FEATURE ARTICLE

Teaching Epigenetic Regulation of Gene Expression Is Critical in 21st-Century Science Education: Key Concepts & Teaching Strategies

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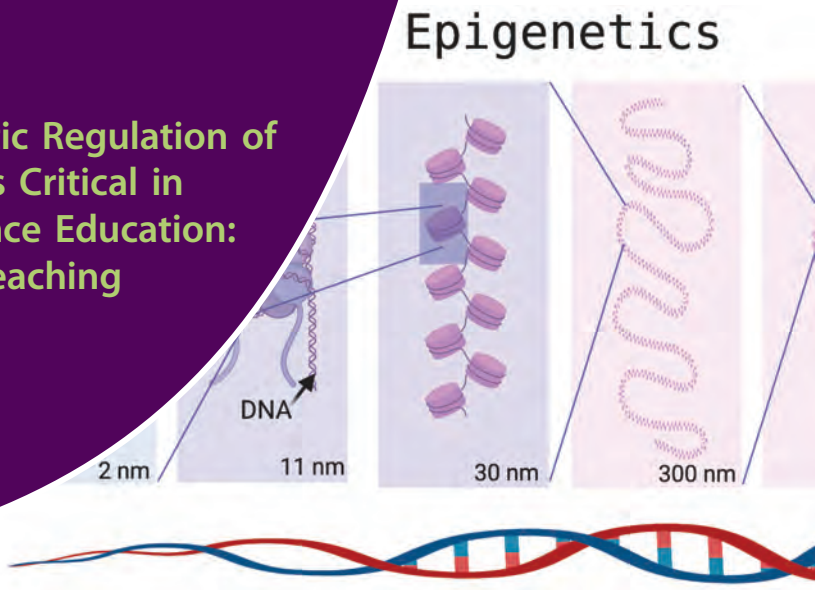

Abstract

The field of epigenetics is progressing rapidly and becoming indispensable to the study of fundamental gene regulation. Recent advances are redefining our understanding of core components that regulate gene expression during development and in human diseases. Scientific knowledge on the importance of epigenetic regulation is now well known and accepted, and it is not surprising to see epigenetics being introduced into many biology curricula at the high school and college levels. Yet the core concepts of epigenetic regulation are differently perceived by the academic communities. Therefore, it is critical that fundamental concepts of epigenetic regulation are taught to the next generation in a simple yet precise manner to avoid any misconceptions. To that end, this article starts by distilling the extensive scientific literature on epigenetic control of gene regulation into a simple primer on the core fundamental concepts. Next and more importantly, it provides suggestions for student-friendly classroom practices and activities that are centered on these core concepts to ensure that students both recognize and retain knowledge on the importance of epigenetic control in eukaryotic gene regulation.

Key Words: *DNA methylation; epigenetics; chromatin; histone modifications.*

Introduction

Over the past 75 years, the field of epigenetics has rapidly evolved along with the functional definition of the term *epigenetics*. Although originally epigenetics was studied in the context of development, today it is at the forefront of gene regulation – involving virtually all biological processes, including neuronal development, regulation of circadian cycle, cancer, stem cell differentiation, homeostasis, and normal body function. It is therefore critical that the core concepts of epigenetics be a part of academic biology curricula from high school to undergraduate education.

> *"As with all fields that experience sudden, rapid growth, the scientific data and literature on epigenetics are ever evolving, and we are learning and relearning core concepts continually."*

As with all fields that experience sudden, rapid growth, the scientific data and literature on epigenetics are ever evolving, and we are learning and relearning core concepts continually. Therefore, we have to be cautious of the approaches we use to introduce this field to our students, and stay alert in order to minimize misconceptions. However, a core set of concepts on epigenetic control of gene regulation has stood both the test of time and rigorous reproducible science, such that it can be taught with certainty to our students. These concepts include knowledge on the basic molecular mechanisms underlying epigenetic modifications that drive changes in gene expression. This fundamental knowledge is both necessary and sufficient to allow students to grasp the core concepts and, hopefully, build on this foundation as they progress academically.

Given that information flow is one of the core concepts of biological literacy (AAAS, 2018), epigenetics can be introduced in secondary and postsecondary biology courses, in a unit on regulation of gene expression. The core concepts that are supported by research can be presented to students at whatever depth is appropriate. To maximize students' learning, instructors can use lifelike examples (some provided here) to take epigenetics teaching out of the realm of the abstract and into the real world.

Historical Perspective

Often called the father of modern epigenetics, Conrad Waddington proposed the term *epigenetic landscape* to verbally conceptualize gene–environment interactions that result in phenotypic changes (Aguilera et al., 2010). As early as 1940, he hypothesized that gene–environment interactions were nontrivial and contributed to phenotypic changes. It was not until 1975 that DNA methylation (a now well-characterized epigenetic modification) was proposed as the molecular mechanism that explains

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Waddington's hypothesis. The rest of the 20th century and the first decades of this century have seen a substantial change in the field. During this time, we have learned that

- both X-chromosome inactivation and genomic imprinting are epigenetic phenomena;
- epigenetic regulation is dynamic and very susceptible to change by the environment; and
- the epigenome is stable in somatic cells—however, it is susceptible to deregulation during gestation, neonatal development, puberty, and old age (Barros & Offenbacher, 2009).

○ What Is Epigenetics & How Does It Work?

Genetic regulation of gene expression is dependent on the underlying DNA sequence around gene regulatory regions. By contrast, epigenetic regulation controls expression by regulating the chromatin structure around gene regulatory regions. How is this achieved?

Eukaryotic DNA does not exist naked; the DNA is wound twice over proteins called histones to form nucleosomes, the smallest unit of the chromatin (Figure 1A). Nucleosomes are sequentially arranged into chromatin, which coils and gets organized into chromatids/chromosome. How tightly the DNA is wound around histones determines the underlying accessibility of genes to transcriptional machinery. When loosely bound, the chromatin is held in a relaxed conformation, which allows easy access to transcriptional machinery and gene activation. By contrast, when tightly wound, the chromatin is rendered inaccessible, thereby blocking subsequent gene activation.

Epigenetic regulation, in contrast to genetic regulation, is highly adaptive and rapidly evolves to control gene expression in response to environmental stimuli. Epigenetic mechanisms are, therefore, in part responsible for biological diversity and the evolution of complex traits (Zhong, 2016). Let us set up a simple argument that conveys the critical role that epigenetics has played

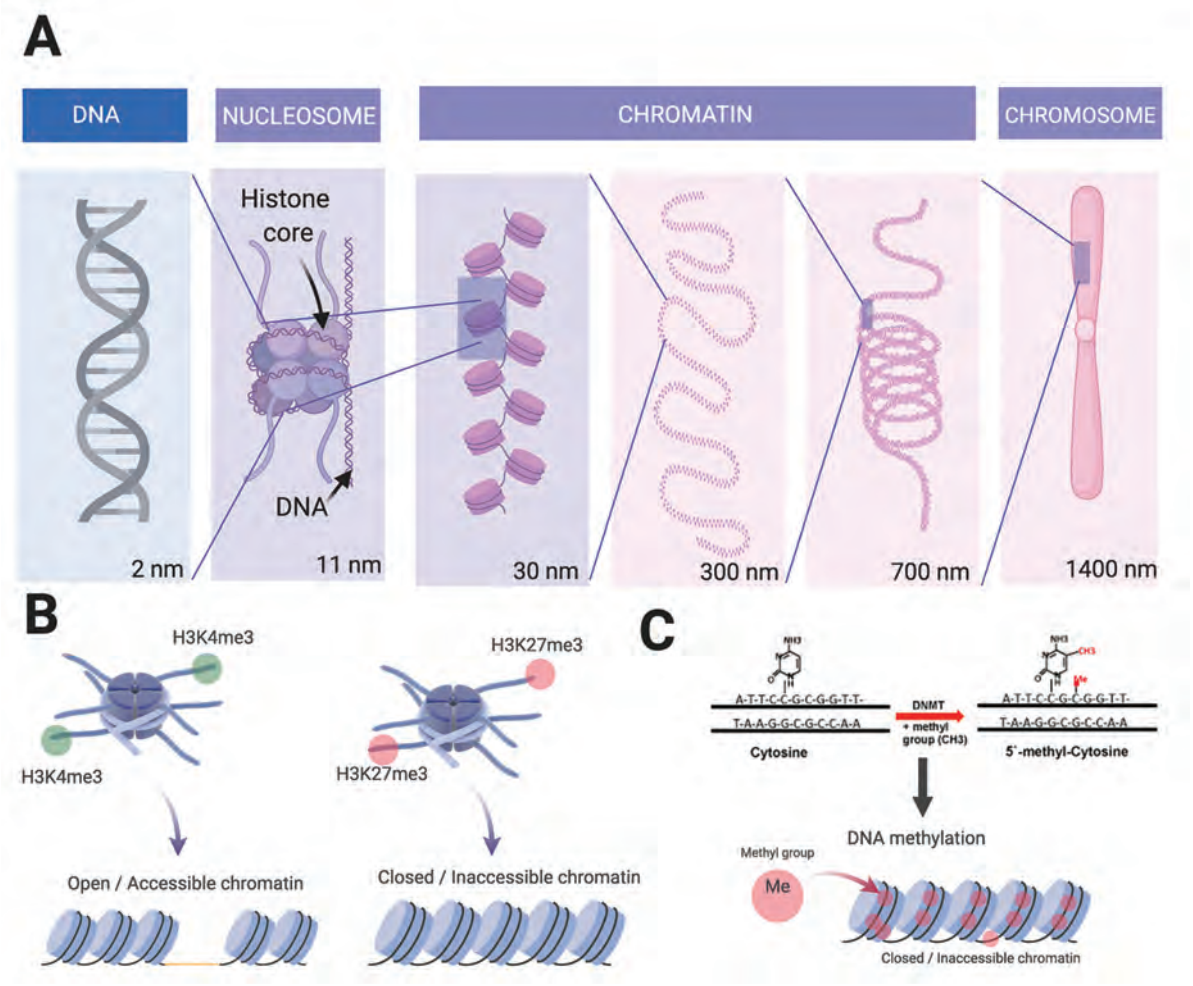

Figure 1. Organization of the genome and common epigenetic modifications. (**A**) DNA in eukaryotes is wrapped around proteins called histones to form nucleosomes. Nucleosomes arranged sequentially form chromatin, which is organized into chromosomes (scale = nm). (**B**) Modifications to histone tails render chromatin into open/closed conformation. (**C**) DNMT enzyme catalyzes the transfer of the methyl group to the cytosine in CpG di-nucleotides, leading to DNA methylation. DNA methylation results in condensed chromatin leading to transcription inactivation.

in shaping human evolution. We know that humans share ~98% of their genome with chimpanzees (Chimpanzee Sequencing and Analysis Consortium, 2005). The human genome encodes for ~30,000 genes, and invertebrates such as *C. elegans* code for ~20,000 genes, the majority of which are human analogues. With so much of the genomic blueprint shared among species, how did we evolve to the complex, unique beings that we are?

An emerging model is that we evolved to gain a complex gene regulatory system, a core part of which is epigenetic regulation. Although most epigenetic modifications are highly conserved across species, some organisms have acquired unique epigenetic regularities. For example, when we compare humans and chimpanzees; the structural changes in gene products are minor, and yet there are significant differences in gene regulation. For instance, the language gene *contactin associated protein-2* (CNTNAP2) is expressed at higher levels during human development than during chimpanzee development. Interestingly, CNTNAP2 promoter analysis has revealed major differences in DNA methylation of the promoter. As a consequence, cntnap2 is highly expressed only in the human brain, in catalyzing human-specific language and in communication traits (Schneider et al., 2014).

Another biological process tightly regulated by epigenetic regulation is the capacity to regrow axons following injuries to the central nervous system. Regeneration-competent neurons respond to injury by undergoing histone relaxation around pro-growth gene loci, leading to subsequent gene activation and axon regrowth (Puttagunta et al., 2014). By contrast, regeneration-incompetent neurons fail to undergo histone relaxation around pro-growth genes, contributing to an abortive growth attempt in mammals (Venkatesh et al., 2016, 2018).

○ Molecular Mechanisms Underlying Epigenetic Regulation

How Does Chromatin Change Conformation?

We have detailed how epigenetic regulation relies on changes made to chromatin conformation. How is this achieved? Intriguingly, simple biochemical reactions drive the chromatin to assume a condensed or a relaxed conformation (Dunham et al., 2012). These biochemical reactions modify or tag DNA and histones with different chemical groups to alter the chromatin conformation. Epigenetic modification of DNA and histones is a complex process, and although many of these modifications are evolutionarily conserved, some are species-specific. For example, plants share many conserved epigenetic modifications with mammals; however, some histone methylation patterns are unique to plants (Feng & Jacobsen, 2011).

To carefully introduce epigenetics in the classroom, this article describes the conserved and well-studied mechanisms for DNA and histone epigenetic modifications. One practical teaching tool to simplify epigenetic modifications and their role in regulating gene expression without altering the DNA sequence is the analogy that epigenetic modifications are like the spaces and punctuation marks in a sentence or a paragraph. Without spaces between words, without commas and periods, the text will be hard to read and can support different meanings. Epigenetic modifications provide structure and formatting to the genetic code. Epigenetic modifications improve interpretation of the genetic code by telling the cells when and where a gene can be expressed (FASEB, n.d.)

Epigenetic Modifications

Two main mechanisms of epigenetic modification are currently well studied in mammals: DNA methylation of the cytosine in CpG dinucleotide area (Maurano et al., 2015), and chromatin remodeling via post-translational modification of histones (Venkatesh et al., 2016). Importantly, these two mechanisms are not mutually exclusive; rather, they synergize to regulate gene transcription.

DNA methylation

In mammals, the de novo methyltransferase (DNMT) enzyme catalyzes the covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide DNA sequences. This form of DNA methylation condenses chromatin and interferes with transcriptional activation (Figure 1C) (Moore et al., 2013).

Differential methylation patterns on different chromosomes directly illustrate the effect of DNA methylation on chromatin conformation and subsequent gene regulation. Genomic areas that are devoid of coding genes and house highly repetitive sequences are usually heavily methylated. These areas are held in a condensed conformation that prevents any ectopic transcription. By contrast, genomic areas around transcriptionally active genes have little or no methylation. Promoter areas upstream of transcription start sites tend to be protected to allow for gene transcription (Saksouk et al., 2015). Furthermore, the pattern of DNA methylation changes in a definite and precise manner during embryonic development, which plays a crucial role in maintaining sexual reproduction.

Histone modifications

In the nucleosomes, histone proteins have exposed N-terminal tails that are subject to a variety of post-translational modifications. The modifications involve covalent attachment of different chemical groups to the N-terminal residues. These modifications lead to changes in chromatin accessibility that cause activation or repression of gene expression, depending on the amino acid residues that are modified and their position (Figure 1B). Three major types of histone modifications have been identified: histone acetylation (Shin & Cho, 2017), histone phosphorylation (Gaspar-Maia et al., 2009), and histone methylation (Mo et al., 2015).

Among these histone modifications, the effect of histone acetylation on chromatin conformation remains the most studied and understood. Histone acetylation is a dynamic process, and it is regulated by histone acetyltransferase (HAT) and histone deacetylase enzyme (HDAC) (Wang et al., 2009). HATs and HDACs are functionally opposing in nature. HATs coordinate relaxed chromatin, promoting the interaction of RNA-polymerase and other transcription factors with DNA and activation of gene expression. By contrast, HDACs mediate closed chromatin conformation, leading to down-regulation of gene expression. The protein families of both HATs and HDACs are evolutionarily conserved, and they play a crucial role in transcription regulation in yeasts, plants, and mammals.

Histone methylation is catalyzed by histone methyltransferase enzyme (HMT). Methylation of histones is associated with activation and repression of gene expression depending on the histone protein and the amino acid residue that is modified. It is a subtype of epigenetic regulation, and patterns of methylation are both highly species-specific and highly genomic-context-specific. Finally, histone phosphorylation has an indirect role in the regulation of

gene expression. In contrast to histone acetylation and methylation, histone phosphorylation works in conjunction with other histone modifications. The cross-talk between histone phosphorylation and other histone modifications results in complex regulation of chromatin conformation and gene expression (Alaskhar Alhamwe et al., 2018). Given the rapidly evolving nature of our understanding of these processes, it is premature to expand on these histone modifications in the classroom at the moment.

○ Examples of Biological Processes under Epigenetic Regulation

This section provides examples of various biological processes that are directly regulated by epigenetic modifications of underlying DNA sequences.

Epigenetic Regulation during Development

Although epigenetic modifications can occur at any point in human life, modifications made during embryonic development are particularly critical (Mo et al., 2015; Venkatesh et al., 2018). Temporal and spatial regulation of gene networks is highly critical for normal embryonic development, in all species (Liscovitch & Chechik, 2013). Each stage of embryonic development is tightly regulated by the expression of specific groups of genes (Miller et al., 2014).

In mammals, including humans, the fusion of the maternal and paternal gametes results in the formation of the single-cell zygote. Gametes are highly specialized cells, and at the stage of fertilization, each gamete has its own unique genomic and epigenomic composition, including specific chromatin structure and epigenetic modifications (Cantone & Fisher, 2013). During development, the zygote transforms from a single cell to a multicellular organism. The transformation of the zygote to a multicellular organism is mediated by cellular processes such as cell division, specification, and differentiation. Figure 2 shows a schematic representation of the cellular development of the zygote. Each cell type has a specific gene expression program, cellular morphology, and function, and they give rise to different cell types. Epigenetic regulation of gene expression is an integral part of this transformation.

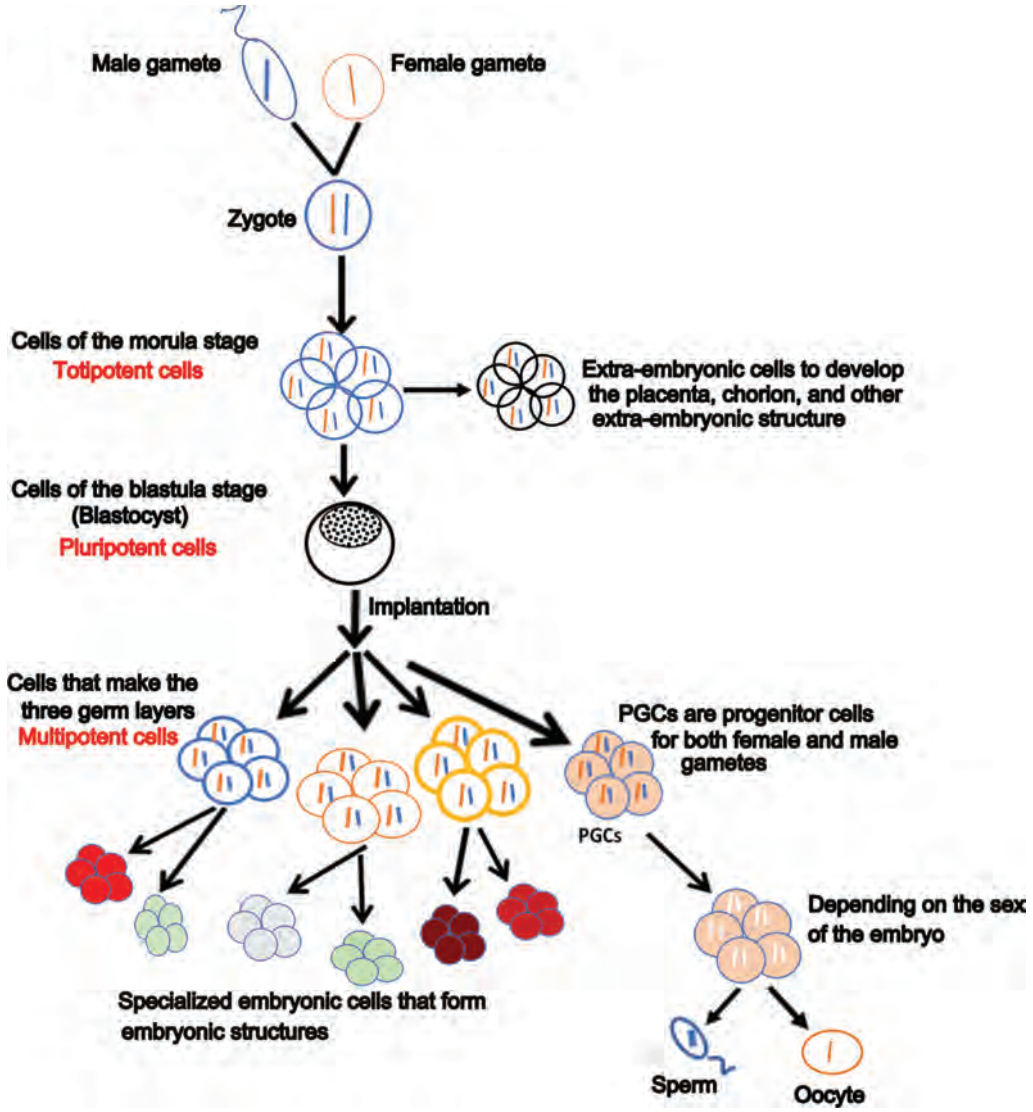

Figure 2. Schematic representation of cellular development in a human zygote.

The resulting zygote inherits the genomic DNA sequences from the paternal and maternal gametes. However, unlike genomic DNA sequences, the epigenome from the gametes is erased during the early stages of development and new programming is established. Epigenetic reprogramming during development is biphasic (Cantone & Fisher, 2013). The first phase occurs early during embryonic development before implantation, and the second phase occurs during gamete formation following sex determination of the fetus. In the first phase, the reprogramming of the epigenome is necessary to reestablish totipotency for cellular specification in embryonic tissues, and organogenesis. The second phase is necessary for the development of the sex-specific gametes. In this regard, epigenetic regulation during development is highly dynamic and mitotically inherited.

The process of rebuilding the embryonic epigenome is highly influenced by the environment and can be vulnerable to environmental insults. The effect of the environment on epigenetic modifications is discussed in the following section.

X-chromosome inactivation

A well-known example of an epigenetically regulated biological process is X-chromosome inactivation. Female mammals, including humans, inherit two X chromosomes. One chromosome in each cell becomes inactive during embryonic development. The inactivation of the X chromosome is a dosage-compensation process that occurs to ensure that the cells of females and males have the same effective dose of genes with loci on the X chromosome. This process occurs during early stages of embryonic development in females and is maintained in all somatic cells. However, due to embryonic epigenetic reprogramming during the development of sex-specific gametes, all female gametes will receive an active X chromosome (Sugimoto & Abe, 2007) (Figure 3A).

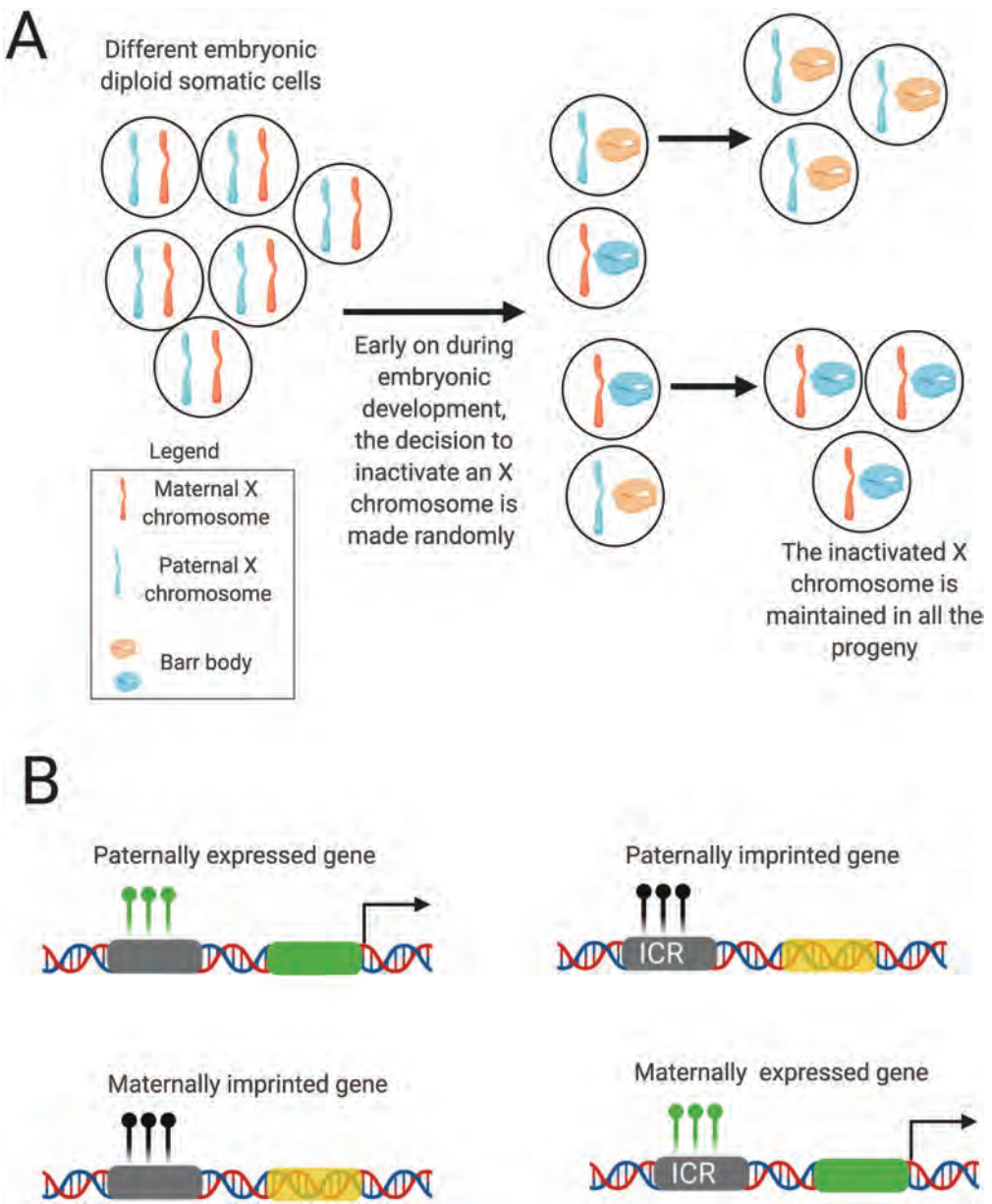

Figure 3. Schematic representation of two epigenetic phenomena, (**A**) X-chromosome inactivation and (**B**) genomic imprinting.

General features of the process of X-chromosome inactivation (Figure 3A) are as follows:

(1) It is an epigenetic phenomenon initiated by the binding of an RNA molecule called *xist*. The binding of *xist* recruits the epigenetic machinery to inactivate the selected X chromosome. Epigenetic marks rapidly accumulate on the X chromosome and convert it into inactive condensed chromatin. The condensed X chromosome is called the Barr body (Balderman & Lichtman, 2011).

(2) It is a random process. All females inherit a paternal and a maternal X chromosome, one of which will be inactivated randomly.

(3) Through the feature of epigenetic mitotic heritability, the progeny of each cell will have the same X chromosome inactive throughout the life of the female.

It has been over 50 years since Mary Lyon first proposed her X-chromosome inactivation hypothesis (Balderman & Lichtman, 2011). Although X-chromosome inactivation is well accepted as an epigenetic phenomenon, a detailed mechanistic understanding is still lacking. Specifically, the mechanisms for (1) how the paternal or maternal X chromosome is selected for inactivation and (2) how X-chromosome reactivation is achieved in the female embryo are still poorly understood. These are essential questions that await answers by future research.

Genomic imprinting

Genomic imprinting is a pattern of epigenetic inheritance that was initially discovered more than two decades ago in mammals. It is a process that derives monoallelic expression (i.e., expression of only one of the two alleles in diploid cells) of a select group of autosomal genes. These genes are differentially expressed depending on the parent of origin. Some genes are expressed if paternally inherited (paternally expressed and maternally imprinted), and some others are expressed if maternally inherited (maternally expressed and paternally imprinted). There are approximately more than 100 such imprinted genes in the human genome (Barlow, 2011; Ferguson-Smith, 2011; Ishida & Moore, 2013).

Expression of imprinted genes is tightly controlled by the methylation of a group of cis-acting regulatory elements called imprint control regions (ICRs). Imprinted genes routinely exist in clusters, and each cluster is controlled by its ICR (Figure 3B). Parent-allele-specific imprinting is mitotically heritable to the daughter cells; however, the epigenetic marks that regulate these genes must be reset during embryonic development by epigenetic reprogramming in each successive generation to establish parent-of-origin imprints.

Parent-of-origin imprint reprogramming can be a challenging concept for students; however, a simple pedigree showing multiple generations and an example of an imprinted gene, as illustrated in Figure 4, can help in deconstructing the concept. The pedigree in Figure 4 illustrates the imprint reprogramming for the IGF-2 gene located on chromosome 11, with the paternal chromosome colored in blue and the maternal chromosome in orange. IGF-2 has a fundamental role in fetal growth, and it is a maternally imprinted gene. Individuals in every generation inherit maternal chromosome 11 with IGF-2 allele controlled by methylated ICR and paternal chromosome with transcriptionally active IGF-2 allele controlled by unmethylated ICR. This process is feasible in mammals primarily due to reprogramming during gametogenesis. The epigenetic modification on the ICR region of imprinted genes stays constant during development until gametogenesis (Figure 2). In male and female embryos, the primordial germ cells receive the parent-of-origin imprint pattern on both paternal and maternal chromosomes. During gender specification and before sex-specific gamete formation, the epigenetic modifications on imprinted genes reset to ensure that all gametes produced by the embryo have the correct parent-of-origin imprint pattern. Therefore, regardless of whether the allele is on the paternal or the maternal chromosome, specific genes are always silenced in the egg, and others are always silenced in the sperm (Figure 4). As shown in Figure 4, the sister (II-2) and brother (II-3) in generation II receive the same combination of chromosome 11 from their parents, but each produces gametes with the correct parent-of-origin imprint pattern for IGF-2 gene for generation III.

The importance of genomic imprinting is best highlighted in the context of human diseases. Several human diseases have a link to genomic imprinting, such as Prader-Willi syndrome (PWS), Angelman syndrome, and Beckwith-Wiedemann syndrome (Rodrigues et al., 2014). The common causational factor among these genetic diseases is a loss of the parent-specific active allele. For instance, the cause of PWS is a deletion on the paternal chromosome 15, and the clinical symptoms are due to the absence of encoded genes on the deleted part of chromosome 15. Affected individuals receive a normal maternal chromosome 15, but these genes are maternally imprinted (Angulo et al., 2015) (Figure 3B). The pedigree in Figure 4 can be used to illustrate how generation III offspring can develop PWS even though none of the generation II parents is affected. PWS in generation III would be due to a de novo deletion of chromosome 15 during paternal gametogenesis.

Classroom activity

We have observed that students find the concept of epigenetic reprogramming for biallelic genes (i.e., genes that are expressed from

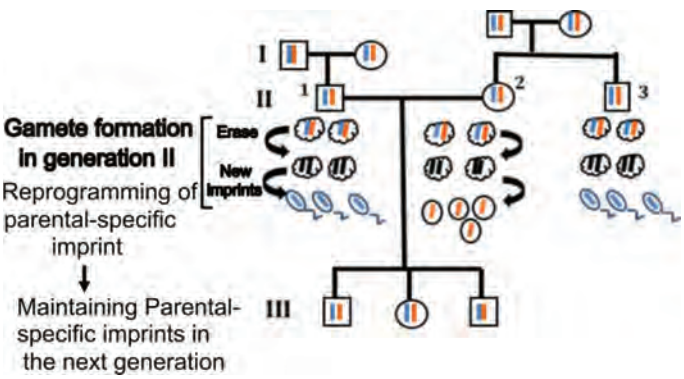

Figure 4. Schematic representation of a three-generation pedigree. The pedigree shows the inheritance of the IGF-2 gene on chromosome 11 across the three generations. The IGF-2 gene is maternally imprinted; therefore, the maternal allele is always silenced. In this example, generation II males and females receive a paternal active allele and a maternal imprinted allele. However, during their gamete formation, the parent-specific imprint is erased, and new imprints depending on the sex of the embryo are established, ensuring the parent-of-origin imprints in generation III.

both maternal and paternal alleles) and monoallelic genes during embryonic development hard to grasp. The activity (see the Supplemental Material available with the online version of this article) has been explicitly designed to help students grasp the fundamentals and significance of epigenetic modification during development. The objective of this exercise is to demonstrate how differential gene expression during development is regulated by DNA methylation.

○ The Effect of the Environment on Epigenetic Modifications

It is now unarguable that our genome makes us who we are. However, it is now increasingly accepted that in addition to our genome, our unique epigenome also contributes heavily to individuality (Tsompana & Buck, 2014). As a classic example, monozygotic twins with identical genomes can have vastly different traits, depending on their epigenomes (Castillo-Fernandez et al., 2014). How exactly is this established? Epigenomic differences affect our behavioral traits, overall health, and responses to diseases. Changes in environmental exposures directly drive differences in the epigenome, particularly in patterns of DNA methylation. Environmental factors affect patterns of DNA methylation during embryonic development, in the early years of childhood, and well into adulthood (Fraga et al., 2005). Such environmental influences on the epigenome can lead to substantial, long-lasting consequences.

Environmental Influences on Epigenetic Programing during Prenatal & Postnatal Development

The effect of the environment on the epigenetic program starts as early as in utero. The intrauterine environment may affect epigenetic programming during development, which may explain the epigenetic variations between monozygotic twins, which ultimately manifest as differences in personality traits and/or in risk for diseases such as cancer (Heyn et al., 2013). Several studies have demonstrated that many monozygotic twins show apparent behavioral differences in risk taking (Kaminsky et al., 2008; Bell & Spector, 2011). Monozygotic twins are also different in their susceptibility to diseases such as cancer (Heyn et al., 2013; Mucci et al., 2016). For instance, in a case published in 2012, one of a pair of monozygotic twins underwent a mutation during development in the BRCA1 gene, leading to multiple types of cancer throughout her life, whereas the other twin was both mutation free and cancer free (Galetzka et al., 2012).

Our overall understanding of the nature of the in utero environment and its effects on epigenetic programming in the fetus is continuously expanding. Many animal and human studies have now shown that the mother's behavior during pregnancy has effects on fetal DNA methylation that increase the risk for diseases later in life (Coussons-Read, 2013; Graignic-Philippe et al., 2014). One well-studied example is chronic stress. The chronic stress of the mother during pregnancy affects the fetus's stress response during childhood and adulthood (Coussons-Read, 2013). This effect is modulated by alteration in the epigenetic modification in the stress response system, such as glucocorticoid receptors (Palma-Gudiel et al., 2015).

A well-studied example of the effect of the environment on postnatal epigenetic modification is how parenting styles can influence behavior in children that can last into adulthood (University of Utah, n.d.). Several animal and human studies showing that high-nurturing parents raise high-nurturing offspring are summarized in a review by Maccoby (2000). It has also been shown by animal and human studies that anxious behavior in adulthood is linked to a low-nurturing environment during childhood (Weaver et al., 2004; Affrunti & Ginsburg, 2012).

○ Teaching Resources & Other Classroom Activities

We believe that understanding the core concepts of epigenetic regulation discussed above is a critical learning objective for students, and below we discuss specific teaching strategies to achieve this. Most of the concepts covered in this review can be used to design a curriculum specific to epigenetics, as a unit in a college biology or human genetics course. This epigenetics unit will be most useful to students who have basic knowledge of chromatin structure and regulation of gene expression from introductory biology courses. Pedagogical tools such as in-class activities are excellent in helping students unpack some of the more challenging concepts in epigenetics. Several examples are provided here and can be scaled down in complexity for lower-division or introductory college courses. Finally, we believe that this review can also aid secondary biology teachers in designing an epigenetic unit and integrating it into their current curriculum.

It is indeed hard to have one comprehensive instructional resource; therefore, we summarized additional resources from previous publications for easy access. For example, several multimedia teaching resources that cover the basics and various key aspects of epigenetic regulation are summarized in the informative review by Stark (2010). Since that review, a few additional teaching resources have been developed:

- The virtual genetic education center at the University of Leicester has provided access to teaching materials targeting either AP-level students or undergraduates, available at https://www2.le.ac.uk/projects/vgec/highereducation/epigenetics_ethics/material-for-teachers. Along with detailed teaching notes that primarily cover epigenetics of human diseases, short ready-to-use presentations and student worksheets are also provided.
- Another useful set of teaching notes is available at http://www.letsgethealthy.org/wp-content/uploads/2013/07/Teacher-Background-on-Epigenetics.pdf. In particular, we find the table summarizing key online resources highly useful.
- A very simple, but very informative and effective, video explaining the epigenome in cells is available at http://www.nature.com/news/epigenome-the-symphony-in-your-cells-1.16955.

Use of Models in the Classroom

Teaching models are great aids for conveying complex concepts. A simple teaching model, available at http://www.pbs.org/wgbh/nova/education/activities/3411_02_nsn.html, effectively illustrates the open vs. closed chromatin conformation. This model has been used in our classroom for many years and has helped students grasp and retain the core concepts effectively. The tools and materials required are readily available. The model can be constructed from three strands of simple laboratory rubber tubing and duct tape. We use colored pushpins to mark areas of DNA methylation when

exemplifying condensed chromatin. This simple model is a powerful strategy that has consistently helped convey the core concepts of the chromatin conformation in a classroom setting.

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