

Chapter 18: Regulation of Gene Expression

1. All genes are not “on” all the time. Using the metabolic needs of *E. coli*, explain why not.

If the environment is lacking in the amino acid tryptophan, which the *E. coli* bacterium needs to survive, the cell responds by activating a metabolic pathway that makes tryptophan from another compound. Later, if the human host eats a tryptophan-rich meal, the bacterial cell stops producing tryptophan, thus saving itself from squandering its resources to produce a substance that is available from the surrounding solution in prefabricated form. Thus, bacteria tune their metabolism to changing environments.

2. What are the two main ways of controlling metabolism in bacterial cells?

Cells can adjust the activity of enzymes already present, a fairly fast response which relies on the sensitivity of many enzymes to chemical cues that increase or decrease their catalytic activity. The activity of the first enzyme in the tryptophan synthesis pathway is inhibited by the pathway's end product. This form of feedback inhibition, typical of anabolic pathways, allows a cell to adapt to short-term fluctuations in the supply of a substance it needs. Cells can also adjust the production level of certain enzymes; that is, they can regulate the expression of the genes that encode the enzymes. Many genes of the bacterial genome are switched on or off by changes in the metabolic status of the cell.

3. Feedback inhibition is a recurring mechanism throughout biological systems.

E. coli regulating tryptophan synthesis is an example of negative inhibition because the operons are switched off by the active form of the repressor protein.

4. What is a promoter?

In the 1930s, Beadle speculated that in *Drosophila*, each of the various mutations affecting eye color blocks pigment synthesis at a specific step by preventing production of the enzyme that catalyzes that step. However, neither the chemical reactions nor the enzymes that catalyze them were known at the time.

5. What is the operator?

A key advantage of grouping genes of related function into one transcription unit is that a single “on-off switch” can control the whole cluster of functionally related genes; in other words, these genes are coordinately controlled. The switch is a segment of DNA called an operator. Positioned within the promoter, or in some cases, between the promoter and the enzyme-coding genes, the operator controls the access of RNA polymerase to the genes.

6. What is an operon?

All together, the operator, the promoter, and the genes they control – the entire stretch of DNA required for enzyme production of the tryptophan pathway – constitute an operon.

7. List the three components of an operon, and explain the role of each one.

The operator controls the access of RNA polymerase to the genes. The promoter is the DNA sequence where RNA polymerase attaches and initiates transcription. The operator and the promoter coordinately control a cluster of functionally related genes.

8. How does a repressor protein work?

The operon can be switched off by a repressor protein, which binds to the operator and blocks attachment of RNA polymerase to the promoter, preventing transcription of the genes. A repressor protein is specific for the operator of a particular operon. The binding of repressors to operators is reversible. An operator vacillates between two states: one without the repressor bound and one with the repressor bound. The relative duration of each state depends on the number of active repressor molecules around. The *trp* repressor, like most regulatory proteins, is an allosteric protein, with two alternative shapes, active and inactive. The *trp* repressor is synthesized in an inactive form with little affinity for the *trp* operator. Only if tryptophan binds to the *trp* repressor at an allosteric site does the repressor protein change to the active form that can attach to the operator, turning the operon off. Tryptophan functions in this system as a corepressor, a small molecule that cooperates with a repressor protein to switch an operon off.

9. *What are regulatory genes?*

A regulatory gene, located some distance from the operon and with its own promoter, is expressed continuously, although at a low rate.

10. *Distinguish between inducible and repressible operons, and give an example of each.*

The *trp* operon is said to be a repressible operon because its transcription is usually on but can be inhibited when a specific small molecule (in this case, tryptophan) binds allosterically to a regulatory protein. In contrast, an inducible operon is usually off but can be stimulated when a specific small molecule interacts with a regulatory protein. The *lac* operon includes two other genes coding for enzymes that function in lactose utilization. The entire transcription unit is under the command of one main operator and promoter. The regulatory gene, *lacI*, located outside the operon, codes for an allosteric repressor protein that can switch off the *lac* operon by binding to the operator. While the *trp* repressor is inactive by itself and requires tryptophan as a corepressor in order to bind to the operator, the *lac* repressor, in contrast, is active by itself, binding to the operator and switching the *lac* operon off. In this case, a specific small molecule, called an inducer, inactivates the response.

12. *Compare and contrast the lac operon to the trp operon.*

The enzymes of the lactose pathway are referred to as inducible enzymes because their synthesis is induced by a chemical signal. Analogously, the enzymes for tryptophan synthesis are said to be repressible. Repressible enzymes generally function in anabolic pathways, which synthesize essential end products from raw materials (precursors). By suspending production of an end product when it is already present in sufficient quantity, the cell can allocate its organic precursors and energy for other uses. In contrast, inducible enzymes usually function in catabolic pathways, which break down a nutrient to simpler molecules. By producing the appropriate enzymes only when the nutrient is available, the cell avoids wasting energy and precursors making proteins that are not needed.

13. *What happens when a repressor is bound to the operator?*

When a repressor is bound to the operator, RNA polymerase cannot bind to the operator, and RNA cannot be synthesized.

14. *What is CAP? How does CAP work?*

The mechanism by which *E. coli* senses the glucose concentration and relays the information to the genome depends on the interaction of an allosteric regulatory protein with a small organic molecule, in this case cyclic AMP (cAMP), which accumulates when glucose is scarce. The regulatory protein, called catabolite activator protein (CAP), is an activator, a protein that binds to DNA and stimulates transcription of a gene. When cAMP binds to this regulatory protein, CAP assumes its active shape and can attach to a specific site at the upstream end of the *lac* promoter. This attachment increases the affinity of RNA polymerase for the promoter, which is actually rather low even when no repressor is bound to the operator.

15. *Explain why CAP binding and stimulation of gene expression is positive regulation.*

By facilitating the binding of RNA polymerase to the promoter and thereby increasing the rate of transcription, the attachment of CAP to the promoter directly stimulates gene expression. Therefore, this mechanism qualifies as positive regulation.

16. *Describe the relationship between glucose supply, cAMP, and CAP.*

When glucose is plentiful and CAP is inactive, the synthesis of enzymes that catabolize compounds other than glucose generally slows down. The ability to catabolize other compounds, such as lactose, enables a cell deprived of glucose to survive. The compounds present in the cell at the moment determine which operons are switched on, the result of simple interactions of activator and repressor proteins with the promoters of the genes in question. If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA coding for the enzymes in the lactose pathway. When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription at a significant rate, even though no repressor is bound.

17. *How can both repressible and inducible operons be negative regulators?*

Regulation of both the *trp* and *lac* operons involves the negative control of genes, because the operons are switched off by the active form of the repressor protein. In the case of the *lac* operon, a chemical signal induces enzyme synthesis not by acting directly on the genome, but by freeing the *lac* operon from the negative effect of the repressor. Gene regulation is said to be positive only when a regulatory protein interacts directly with the genome to switch transcription on.

18. *What is differential gene expression?*

Almost all the cells in an organism contain an identical genome. (Cells of the immune system are one exception.) However, the subset of genes expressed in the cells of each type is unique, allowing these cells to carry out their specific function. The difference between cell types are due to differential gene expression, the expression of different genes by cells with the same genome, *not* by the presence of different genes. The function of any cell depends on the appropriate set of genes being expressed.

19. *What percentage of the genes of a typical human cell is expressed at any given time?*

A typical human cell might express about 20% of its protein-coding genes at any given time. Highly differentiated cells, such as muscle or nerve cells, express an even smaller fraction of their genes.

20. *What is the common control point of gene expression for all organisms?*

The nuclear envelope separating transcription from translation in eukaryotic cells offers an opportunity for post-transcriptional control in the form of RNA processing that is absent in prokaryotes. In addition, eukaryotes have a greater variety of control mechanisms operating before transcription and after translation. Gene expression is commonly controlled through chromatin modification, transcription, RNA processing, transport of mRNA to the cytoplasm, translation, protein processing (such as cleavage and chemical modification), transport of an active protein to its cellular destination, and degradation of a protein.

21. *Distinguish between heterochromatin and euchromatin.*

Genes within heterochromatin, which is highly condensed, are usually not expressed. In contrast, euchromatin's looser structure allows RNA polymerase to bind to the DNA strands and proceed with transcription. Certain chemical modifications to the histone proteins and to the DNA of chromatin can influence both chromatin structure and gene expression.

22. *What occurs in histone acetylation? How does it affect gene expression?*

In histone acetylation, acetyl groups ($-\text{COCH}_3$) are attached to lysines in histone tails; deacetylation is the removal of acetyl groups. When the lysines are acetylated, their positive charges are neutralized and the histone tails no longer bind to neighboring nucleosomes. Such binding promotes the folding of chromatin into a more compact structure; when this binding does not occur, chromatin has a looser structure. As a result, transcription proteins have easier access to genes in an acetylated region. Some enzymes that acetylate or deacetylate histones are closely associated with or even components of the transcription factors that bind to promoters, suggesting that histone acetylation enzymes may promote the initiation of transcription not only by remodeling chromatin structure, but also by binding to and thus "recruiting" components of the transcription machinery.

23. *What is DNA methylation? What role may it play in gene expression?*

While some enzymes methylate the tails of histone proteins, a different set of enzymes can methylate certain bases in the DNA itself, usually cytosine. Such DNA methylation occurs in most plants, animals, and fungi. Long stretches of inactive DNA, such as that of inactivated mammalian X chromosomes, are generally more methylated than regions of actively transcribed DNA, although there are exceptions. On a smaller scale, individual genes are usually more heavily methylated in cells in which they are not expressed. Removal of the extra methyl groups can turn on some of these genes.

24. *The inactive mammalian X chromosome is heavily methylated. What is the result of this methylation?*

Individual genes are usually more heavily methylated in cells in which they are not expressed. At least in some species, DNA methylation seems to be essential for the long-term inactivation of genes that occurs during normal cell differentiation in the embryo. For instance, experiments have shown that deficient DNA methylation (due to lack of a methylating enzyme) leads to abnormal embryonic development in organisms such as mice and mustard plants.

25. *What is genomic imprinting, and how is it maintained? Give an example discussed earlier in human genetics.*

Once methylated, genes usually stay that way through successive cell divisions in a given individual. At DNA sites where one strand is already methylated, enzymes methylate the correct daughter strand after each round of DNA replication. Methylation patterns are thus passed on, and cells forming specialized tissues keep a chemical record of what occurred during embryonic development. A methylation pattern maintained in this way also accounts for genomic imprinting in mammals, where methylation permanently regulates expression of either the maternal or paternal allele of particular genes at the start of development.

26. *Explain what is meant by epigenetic inheritance, and give an example of epigenetic changes.*

Inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called epigenetic inheritance. Epigenetic variations might help explain why one identical twin acquires a genetically based disease such as schizophrenia, while the other does not.

27. *Explain how enhancers and activators interact with transcription factors to affect gene expression.* ✍

Bending of the DNA by a protein enables enhancers to influence a promoter hundreds or even thousands of nucleotides away. Specific transcription factors called activators bind to the enhancer DNA sequences and then to a group of mediator proteins, which in turn bind to general transcription factors, assembling the transcription initiation complex. These protein-protein interactions facilitate the correct positioning of the complex on the promoter and the initiation of RNA synthesis. A gene may have several enhancers that act at different times or in different cell types.

28. *In prokaryotes, functionally related genes are usually clustered in a single operon.*

In eukaryotes, co-expressed genes are typically scattered over different chromosomes. In these cases, coordinate gene expression depends on the association of a specific combination of control elements with every gene of a dispersed group. Copies of the activators that recognize the control elements bind to them, promoting simultaneous transcription of the genes, no matter where they are in the genome.

29. *What is a plausible mechanism for the coordination of gene expression?*

Coordinate control of dispersed genes in a eukaryotic cell often occurs in response to chemical signals from outside the cell. Many signaling molecules, such as growth factors, bind to receptors on a cell's surface and never actually enter the cell. Such molecules can control gene expression indirectly by triggering signal transduction pathways that lead to activation of particular transcription activators or repressors.

31. *Posttranscriptional control includes regulation of mRNA degradation. Explain how this affects translation.*

Nucleotide sequences that affect how long an mRNA remains intact are often found in the untranslated region (UTR) at the 3' end of the molecule. Translation cannot occur when an mRNA molecule lacks a poly-A tail of sufficient length to allow initiation.

32. *How can proteins be activated, processed, and degraded? Give an example.*

Often, eukaryotic polypeptides must be processed to yield functional protein molecules. For instance, cleavage of the initial insulin polypeptide (pro-insulin) forms the active hormone. Many proteins also undergo chemical modifications that make them functional. Regulatory proteins are commonly activated or inactivated by the reversible addition of phosphate groups, and cell-surface proteins must be transported to target destinations in the cell in order to function. The length of time each protein functions in the cell is strictly regulated by means of selective degradation.

33. *An article in Scientific American about proteasomes was titled "Little Chamber of Horrors." ☺ Explain how proteins are targeted for degradation, and give a specific example of when this might occur.*

Many proteins, such as the cyclins involved in regulating the cell cycle, must be relatively short-lived if the cell is to function appropriately. To mark a particular protein for destruction, the cell commonly attaches molecules of a small protein called ubiquitin to the protein. Giant protein complexes called proteasomes then recognize the ubiquitin-tagged proteins and degrade them. Mutations making specific cell cycle proteins impervious to proteasome degradation can lead to cancer.

34. *How do these "little chambers of horrors" function? Explain their role in regulation of gene expression.* ✍

First, multiple ubiquitin molecules are attached to a protein by enzymes in the cytosol. Next, the ubiquitin-tagged protein is recognized by a proteasome, which unfolds the protein and sequesters it within a central cavity. Finally, enzymatic components of the proteasome cut the protein into small peptides, which can be further degraded by other enzymes in the cytosol.

35. *Much of the RNA that is transcribed is not translated into protein. These are called noncoding RNAs. What is their role?*

Regulation by both small and large ncRNAs is known to occur at several points in the pathway of gene expression, including mRNA translation and chromatin modification. A class of small ncRNAs called piwi-associated RNAs also induces formation of heterochromatin, blocking expression of some parasitic DNA elements in the genome known as transposons.

36. *Explain the two modes of action of microRNAs.* ✍

Small single-stranded RNA molecules, called microRNAs, are capable of binding to complementary sequences in mRNA molecules. An miRNA-protein complex then either degrades the target mRNA or blocks its translation.

37. *What three processes lead to the transformation of a zygote into the organism?*

Development of a zygote into a fully fledged organism results from cell division, cell differentiation, and morphogenesis.

38. *Explain what occurs in cell differentiation and morphogenesis.*

During embryonic development, cells not only increase in number, but also undergo cell differentiation, the process by which cells become specialized in structure and function. Moreover, different kinds of cells are not randomly distributed but are organized into tissues and organs in a particular three-dimensional arrangement. The physical processes that give an organism its shape constitute morphogenesis, meaning “creation of form.”

39. *Differential gene expression results from different activators in different cells. How do different sets of activators come to be present in two cells?*

The specific genes expressed in any particular cell of a developing organism determine its path. Two sources of information, used to varying extents in different species, “tell” a cell which genes to express at any given time during embryonic development. Firstly, the cytoplasm of an unfertilized egg is not homogeneous. Maternal substances in the egg that influence the course of early development are called cytoplasmic determinants. After fertilization, early mitotic divisions distribute the zygote’s cytoplasm into separate cells. The nuclei of these cells may thus be exposed to different cytoplasmic determinants, depending on which portions of the zygotic cytoplasm a cell received. The combination of cytoplasmic determinants in a cell helps determine its developmental fate by regulating expression of the cell’s genes during the course of cell differentiation.

Secondly, the environment around a particular cell becomes increasingly important as the number of embryonic cells increases. Most influential are the signals impinging on an embryonic cell from other embryonic cells in the vicinity, including contact with cell-surface molecules on neighboring cells. Such signals cause changes in the target cells, a process called induction. The molecules conveying these signals within the target cell are cell-surface receptors and other proteins expressed by the embryo’s own genes. In general, signaling molecules send a cell down a specific developmental path by causing changes in its gene expression that eventually result in observable cellular changes.

40. *What is meant by determination?*

Once it has undergone determination, an embryonic cell is irreversibly committed to its final fate. If a committed cell is experimentally placed in another location in the embryo, it will still differentiate into the cell type that is its normal fate.

41. *What process ensures that all the tissues and organs of an organism are in their characteristic places? Where do the molecular cues that control this process arise?*

Cytoplasmic determinants and inductive signals both contribute to the development of a special organization in which the tissues and organs of an organism are all in their characteristic places, a process known as pattern formation. The molecular cues that control pattern formation, collectively called positional information, are provided by cytoplasmic determinants and inductive signals. These cues tell a cell its location relative to the body axes and to neighboring cells and determine how the cell and its progeny will respond to future molecular signals.

42. *What is controlled by homeotic genes?*

Homeotic genes, “discovered” by Edward B. Lewis, control pattern formation in the late embryo, larva, and adult.