Chapter 5

Gene Expression and Epigenesis

Virtually every cell in your body contains all the genetic information about making a complete human being that would be your identical twin. This fact makes cloning you a theoretical possibility. A mad geneticist could try this by extracting the DNA from one of your cells, placing it into the nucleus of a human egg where the DNA has been removed, inducing the egg to start dividing, and then inserting it into the uterus of a woman. If the resulting zygote were viable, the organism would be your identical twin, albeit in a different phase of the life cycle.

But if every cell has the same genetic code, then why are some cells liver cells while others are neurons? Another problem arises from the consideration of cell division. You and I begin as a single fertilized egg. This egg divides into two cells that contain the same genetic material. These two genetically identical cells each divide, giving four genetically identical cells; these four divide, giving eight and so on. Why were our parents not rewarded for nine months of pregnancy by bouncing, seven pound blobs of identical cells?

Although the answers for these questions are complicated and not well understood, a major reason is that genes are differentially expressed in some tissues and are also regulated over time even within the same tissue. To oversimplify, even though a liver cell has all the genetic information to make a neuron, only those “liver cell” genes are working in the liver. The “neuron” genes in the liver are in some way shut down. This phenomenon is called genetic regulation, gene expression, epigenesis, or epigenetic control. In common sense terms, epigenesis is a pedantic word for the genetic dimmer switches spoken about in previous chapters.\footnote{In classic biology, the term epigenesis refers to embryonic development. Specifically, it was the theory that an organism arises from successive cell differentiation from an initially undifferentiated zygote (fertilized egg). Today, however, the term is often used as a synonym for the regulation of a gene.}
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Progress in unraveling the mechanisms of epigenesis is occurring at a blistering pace, so this review must be selective. Hence, only a few of these mechanisms will be discussed. We will first layout the different mechanisms for gene expression and then give some examples to solidify learning.

5.1 Two types of genetic transmission

Before discussing genetic regulation, we note that there are two types of genetic transmission. The first is transmission of the DNA sequence of nucleotides, packaged in chromosomes. For example, a mother transmits the sequence AGCTGCCTA at position 1,207,915 of chromosome number 3. In common sense terms, this is the transmission of DNA spellings. There is no universally accepted term for this type of genetic transmission, so let us arbitrarily call it sequence transmission.

The second type of genetic transmission is epigenetic transmission. Here, some mechanism(s) of gene regulation is acquired by a parent and information about that regulation is transmitted in the sperm or egg. The offspring inherits this modification. Although the DNA sequence is not changed, gene expression may be changed. We call this epigenetic transmission. We will discuss epigenetic transmission after we have examined the varied mechanisms for genetic regulation.

5.2 X chromosome inactivation (Lyonization)

Females have two X chromosomes while males have only one. Thus, one might expect that females should have twice the level of X-chromosome proteins and enzymes than males. Empirically, however, this does not happen—the levels are equal in men and women. The reason for this is that in the cells of a human female, one and only one X chromosome is active.

The other X coils and condenses into a small ellipsoid structure that is called a Barr body and is functionally deactivated—the genes on that chromosome are not transcribed. The geneticist Mary Lyon (1961) hypothesized this almost 50 years ago, so the phenomenon is often called Lyonization.

During the very early embryonic development of a female, both her maternal and paternal X chromosomes are active. After 12 days of development, when the embryo has about 5,000 cells, one of these chromosomes is randomly deactivated in each cell. Once a chromosome is inactive in a given cell, all its daughter cells will have the same chromosome deactivated. That is, if “cell number 2,317” has the paternal X deactivated, then all descendants of cell 2,317 will also have the paternal X deactivated. The particular X chromosome deactivated in the original cell is random. Consequently, half of a female’s cells will express her paternal X chromosome while the other half will express her maternal X. Thus, females are genetic mosaics.

Fur color in the calico cat (AKA tortoise shell cat) is a classic example of Lyonization (see Figure 5.1). An allele on one X chromosome of the calico con-
Figure 5.1: A calico (tortoise shell) cat

The gene responsible for X chromosome inactivation, the XIST locus, has recently been localized to the long arm of the X, but the precise mechanism for achieving inactivation is not totally understood.

Certain data suggest that the major reason for Lyonization is “dosage compensation”—making certain that the same levels of proteins and enzymes are expressed in males and females. Females with Turner’s syndrome (only one X chromosome) do not have Barr bodies, females with three X chromosomes have two Barr bodies in each cell, and males with Klinefelter’s syndrome (two X chromosomes and one Y chromosome) have one Barr body. It appears that the process evolved to guarantee that one and only one X chromosome is active in any given cell.

5.3 Transcriptional control mechanisms

There are three major mechanisms that influence gene expression during the process of transcription. They are DNA methylation, histone modification (chromatin remodeling), and transcription factors. Each is discussed in turn.
5.3.1 DNA methylation

In biochemistry, methylation is the process of adding a methyl group \((\text{CH}_3)\) to a molecule. When that molecule is DNA, then the dimmer switch may be affected. In DNA, methylation preferentially occurs in areas rich in cytosine, especially those with a long string of CG dinucleotide repeats. Methylation of areas near of in a gene turns the dimmer switch down. That is, it reduces transcription usually to the point of shutting the gene down (Bird, ; Suzuki & Bird, 2008). Figure 5.2 gives an example of how methylation near a promoter region can influence transcription. In panel A, the DNA is unmethylated, permitting the transcription stuff to bind to the promoter and initiate transcription. When methyl groups are added to the DNA near the promoter (panel B), the transcription stuff can no longer bind to the DNA. With no transcription, there is no biologically active molecule. The gene is “turned off”.

DNA methylation is a key mechanism in embryonic development and tissue differentiation. Some DNA areas are methylated in both sperm and egg, although not always the same areas. After fertilization, the DNA undergoes rapid demethylation (the methyl groups are removed from the DNA). As cell division progresses, methylation increases and becomes tissue specific. Usually—but not always—once a gene becomes methylated in a cell, it remains methylated in all of the daughter cells originating from that cell. In a gross way then, embryonic development is a process of selectively silencing genes. A kidney cell is a kidney cell not because kidney cell genes are turned on. Rather, it is a kidney cell because non-kidney cell genes are turned off.

There are still many unknowns about methylation. Why do some CG rich areas become methylated while others remain unmethylated? In the early embryo, why is an area methylated in one cell type but unmethylated in an adjacent
5.3.2 Histone modification (chromatin remodeling)

Remember the nucleosome, a section of DNA wound around histone proteins? Well, the histones are proteins and as such have their blueprint in DNA. They can undergo post-translational modification by a number of “ations” (methylation\(^2\), acetylation, phosphorylation, ubiquitination, and several others). These modifications influence how the nucleosomes are packed together and, in turn, influence gene expression by regulating transcription. A few modifications enhance the transcription of a gene. Others inhibit transcription, often to the point of completely silencing the gene (Bannister and Kouzarides, 2011).

The various ways modifications of the histones are collectively termed chromatin remodeling. (Chromatin is the term used for the DNA-protein complex that constitute the chromosome). Usually, the chromatin remodeling involves “opening up” nucleosomes within or close to the promoter region so that the proteins and enzymes constituting the “transcription stuff” can bind to the DNA and do their work. Remodeling can also have the opposite effect by “bunching up” the nucleosome, making it difficult for the transcription stuff to bind.

Not all chromatin remodeling, however, occurs in the proximity of a promoter. There are DNA regions that are far away from the promoter, perhaps even on a different chromosome, that can increase the rate of transcription of a gene. These regions are known as enhancers. Repressors or silencers are DNA regions not in a promoter that inhibit transcription of a gene.

There are several mechanisms whereby enhancers and repressors perform their action on transcription (Dean, 2006). One is depicted in Figure 5.3. Recall from Chapters 2 and 3 that in the cell’s ordinary state, the chromosomes are thin structures that resemble a jumbled ball of string. This jumbling, however, is not random. As a result, two DNA sections on the same chromosome that are far away were the chromosome stretched out into a straight line (the upper panel in Figure 5.3) might be close together in the jumbled state (the lower

\(^2\)Be careful not to confuse DNA methylation with histone methylation. Methylation is a generic process that can happen to a number of different molecules.
panel). Figure 5.3 depicts an enhancer, but different histone modification could make the area a repressor.

For a large number of DNA areas, both DNA methylation and histone modification work together. In these sections, the DNA is methylated and the histones are modified (Cedar and Bergman, 2009). This is the mechanism responsible for X chromosome inactivation in mammals. The histone modification together with DNA methylation coils sections of the X chromosome into a “ball,” making them inaccessible to the proteins and enzymes required for transcription.

5.3.3 Transcription factors

Typically, DNA methylation and histone modification are long term control mechanisms. Especially in development, once these mechanisms are in place, they remain in place. The use of transcription factors is a mechanism that can influence the dimmer switch in the short run. The transcriptional dimmer switch can be turned up or down as needed by the cell. We also know more about the role of transcriptional factors in behavior than we do about the effects of DNA methylation and histone modification. Hence, we will discuss spend some time overviewing this mechanism. Detailed case examples are given later in Sections 5.8 (cortisol and stress) and 5.9 (CREB, learning and memory).

A transcription factor is a protein or protein-based complex that binds to the DNA and can increase or decrease the rate of transcription for one or more genes. A classic example is the SRY gene responsible for sex differentiation in mammals and marsupials. Let us examine sex development for its lessons about transcription factors.

SRY stands for sex-determining region of the Y, a gene on the short arm of the Y that produces a protein of 203 amino acids (Su and Lau, 1993). That is quite small as proteins go, but the effects of the tiny molecule are very profound. Before seven weeks of gestation, the tissues for sex development are the same in both males and females. When a Y chromosome is present, the SRY gene is expressed at that time. The SRY protein produced from the gene blueprint binds to the DNA at certain regions and “bends” the DNA. The change in physical structure of the DNA alters the way in which the DNA loops and folds in the cell. This, in turn, opens up certain genes for transcription. The result is a cascade of transcription and further epigenesis that deflects certain cells away from developing into ovaries and towards the development of testes.

The story of sexual development—as well as our lesson on transcription factors—does not end here. Before testes development, both females and males produce feminizing and masculinizing hormones. With testes development the dimmer switch for masculinizing hormones in the testes cells get turned up into overdrive. One of the several hormones that is produced is testosterone. Testosterone and its cousin, 5α-dihydroxytestosterone, slip into cells and bind with a receptor protein—the androgen receptor. That hormone-receptor complex acts as a transcription factor that regulates a number of different genes, turning the dimmer switch up on some but down on others. The effect is the development of male external genitalia and the prostate (Gilbert, 2010).
Abnormalities in the genes that influence sex development illustrate the importance of transcription factors in epigenesis. Chromosomal XY individuals with mutations in the SRY gene that prevent the protein from binding properly with the DNA will develop as females (Cameron and Sinclair, 1997). Some mutations in androgen receptor, however, produce a different outcome in XY individuals—a syndrome previously called testicular feminization but now know as androgen insensitivity syndrome or AIS (Gilbert, 2010; Hughes et al., 2012). Here, the SRY gene is perfectly fine, so they will develop testes. Those AIS individuals with mutations that fail to produce an androgen receptor or produce one that is totally nonfunctional have complete AIS. Here, the testes cells will produce testosterone but there will be no functional hormone-receptor transcription factor. As a result, they will fail to develop male genitalia. The testes will remain internal, but the estrogen produced in other cells will result in female external genitalia. They will lack, however, ovaries, the ovarian tract and a uterus.

XY individuals with mutations that produce androgen receptor proteins that function but do not function well develop mild or partial AIS. Here, the extent of masculinization is variable. Usually, the external genitalia are ambiguous or poorly developed. Over 800 different mutation in the androgen receptor gene are known to cause a form of AIS. Most of these are located in the area of the protein that binds to the DNA (Hughes et al., 2012).

Because of the appearance of the external genitalia, XY individuals with complete AIS are raised as girls. The primary reason they come to medical attention is primary amenorrhea (failure to have monthly periods) and problems with fertility. Behaviorally, they identify as women and on most psychological measures that traditionally show gender differences, they show no mean differences from normal women (Hines et al., 2003).

Two examples of transcription factors and behavior are given later in Sections 5.8 (stress and cortisol) and 5.9 (CREB, learning and memory).

5.4 Alternative RNA splicing

Alternative RNA splicing is an epigenetic mechanism that operates in the editing phase of protein synthesis. The editing step of protein synthesis removes introns and splices exons together and it might appear that every RNA transcript has the same introns removes and the same exons spliced together. That, however, is not the case.

For most long human genes, different exons are spliced together resulting in different mRNA nucleotide sequences after transcription. This phenomenon is termed differential RNA splicing (or simply RNA splicing) and is a major epigenetic event in regulating the amino acid sequence that can result from a single gene.

Figure 5.4 illustrates RNA splicing. Here, the gene of interest has five exons and four introns. All five exons and four introns are copied into RNA during the transcription step. During editing, however, two different types of mRNA
result from RNA splicing. The first mRNA, depicted in the lower left, spliced together exons 1, 2, 3 and 4. The second mRNA, illustrated in the lower right side of the figure, spliced together exons 1, 2, 3 and 5. As a result, two different polypeptide chains will result from the same genetic blueprint.

Differential RNA splicing is the rule, not the exception (Grabowski and Black, 2001). Most geneticists speculate that this is the reason why there are so few human genes. It occurs in over 90% of long human genes and on average each gene generates between three and five splice variants. It is particularly prevalent in the human brain. Grabowski and Black (2001) argue that RNA splicing is the most important mechanism in generating the hundreds of thousands of human proteins.

The GABA\(_A\) receptor is an example of how alternative RNA splicing influences behavior. \(\gamma\) amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and GABA\(_A\) is one of its receptors. The GABA receptors are involved in the action of class of drugs known as benzodiazepines (e.g., diazepam or Valium\(^\text{®}\), lorazepam or Ativan\(^\text{®}\)) that have important anticonvulsant and anti-anxiety effects. Hence, they are the subject of considerable research. Alternative RNA splicing can generate different types of GABA\(_A\) receptors that respond differently to alcohol and to the benzodiazepines (Grabowski and Black, 2001).

5.4.1 Case Study: Alternative RNA splicing and tau proteins

What do repeated concussions and Alzheimer’s disease have in common? Both lead to abnormalities in brain neurons called neurofibrillary tangles or NFTs. NFTs arise in microtubules, structures present in all types of cells. In neurons, microtubules give structural support to the axon and also act as “railway lines” providing transportation of substances to the dendrites and terminal buttons. Proteins called tau proteins act as chemical railroad ties that maintain the structural integrity of the microtubules. For unknown reasons, these tau proteins sometimes become chemically “sticky” and clump together forming the
NFTs. In the process, the microtubule disintegrates. NFTs characterized a wide number of neurological syndromes known as **tauopathies**, the most famous of which is Alzheimer’s disease.

The genetics of tau proteins provide a learning exercise in alternative RNA splicing. The technical details about tau are unimportant. Instead, use this section to reinforce your understanding of transcription, editing, and alternative RNA splicing.

In humans, the blueprint for tau proteins is in the MAPT gene (**M**icrotubule-**A**ssociated **P**rotein **T**au) located on 17q21. The gene is approximately 150kb in length and has the 16 exons illustrated by the top part of Figure 5.5 labeled “DNA.” Note the numbering of the exons. Initial reports of gene structure were not completely accurate, so the numbering required the addition of exon -1 and 4a to maintain consistency with the published literature.

Exons -1 and 14 are included in the transcript but these sections are not translated into the polypeptide chain. In the human central nervous system, exons 4a, 6 and 8 are not transcribed. As a result, the structure of the primary transcript is shown under the rubric “RNA transcript” in Figure 5.5.

In editing the transcript, alternative RNA splicing involving exons 2, 3 and 10 will result in six different messenger RNAs. These are shown at the bottom of Figure 5.5. These variants, called **isoforms**, are referred to by the exon number followed by a plus or minus sign to denote whether the exon is present or absent.
Figures 5.6: RNA interference (simplified).

For example, isoform 2+, 3−, 10+ denotes that exons 2 and 10 are present and exon 3 is absent.

Note that some alternative RNA splicing forms that are logically possible are not biologically observed. For example, it is logically possible to have a splice variance with exon 3 present but exons 2 and 10 absent. Such a variant, however, does not occur in the human brain.

Mutations that affect the splicing mechanism for exon 10 have been implicated in a number of neurological syndromes (Pittman et al. (2006); BuÅ©e et al. (2000)). Although clinical manifestations are highly variable, many of these syndromes involve dementia. While this suggests that aberrant tau can cause dementia in some cases, the role of tau and NFTs in other dementing illnesses, including Alzheimer’s disease, is not well understood.

We will learn more about tau proteins when we discuss disorders with complex genetics and Alzheimer’s disease in Chapter X.X.

5.5 RNA interference

RNA interference (RNAi) is a regulatory mechanism that occurs after transcription but before translation. The process is depicted in Figure 5.6. It begins with a short segment of RNA which, for simplicity’s sake, we will call iRNA for “interfering” RNA. The iRNA joins with a series of proteins (the interfering “stuff”) forming a complex. The RNA in this complex will being to a molecule of mRNA if the mRNA has the complementary nucleotide sequence to the iRNA. Enzymes in the interfering complex then slice the mRNA.

You should be able to predict the consequences of this cleavage. The “head”
part of the cleaved mRNA may be able to bind with a ribosome and initiate the translation process. Without the “tail” information, however, the resultant polypeptide chain will be shortened and, with possible rare exceptions, non-functional. The “tail” section of the mRNA lack the requisite header information to allow it to bind with a ribosome. Hence, no translation occurs. The net effect is to reduce translation and the amount of polypeptide. The effect can be so large as to effectively silence a gene that is being actively transcribed.

RNAi has several functions (Wilson and Doudna, 2013). It is an important regulatory mechanism during development and also functions to combat viral infections by manufacturing iRNA complementary to the mRNA produced by the virus. The role of RNAi in mammals, however, is poorly understood.

RNAi, however, is becoming a powerful tool in research. It is possible to manufacture iRNA molecules specific for a single gene and then microinject them into an area of, say, the brain. With a large enough amount of iRNA, this will silence the gene, so one can examine its role for a phenotype. This strategy has also spawned a vibrant biotech industry focusing on RNAi as a therapeutic mechanism for diseases. For example, Zimmermann et al. (2006) used RNAi in an animal model to reduce cholesterol levels by slicing the mRNA for a protein that transports cholesterol.

5.6 Epigenetic transmission

Epigenetic transmission has been well established in a variety of plant and animal species (Jablonka and Lamb, 2005). Because it has been systematically studied in only recent decades, however, there is still much to be discovered. This pertains especially to human behavioral phenotypes. Outside of some forms of pathology, there is no universally accepted example of epigenetic transmission influencing normal human behavior. That should not be interpreted as “epigenetic transmission is unimportant for human behavior.” Rather, the recency of the discovery of this mechanism, coupled with the long generation time for humans compared to those species used in laboratory studies, means that a large body of empirical data have yet to be gathered on humans.

5.6.1 Epigenetic transmission and behavior

The best examples of epigenetic transmission that may relate to human behavior are early handling and maternal behavior in rodents (see Meaney, 2001a, for a review of this area). In nature, mice and rats are born blind and immobile. Mothers leave their litters in nesting material for periods up to 30 minutes to forage. In early handling experiments, pups are removed from the nest for periods of 3 to 15 minutes and then replaced. Usually, such handling occurs daily until the pups are weaned at c. three weeks. This simple handling produces long-term changes in the response to stress (Levene, 1957). As adults, handled rats adapt better and have lowered hormonal responses to stressful situations than non-handled rats.
Denenberg and Rosenberg (1967) published one of the first reports of the epigenetic transmission for a behavior–activity in rats. Adult females who were to become the grandparents of the test rats were randomly handled or not handled as pups. Their female pups (the mothers of the test pups) were raised in either the same housing before and after weaning or different housing. There was a significant interaction between handling and housing. The pups of handled grandmothers and mothers experiencing different housing were more active (and less stressful) as adults. This study demonstrated that in the right circumstances, an environmental experience in one generation can influence behavior in grandchildren.

The epigenetic transmission of handling effects is not restricted to activity. Francis et al. (1999) demonstrated that handling of rats influences changes in offspring mRNA for two proteins associated with the hypothalamic-pituitary-adrenal axis, a hormonal response associated with stress (see Section 5.8).

5.6.2 Genomic imprinting

Genomic imprinting (aka parental imprinting) is a special case of epigenetic transmission (Mannens and Alders, 1999). It refers to the fact that the expression of a gene depends on whether it is inherited from the mother or the father. Imprinting is a functional change—the actual DNA is not altered in any way; it is just that the expression of the gene product is changed. The imprinting occurs before or soon after conception and once it occurs, all daughter cells seem to be imprinted in the same way.

The textbook example of imprinting concerns a genetic syndrome that arises when a small section of chromosome 15 is deleted. If the chromosome deletion is inherited from the father, then the offspring will have the Prader-Willi syndrome, characterized by an insatiable appetite, obesity and mental retardation. But when the deletion is inherited from the mother, then the child will exhibit the Angelman syndrome involving severe mental retardation, loss of motor coordination (ataxia), lack of speech, and seizures. It is suspected that when the father transmits the deletion, then the mother’s genes on chromosome 15 are in some way inhibited from expressing their polypeptides. The opposite occurs in Angelman’s syndrome.

Imprinting is gene specific. That is, only certain loci are imprinted. In mammals, fewer than 1% of genes are subject to imprinting, but those few genes can still influence brain development (Wilkinson et al., 2007b). Most studies of imprinting use rodent models and “knock out” the imprinted gene (the one that is ordinarily active). For example, if a gene is paternally expressed, then what is the effect of eliminating the effect of that paternal gene? As a result, rodent models may be looking at conditions akin to a recessive disorder where the DNA blueprints from both mother and father’s are both faulty.

The importance of genomic imprinting for normal variation in behavior is still unknown. Limited evidence from animal models and from Turner’s syndrome (discussed later in Chapter X.X) is being interpreted as suggesting that imprinting may occur for some aspects of sociality (Skuse et al., 1997) and cogni-
There is evidence from rodents that imprinting of the Peg3 gene influences rodent maternal behavior (Champagne et al., 2009). For normal human behavior, the majority of publications genomic imprinting and human behavior have been theoretical (e.g., Crespi and Badcock, 2008; Gorelik et al., 2010; Haig, 2011).

5.7 Case study: Addiction and chromatin remodeling

Why can substances like nicotine, alcohol, amphetamine, and heroin be addictive? In some people at least, repeated use of one of these substances leads to the classic addiction triad of craving, tolerance, and, when the substance is not available, withdrawal. Clearly, the drugs are modifying something in the brain and possibly other organs. But what is being modified and what modifications are associated with addiction?

Considerable evidence indicates that both acute and chronic administration of drugs of abuse have epigenetic consequences (Nielsen et al., 2012). Researchers are now pursuing these leads with an eye that some epigenetic mechanisms may play a role in chemical dependencies. Let’s focus on one class of drugs, the opiates.

Humans first use of opium is lost to history but deliberate cultivation of the opium poppy was being done by at least 3,000 BCE by the Sumerians who called it the “plant of joy” (Brownstein, 1993). Opium preparations have been used medicinally for thousands of years and are still used today for cough suppression (codeine) and pain relief (morphine). Today’s derivatives—heroin (a morphine derivative) and semi-synthetic opioids like oxycodone—are highly addictive.

Our bodies naturally produce several classes of small opioid-like peptides that act as neurotransmitters and neuromodulators and play a role in pain relief. There are several types of receptors for these endogenous opioids but one, the \( \mu_1 \)-opioid receptor, is associated with opioid dependence. Morphine binds to this receptor (the \( \mu \) is for morphine) as does the drug naltrexone which is used in maintenance therapy because it blocks withdrawal symptoms.

The \( \mu_1 \)-opioid receptor is coded for by the OPRM1 gene. In the test tube at least, transcription of this gene can be modified by methylation of the promoter (Andria and Simon, 1999) and by histone modifications (Andria and Simon, 1999; Hwang et al., 2007). Also, manipulation of methylating and histone modification agents results in different distributions of the \( \mu_1 \)-opioid receptor in rat brains (Hwang et al., 2009).

Fine, but what about people? Two recent studies have examine the OPRM1 promoter region in opioid addicts. Both report hypermethylation in the promoter region in white blood cells (Nielsen et al., 2009a) and in blood and sperm (Chorbov et al., 2011). The Chorbov study also reported lower levels of the \( \mu_1 \)-opioid receptor in the blood of participants with heroin dependence than in controls.
Although these results are new and required replication and further exploration, they suggest that heroin use may result in a negative feedback mechanism with the $\mu_1$-opioid receptor through chromatin remodeling. It is as if the body is saying to itself, “Hey, we are experiencing too much opioid stimulation, let’s cut down on the receptors to get us back to an equilibrium.” Could such a mechanism be involved in tolerance (the need to take more and more of the substance to achieve the same effect)? It is much too early to tell, but this story shows how research into epigenetics can play an important role in answering such questions.

5.8 Case study: Stress and cortisol

Here we will describe what is known about one physiological response to stress, the excretion of cortisol, and how this influences gene regulation. We will explore this in considerable detail—and risk losing the reader in the process—to illustrate an important phenomenon about genes, physiological systems, and behavior. It will also serve to reinforce learning about genes, cell communication, protein synthesis and transcription factors.

Imagine that a good friend convinces you to try a parachute jump. You pay the money, go through the ground training, and receive considerable reinforcement from your friend, your instructor, and your fellow virgin parachutists about how thrilling and exciting an experience this is going to be. After donning the appropriate regalia and entering the airplane, you are flown to a point over a mile above the solid earth. The green light flashes, the instructor points to you, you move to the door, look down, and ask yourself the proverbial question, “Why am I jumping out of a perfectly good airplane?”

It is easy for the social scientist to focus on the obvious—and very important—environmental circumstances that produce stress in this situation. But this clear environmental stressor also has a genetic side, albeit a poorly understood one. A generic view of the process begins with the endocrine (i.e., hormonal) system referred to as the hypothalamic-pituitary-adrenal axis (HPA) that is depicted in Figure 5.7. The stress and anxiety of your parachute jump are accompanied by the release of a hormone called corticotropin releasing hormone (CRH). CRH stimulates the production of another hormone, adrenocorticotropic hormone (ACTH), which in turn initiates excretion of cortisol and
simulates cells to increase the production of cortisol. The cortisol response is now engaged. As cortisol builds up and plays its part in informing cells that stress is present, it also inhibits the production of ACTH. As ACTH levels drop, so does cortisol, and the system shuts down. This is a classic case of negative feedback.

Let us now put this process under a more powerful microscope to see how genes play a role in it. Everyone has a structure in the hypothalamus of the brain called the paraventricular nucleus (PVN) that has nerve inputs from other parts of the brain. Within the cells of this nucleus are a large number of vesicles that contain the hormone CRH. At various stages in your jump, the neurons from the central nervous system that impinge on the PVN fire. In response, the cells release CRH. The stress response has just begun.

CRH soon encounters the cells of the anterior pituitary gland located below the hypothalamus. There, it binds to a receptor molecule sitting in the plasma membrane and starts chemical messaging system. The message has two important consequences. The first and most immediate consequence is to stimulate the release of ACTH that is stored in vesicles in the cell. This ACTH leaves the cell and enters the bloodstream.

The second consequence of CRH is to initiate the synthesis of more ACTH to replace the ACTH that was released. This process begins with transcription of the pro-opiomelanocortin (POMC) gene. The POMC polypeptide chain synthesized after translation undergoes an important post-translational process. Enzymes literally cut out a section of the POMC polypeptide. This section of amino acids cleaved from POMC is the peptide hormone ACTH. The newly manufactured ACTH is transported and stored in vesicles in the cells of the anterior pituitary.

Our attention now switches away from the head and to the adrenal gland, located on the top of the kidney. Like most cells, adrenal cells both manufacture cholesterol and “import” it from the blood. The adrenal gland contains receptors specific for ACTH within its plasma membrane. The ACTH that is circulating in your blood as a result of the stress of your first parachute jump blood binds to this receptor. There, the resulting ACTH-receptor complex initiates another messaging system to the adrenal cells. One result of the message leads to the excretion of cortisol into the bloodstream.

A second consequence is to activate the five different enzymes that produce cortisol from cholesterol. (Remember, protein/enzyme activation is an example of post-translational modification.) The newly manufactured cortisol is excreted from the adrenal cells, enters the bloodstream, and is diffused throughout the body. Cortisol enters the cells of a wide variety of organs and cells—pancreas, liver, kidney, neurons, and many immune cells. There, it binds to its receptors and the cortisol-receptor complex acts as a transcription factor for a large number of genes. In some cases, the cortisol-receptor complex initiates transcription while in other cases it inhibits transcription. The stress and anxiety of your parachute jump are now influencing your genetic dimmer switches!

Among all the cells that cortisol enters are those of the anterior pituitary, the very cells that released ACTH and sparked the production of cortisol in
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the first place. There, cortisol binds with another receptor, and this cortisol-receptor complex stops transcription of the POMC locus. With no more POMC polypeptides, there is no more ACTH being produced. With no more ACTH, the enzymes that synthesize cortisol from cholesterol are no longer activated, so cortisol production stops. The system is now shut down.

The HPA axis illustrates two major points. First, gene regulation can occur in response to environmental cues and circumstances. Although regulation occurs within the cell, it is not some closed, endogenous, biological process immune to events that physically occur outside of the organism. In the case of cortisol, a wide variety of environmental cues can influence gene regulation. Such cues range from the anticipation of taking a final exam to downright, life-threatening events.

As an engineering standpoint, the cortisol response resembles a nightmarish Rube Goldberg mousetrap. The ultimate goal is to produce cortisol when nerves in the brain signal that something important is happening and cortisol is needed. However, both the neurons in your central nervous system and those in the paraventricular nucleus of your hypothalamus contain cholesterol and the genes for the enzymes necessary to metabolize the cholesterol into cortisol. One highly efficient system would be for these neurons to produce the cortisol once the neurons fire. Just think of the waste of the precious biological resources that this system would overcome: (1) there would be no need to make and store CRH and its receptors; (2) there is no need to transcribe the POMC gene and produce POMC polypeptide only to throw much of it away to get ACTH; (3) there is no need to store ACTH; (4) and there is no need to release ACTH and diffuse it throughout your blood only to have it influence only one, small organ. The HPA axis makes as much engineering sense as plugging a monitor into a computer with a cord that passes through each room in the building. We will return to this topic later.

5.9 Case study: CREB, learning and memory

This text focuses on human genetics and human behavior. Much behavioral genetic research, however, must be done using animal models. Here, we can explore some of the genetic techniques used in animal models to examine important questions about behavior. The specific case we explore is the importance of a protein, the cyclic AMP response-element binding protein (mercifully acronymized as CREB) for learning and memory. Technically, there is a small family of genes that can generate CREB-like proteins, but we will oversimplify and treat the system as a single gene and a single protein.

It has long been known that the inhibition of mRNA synthesis and protein synthesis can influence memory (Davis and Squire, 1984; Flexner et al., 1963). In the early 1990s, studies in the sea slug Aplysia (Dash et al., 1990) and the fruit fly (Yin and Tully, 1996) suggested a role for the CREB pathway in learning and memory. Bourtchuladze et al. (1994) and Gass et al. (1998) extended that
to mammals by using knockout mice. A knockout organism is one that has been genetically engineered to prevent a specific gene from functioning. In the Bourtchuladze et al. study, the knockout strains had a nonfunctioning gene for certain forms of CREB. Both knockout and control mice learned certain tasks and were then tested for retention of these tasks at various time points after task acquisition. Both strains retained the task equally well a short time after acquisition. Retention in the knockouts, however, was much poorer as the time interval increased from learning to testing.

These results suggest that CREB might be involved in the process of forming long-term (as opposed to short-term) memories. The simple knockout strategy, however, is not ideal technique because the gene product is missing over the whole developmental period of organism. Perhaps CREB has little to do directly with memory but creates damage to certain structures in the brain during a sensitive period of neuronal development.

A superior strategy is to experimentally manipulate the gene or gene product around the time of the learning task. This can be done with an RNA antisense oligonucleotide. This is a single RNA strand comprised of a short sequence of nucleotides that is complementary to mRNA for a specific gene. The oligonucleotide will bind to its complementary section of the mRNA molecule and inhibit the translation step in protein synthesis. Guzowski and McGaugh (1997) synthesized an antisense RNA strand for CREB and infused it into a section of the hippocampus previously implicated in memory in rats. (Antisense RNA is an implementation of RNA interference described in Section 5.5. It will reduce the number of CREB protein molecules in the relevant brain area.) The controls were rats who received an identical infusion but with a random antisense RNA molecule that had no mRNA complementary sequence. Both groups of rats were given a spatial learning task. They performed equally in both the acquisition of the task and in a retention trial four hours after the learning phase. Two days after learning, however, the CREB-inhibited rats performed more poorly than the controls. This agrees well with the notion that CREB is involved in the formation of long-term but not short-memory.

Another genetic technique involves the use of pseudovirions. This is a genetically engineered virus that lacks key viral functions, notably replication, but can enter a cell and inject a desired nucleotide sequence into that cell. The sequence is designed to increase or decrease the manufacture of a desired polypeptide. Dong et al. (2006) used neuronal cell cultures and pseudovirions to demonstrate that CREB can influence the firing rate of certain neurons. (A neuronal cell culture is a thin slice of tissue from a desired area of the brain. The slice is kept or “cultured” in a soup of nutrients that...Case study: Stress and cortisol delays cell death.) In the Dong et al. study, two pseudovirions were used (in addition to controls). One enhanced CREB while the other inhibited it. Enhancing CREB increased the rate of firing while inhibiting it decreases firing. Hence, CREB some influence on neuronal excitability.

How does CREB influence memory? We really do not know but most neuroscientists speculate that its action is due to CREB’s function as a transcription factor. Let’s explore this for a moment. Once again, do not worry about the
small details. The purpose of this exercise is to reinforce knowledge of how genes work to influence behavior.

Figure 5.8 is a highly simplified view of CREB and its action. The action begins when the neuron is stimulated. The figure depicts five different mechanisms for this stimulation. Four are from classical neurotransmitters: serotonin, glutamate, acetylcholine, and dopamine. The fifth depicts a neurotrophic factor—brain-derived neurotrophic factor. (A neurotrophic factor is a protein that affects the survival and development of a neuron). In reality, some of these stimulators will be important for some cell types more than other cell types.

When the neurotransmitters or BDNF bind to their receptors two things may occur. In the case of the glutamate and acetylcholine receptors in Figure 5.8, ion channels open and calcium, specifically Ca^{2+}, enters the cell. Not only does this assist in depolarization, but it also passes through a metabolic pathway that acts as a signal to the cell that it is being stimulated. For the three others, binding of the molecules with their receptors initiates different second messenger systems that also inform the cell of the signal. The signaling pathways can be long and complex. They may also interact with one another.

For our purpose, the result of the messenger pathways is to activate CREB. This is done by gluing a phosphate molecule (the round green shape in Figure 5.8) onto the CREB protein. The CREB, now activated, acts as a transcription factor. It binds with the DNA in the promoter regions of a large number of different genes and enhances transcription. Figure 5.8 three such genes. The real number of genes influenced by CREB is in the hundreds (Benito and Barco,
2010) and the genes influenced vary by the type of cell.

In Figure 5.8 enhances transcription of the enzyme tyrosine hydroxylase. This enzyme is important for the synthesis of the neurotransmitters dopamine and norepinephrine. In a sense, CREBs action is akin to saying to the neuron “Hey, you are being stimulated. Make more neurotransmitter to pass the message on.”

The second gene whose transcription CREB influences is brain-derived neurotrophic factor (BDNF). This is a protein that helps neurons to survive, grow, and extend their dendrites and synapses. It has been linked to long term memory (Bekinschtein et al., 2007), so increasing it is likely to strengthen the memory.

The final gene codes for one of the glutamate receptors. Since glutamate is a neurotransmitter that may have stimulated the neuron in the first place, the role of CREB is to facilitate positive feedback. In effect, neuron is saying “I’m being stimulated, so I better make more receptors in case the simulation increases.”

5.10 Why the complexity?

Indeed, at this point let us postpone discussion of genetic regulation and development and return to the statement made earlier about how the HPA axis is, from an engineer’s perspective, a complicated and inefficient system. A moment’s reflection on the genetic code, the organization of the human genome, and the process of protein synthesis reveals that they are also quite disorganized.

Each of us humans has over a trillion cells, and each cell contains over 12 billion nucleotides. Thus, we each have a minimum of 12,000,000,000,000,000,000,000 nucleotides, the majority of them unneeded. Why should bone marrow cells spend energy and resources maintaining all those nucleotides for enzymes that produce skin pigment, fingernails, or hair?

Clearly, any bioengineer who designed a protein synthesis system by placing introns into the blueprint would be fired very quickly. So would a chip designer who insisted that the manufacturer keep the nonfunctioning β hemoglobin “pseudo-chip” in the computer along with its perfectly functioning counterpart.

Some DNA currently suspected as junk undoubtedly will turn out to be important as knowledge of genetics expands. But it defies reason to imagine that human DNA and each and every anatomical and physiological entity influenced by the DNA is perfectly optimized in terms of simplicity, elegance, efficiency, and reliability of design. Otherwise, we would all look and act the same.

The intriguing question is, “Why is the genome so complicated and inefficient?” Truthfully, the reason is unknown, but the answer that first comes to the geneticist’s mind is evolution. Evolution never anticipates or thinks ahead. There is never any ultimate or teleological goal that evolution strives for when it alters an organism’s DNA. Instead, evolution is the ultimate pragmatist. Evolution cares—actually demands—that a problem gets solved. It does not care HOW the problem gets solved.

When there is a difficulty, evolution solves it in the here and now. It will
tinker with and adopt any ad hoc solution with no consideration as to how
elegant the solution is designed and no forethought about whether that solution
will be beneficial or detrimental to the organism after the problem is solved.
Evolution’s motto is ‘if it ain’t broke, don’t fix it.” As long as we humans
reproduce at an acceptable rate, it does not care if we all waste extra energy
and nucleotides carrying around a β hemoglobin pseudogene that does nothing.

The only reason for evolution to be concerned about simplicity and efficiency
is when the lack of them interferes with reproduction. But even here, evolution
retains its pragmatism. If an inefficient and complex system impedes repro-
duction, evolution could actually make the organism more complicated with a
series of string and bubble gum patches as long as those patches overcome the
problems with reproduction.

The purpose for this digression into evolution is not to merely point out the
complexity of the genome. It is crucial to recognize that our human behavior
has gone through the same pragmatic process of evolution as our DNA. It is
likely that many aspects of human behavior are not simple, logical, efficient, and
parsimonious, or for that fact, even nice from a moral standpoint. Perhaps much
of our behavior is illogical, inefficient, and complicated when viewed through the
faculty of reason. Perhaps we are all Rube Goldberg contraptions in more ways
than our DNA and hypothalamic-pituitary-adrenal axes.3

5.11 References

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3Rube Goldberg was an early 20th century cartoonist well known for drawings of ridicu-
ously complicated machines designed to accomplish a simple task. A contemporary example
of a Rube Goldberg apparatus example is the children’s game Mouse Trap.
CHAPTER 5. GENE EXPRESSION AND EPIGENESIS


