

Introduction

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To correctly read the information stored in our DNA genomes (the genetic code), cells must read another language that overlays it, **the epigenetic code**, which controls access to that information. A process such as transcription can only retrieve this information according to the access granted by the epigenome. The term epigenetics was coined in the 1940s by British embryologist and geneticist Conrad Waddington to describe “the interaction of genes with their environment...which bring the phenotype into being”. Now the term epigenetics (literally over or above genetics) refers to the extra layers of instructions that influence gene activity without altering the DNA sequence. There are three main components to the epigenetic code: (i) methylated cytosine residues in DNA¹; (ii) the range of post-translational modifications to the core histone proteins within the nucleosomes (referred to as the histone code)^{2,3}; and (iii) RNA molecules, often non-coding RNA⁴.

Small RNAs (sRNAs; 20–30 nt) guide the modification of DNA or histones at specific sites in the genome. Long non-coding (lnc) RNAs are associated with differential epigenetic marking of whole chromosomes (for example, Xist lncRNA guides the repression of one of the X-chromosomes in females) as well as specific imprinting (silencing) of individual genes on one of the two autosomes depending on its parental origin. Imprinting often results in one active allele and one repressed allele and is very important for development. Given that our genomes are ubiquitously transcribed on both strands of DNA, lncRNA, including sRNA and antisense transcripts, are likely to play a more central role in epigenetic regulation than is currently appreciated.

DNA methylation occurs primarily at cytosines when followed by guanosine (CpG methylation) to yield 5'-methylcytosine. Areas of the genome free from CpG methylation are known as CpG islands and are often found at gene promoters. CpG methylation tends to be associated with regions of the genome that are less transcriptionally active, and methylation changes to reflect these alterations to gene expression.

Post-translational modifications to the two copies of the four core histones in a nucleosome include reversible lysine and arginine methylation, lysine acetylation, lysine ubiquitylation and serine/threonine/tyrosine phosphorylation. The combination of modifications to nucleosomes may alter the activity of the DNA wrapped around them. For example, particular modifications might mask a transcription factor (TF)-binding site in the DNA, resulting in competition between a nucleosome and TF for occupancy of that site. The actions of chromatin-remodelling ATPases, enzymes that use ATP hydrolysis to move or remove nucleosomes, make occupancy by the TF more or less likely⁵. Histone modifica-

tions also act to recruit proteins, for example the repressive polycomb (Pc) complex, leading to gene silencing, or TFs that direct the assembly of the pre-initiation complex at the promoter of a gene destined to be expressed⁶. The language of histone modifications is complex, but it is clear that context defines the function associated with a modification such as Lys⁴ methylation (K4me) on histone H3. K4me can be found at the 5' region of active genes. However, K4me is also found at poised genes (an intermediate state, neither active nor repressed) as part of a bivalent mark with Lys²⁷ methylation (a modification usually associated with repressed genes). Bivalent marks are commonly found at promoters in embryonic stem cells and are important for maintaining pluripotency. Promoters carrying a bivalent mark become activated during development, after removal of K27me, to produce the TFs that determine cell fate.

An epigenetic state may remain for the life of a cell, surviving cell division and lasting for many generations, but it is also plastic and can change in disease or during aging. Thus we are more than our genes, as the genetic code in the DNA (our genotype) combines with the epigenetic code on histones and the DNA (our epigenotype) and inherited RNA and protein, for example prions, to define our external characteristics (our phenotype). This is strikingly illustrated in the following examples.

In a disease such as cancer, tumour cells undergo a major disruption of their DNA methylation and histone-modification patterns. The aberrant epigenetic landscape leads to repression of genes that normally control cell growth (tumour-suppressor genes) by hypermethylation of the DNA and an altered histone code. In addition, genes that would normally be silenced become hypomethylated and decorated with a histone code permissive for transcription.

Some individuals have a genetic predisposition to cancers of the breast or prostate. However, monozygotic twin studies reveal that not every individual who carries such a mutation will go on to develop the cancer⁷. Only one in three sets of identical twins showing a genetic predisposition will both go on to develop prostate cancer. For the BRCA-dependent breast cancers, the number is even lower, at around 1 in 10. As identical twins age, their epigenomes change and diverge, strongly implicating an epigenetic basis controlling the development of these diseases⁸. Our chromosomes adopt higher-order structures, for example an enhancer–promoter interaction over tens of kilobases, that can be revealed by techniques such as chromosome conformation capture (3C) that analyse the DNA involved in these interactions. 3C interactions change as the epigenome changes. These ‘epigenetic switches’ can be used to diagnose a cancer long before other symptoms are evident.

In addition to cancer, it is clear that many other diseases, such as multiple sclerosis, Type 1 and Type 2 diabetes, schizophrenia and dementia, also have a strong epigenetic basis to their development. Epigenetics has become a central topic in several neurobiology fields such as long-term memory, drug addiction, and several psychiatric and mental disorders.

The link between epigenetics and our brains markedly influence us by strengthening and maintaining the synaptic connections required for long-term changes in behaviour. This is illustrated by post-partum maternal care, in rats known as ‘licking and grooming’, that influences brain development in the pups (particularly the density of synapses in the hippocampus), reduces anxiety and improves resistance to stress. At day 1 after birth, DNA-methylation patterns are similar in pups born to caring or non-caring mothers. Within the first week of life, changes develop in the patterns and levels of DNA methylation, depending on the care received, and remain stable thereafter. This is a typical epigenetic trait as it is stable and inheritable, yet dynamic and plastic as it can be reversed. Rats deprived of maternal care can have their anxiety reduced by flooding their brains with chemical activators, inhibitors or nutrients such as methionine that directly alter the epigenome and patterns of gene expression in the brain. Similar effects of maternal behaviour in primates and humans on the development of their offspring are likely to be manifested through the epigenome. Abusive behaviour in macaques can be transmitted from mother to daughter, and is manifest in many behavioural and neurobiological characteristics. Similarly, in humans, lack of parental care can contribute to subsequent criminal behaviour, and is a risk factor for depression, adult antisocial personality traits, anxiety disorders, drug abuse, obsessive–compulsive disorder and attention-deficit disorders⁹. Individuals who

received high levels of maternal care tend to have high self-esteem and low anxiety.

The diet of a pregnant mouse can influence the phenotypes displayed by her offspring, such as coat colour and propensity for obesity, and this is reflected in a distinct epigenome, both in patterns of DNA methylation and histone modifications, on an otherwise identical genome. These and other experiments raise the possibility that diet affects the phenotype by shaping the epigenotype, and that epigenetic damage may be partly reversed by nutrition that directly influences levels of histone acetylation or DNA and histone methylation, particularly l-methionine, folates and sulforaphane. Your mother was right: broccoli is good for you! The link between diet and the epigenome is not surprising given that the cofactors for processes such as acetylation (acetyl-CoA), methylation [S-adenosylmethionine (SAM)] and deacetylation (NAD⁺) are central intermediates in metabolism or synthesized from essential amino acids. Thus diet directly influences epigenetic modifications and plays a major role in determining behaviour and aging.

Given that the epigenome plays such a central role in disease, development and cell fate, a great deal of groundbreaking research focuses on understanding its influence on these processes. Chromatin immunoprecipitation (ChIP), used to map histone modifications, and bisulfite mapping, used to map sites of DNA methylation, coupled with high-resolution tiling arrays and the rapidly improving deep sequencing technologies are being harnessed to define the human epigenome in various tissues and developmental stages, and the environmental factors that influence it. In this special issue of *The Biochemist*, edited by students from the University of Oxford, seven researchers provide a flavour of their current research in this exciting field. ■

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