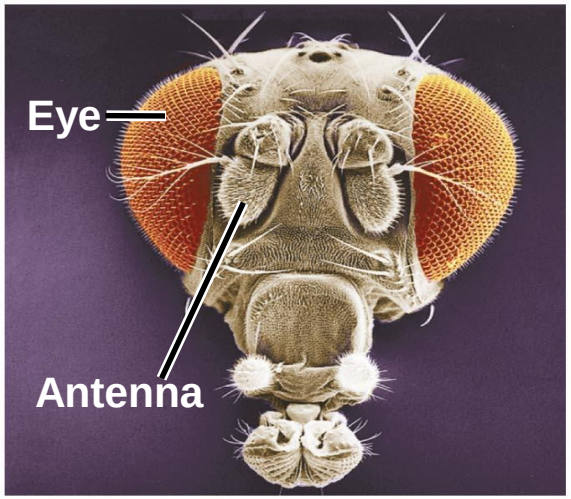
- Fruit flies have segmented bodies which make up the head, thorax, and abdomen. The body can be divided by three major axes.
- In *Drosophila*, cytoplasmic determinants in the unfertilized egg determine the axes before fertilization.
- After fertilization, the embryo develops into a segmented larva with three larval stages.



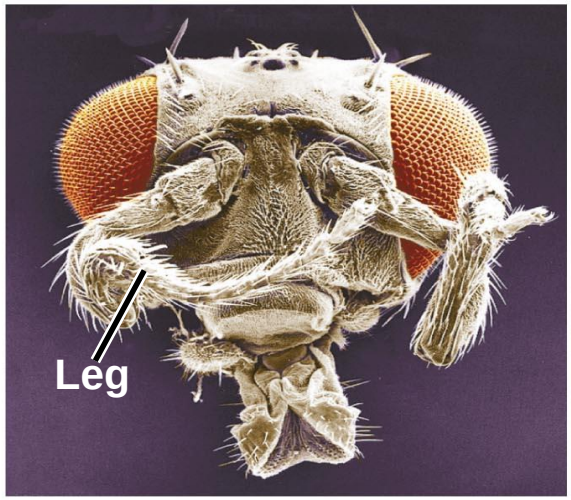
(a) Adult

Genetic Analysis of Early Development

- Edward B. Lewis was a biologist that studies developmental defects in *Drosophila* to link developmental abnormalities to specific genes.
- He discovered genes, called homeotic genes, which control pattern formation in the late embryo, larva, and adult.



Wild type



Mutant

Genetic Analysis of Early Development

- Another 30 years later, Christiane Nüsslein-Volhard, and Eric Wieschaus were able to determine genes involved with pattern formation during early embryonic development.
- They created mutants, conducted breeding experiments, and looked for corresponding genes.
- Breeding experiments were complicated by **embryonic lethals,** embryos with lethal mutations. These mutants could not be bred for study.
- They found 120 genes essential for normal segmentation and were able to map and clone them for further study.

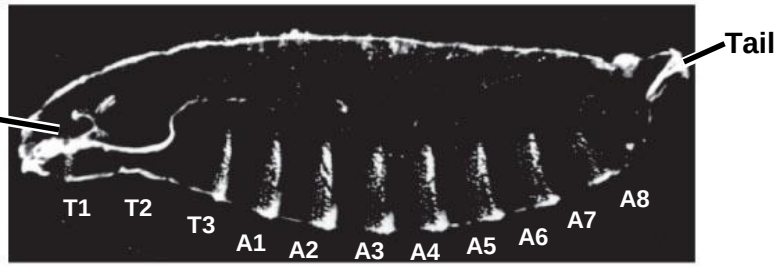
Axis Establishment

- Cytoplasmic determinants in the egg are the substances that initially establish the axes of the *Drosophila* body.
- These cytoplasmic determinants are coded for by **maternal effect genes**. When these are mutated in the mother, it results in a mutant phenotype in the offspring. This is regardless of the offspring's own genotype.
- These maternal effect genes are also called **egg-polarity genes** because they control orientation of the egg and help set up the axes.

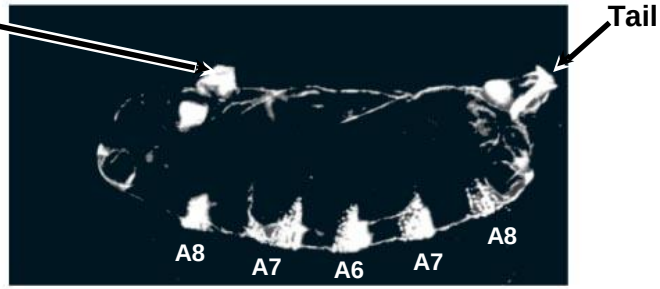
Bicoid: A Morphogen Determining Head Structures

- *Bicoid* is a maternal effect gene. When mutated in the mother, the embryo doesn't develop an anterior end and instead has two posterior ends.
- This phenotype suggests that the product of the mother's *bicoid* gene is concentrated at the future anterior end.
- This hypothesis is an example of the morphogen gradient hypothesis, in which gradients of substances called **morphogens** establish an embryo's axes and other features.
- It was then shown that mRNA for *bicoid* is more concentrated at the anterior end.
- If injected into early embryos, anterior structures formed at the site of injection.

EXPERIMENT

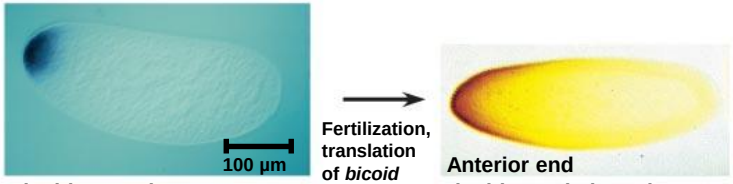


Wild-type larva



Mutant larva (*bicoid*)

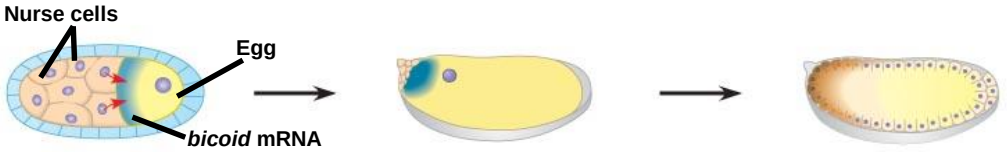
RESULTS



Bicoid mRNA in mature unfertilized egg

Bicoid protein in early embryo

CONCLUSION



Developing egg

Bicoid mRNA in mature unfertilized egg

Bicoid protein in early embryo

Importance of *bicoid* research

- It identified a specific protein required for some early steps in pattern formation
- It increased understanding of the mother's role in embryo development
- It demonstrated a key developmental principle that a gradient of molecules can determine polarity and position in the embryo