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Introduction

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Epigenetics: the first 25 centuries

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Epigenetics is a natural progression of genetics as it aims to understand how genes and other heritable elements are regulated in eukaryotic organisms. The history of epigenetics is briefly reviewed, together with the key issues in the field today. This themed issue brings together a diverse collection of interdisciplinary reviews and research articles that showcase the tremendous recent advances in epigenetic chemical biology and translational research into epigenetic drug discovery.

This article is part of a discussion meeting issue 'Frontiers in epigenetic chemical biology'.

1. Introduction

Within the European Union, the COST programme funds scientists to set up interdisciplinary research networks. Since 2015, one of these initiatives is the Action CM1406 'Epigenetic Chemical Biology' chaired by myself. We currently have over 150 principal investigators from 36 countries in our network, coming from diverse backgrounds such as academia in universities and research institutes as well as industrial scientists working in small and medium enterprises and large multinational pharmaceutical companies. As part of our activities, we thought it would be an excellent idea to organize an international conference and are indebted to the Royal Society for giving us this opportunity. The resulting Hooke discussion meeting 'Frontiers in epigenetic chemical biology' on the 22 and 23 May 2017, and the following scientific meeting 'Readers, writers and erasers' on the 24 and 25 May were a great success, with an excellent line-up of international experts in the field and enthusiastic participation from the audience. Although this themed issue cannot recreate the full experience of being there, the following reviews and research publications will provide a flavour of the excitement around epigenetic chemical biology and showcase some of the topics that were discussed.

2. What is heritable and what is not?

Epigenetics as a science is currently in vogue and has attracted intense scientific efforts as well as media attention. It is rooted, however, in the dawn of scientific inquiry into reproduction and how form and function is encoded and transmitted between generations. In his 350 BC treatise *Περὶ ζῴων γενέσεως* (*On the generation of animals*) [1], the Greek philosopher Aristotle discoursed on this topic at length, including speculation on whether pleasure in the sexual act was a precondition for producing the life force:

> 'Does the female contribute nothing whatever to generation, merely providing a place where generation may happen; or does it contribute something else, and if so, how and in what manner does it do so?' ... 'Very often the female conceives although she has derived no pleasure from the act of coitus; Women sometimes derive pleasure from it comparable to the male and also produce a fluid secretion. This fluid, however, is not seminal; There is a discharge from the uterus, which though it happens in some women does not in others. Speaking generally, this happens in fair-skinned women who are typically feminine, and not in dark women of a masculine appearance. This discharge is sometimes on quite a different scale from the semen discharged by the male, and greatly exceeds it in bulk.'

While the discussion appears antiquated from our modern viewpoint, Aristotle was ahead of his time in describing the female ejaculation which was only rediscovered recently! Meanwhile, he correctly identified the male semen as an important contributor to heredity. Subsequent generations of scientists refined these hypotheses, culminating in the elegant experiments by Mendel, and Bateson's coinage of the new word genetics (from the Greek '*γένεσις*' for 'origin') in the early twentieth century. Genetics was able to provide an accurate and mathematically precise depiction of heredity, achieved without actually knowing the physical manifestation of a gene.

The next major revolution came with the unravelling of the three-dimensional structure of proteins and nucleic acids. Knowledge at the molecular level brought about a sophisticated understanding of heredity, and more recently the ability to comprehensively curate an organism's heritable elements through whole genome sequencing. A central dogma became popular in biology that equates life with the sequence DNA → RNA → protein. While the central dogma is fundamentally correct, it is a reductionist statement and clearly there are additional layers of subtlety in 'how' it is accomplished. Not surprisingly, the answers have turned out to be far more complex than originally imagined, and we are discovering that the phenotypic diversity of life on Earth is mirrored by an equal diversity of hereditary processes at the molecular level. This lies at the heart of modern day epigenetics, which is classically defined as the study of heritable changes in phenotype that occur without an underlying change in genome sequence. The central dogma's focus on genes obscures the fact that much of the genome does not code for genes and indeed such regions were derogatively lumped together as 'junk DNA'. In fact, these non-coding regions increase in proportion as we climb up the evolutionary tree and clearly play a critical role in defining what makes us human compared with other species. Another key point in the post-genomic world is the dynamism of genetic material. The genomic DNA is not static, with a proportion of cytosine residues undergoing chemical modification to 5-methylcytosine. The occurrence of 5-methylcytosine is sufficiently high for it to be referred as 'the fifth base in DNA' and the story is further complicated by the recent detection of further oxidized forms of 5-methylcytosine, viz. 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine. These DNA modifications provide the clearest evidence of epigenetic heritable transmission, although their relative importance varies from species to species. How are nucleic acid modifications introduced, how are they recognized or targeted, and to what extent are they heritable?—these are all burning issues in epigenetics at the moment. In this issue, Klimašauskas [2] describes studies with the doubly methylated base N^4,5-dimethylcytosine using enzymes from *Escherichia coli*, while the potential for epigenetic modifications to influence alternative splicing is reviewed by Koziol [3]. Gene-specific editing *in vitro* and *in vivo* is a hot topic, and the different methodologies available are compared by Rots [4]. One of these, the selective recognition of *N*4-methylcytosine by engineered transcription activators, is reported by Summerer [5]. While DNA is relatively faithful to the four Watson–Crick bases and variants are relatively rare, the opposite is true with non-coding RNAs, which rarely consist of only the canonical adenine, guanine, cytosine and uracil bases. In epigenetics, non-coding RNAs are less well studied than DNA and histone proteins but they clearly have important functions and are potentially heritable. Berdasco [6] explores the importance of long non-coding RNAs in cancer, and Jeronimo [7] reports the expression of a specific microRNA in germ cell tumour subtypes.

3. Which gene and when?

Besides the question of heritability, the other major concern of epigenetics is the process of regulated gene expression in time and space in multicellular eukaryotic organisms. Once again, this dates back to Aristotle's philosophical treatise [1]:

> For nobody would put down the unfertilized embryo as soulless or in every sense bereft of life (since both the semen and the embryo of an animal have every bit as much life as a plant), and it is productive up to a certain point. That then they possess the nutritive soul is plain (and plain is it from the discussions elsewhere about soul why this soul must be acquired first). As they develop they also acquire the sensitive soul in virtue of which an animal is an animal. For e.g. an animal does not become at the same time an animal and a man or a horse or any other particular animal. For the end is developed last, and the peculiar character of the species is the end of the generation in each individual.

In other words, Aristotle distanced himself from the prevailing notion of preformation in which the adult features are fully formed in the zygote and instead advocated a gradual development from an undifferentiated origin which he called 'epigenesis' or 'after genesis'. Despite this ancient history, the term fell out of favour and was forgotten for over 2000 years until it was resuscitated by Conrad Waddington [8]:

> Many geneticists have recognized this and attempted to discover the processes involved in the mechanism by which the genes of the genotype bring about phenotypic effects. The first step in such an enterprise is—or rather should be, since it is often omitted by those with an undue respect for the powers of reason—to describe what can be seen of the developmental processes. For enquiries of this kind, the word 'phenogenetics' was coined by Haecker. The second and more important part of the task is to discover the causal mechanisms at work, and to relate them as far as possible to what experimental embryology has already revealed of the mechanics of development. We might use the name 'epigenetics' for such studies, thus emphasizing their relation to the concepts, so strongly favourable to the classical theory of epigenesis, which have been reached by the experimental embryologists. We certainly need to remember that between genotype and phenotype, and connecting them to each other, there lies a whole complex of developmental processes. It is convenient to have a name for this complex: 'epigenotype' seems suitable.

Thus, Waddington, needing to distinguish between Haecker's 'phenogenetics' and studying the mechanisms of differentiation, returned to the long disregarded 'epigenetics' of Aristotle. In Waddington's time, it was impossible to gain a detailed picture of how epigenetics is actually accomplished. The great enlightenment of epigenetics in the last 20 years comes from the discovery of nucleosome dynamics and how reversible histone modifications influence gene transcription. As stated by John D. Watson [9], the co-discoverer of the DNA double helix: 'What determines whether a given piece of DNA along the chromosome is functioning, since it's covered with the histones? You can inherit something beyond the DNA sequence. That's where the real excitement of genetics is now.' Meanwhile, for a concise definition of epigenetics, it is hard to improve upon the pithy description by Medawar & Medawar [10] in their entertaining dictionary of biology, 'Genetics proposes; epigenetics disposes.'

4. Altered states through epigenetics

How do eukaryotic organisms adapt their hard-wired genetic code to the specific features of their environment? The answer lies in epigenetics: while the genome remains invariant, the epigenome is dynamic and responding to external cues. Regardless of whether any of these epigenetic changes are heritable, they play a vital role in the fitness of the individual, both for benefit and for harm. Gerhauser [11] and Tellez-Plaza [12], for example, respectively report on the effects of microbial gut metabolites and metal exposure on the epigenome. Virtually every human disease has an epigenetic component and particularly so in the complex multifactorial conditions that are more pronounced with ageing. Herein lies one of the most exciting aspects of epigenetics: If the process is dynamic, it is potentially possible to reprogramme a defective epigenome back to a healthy state. Within the last decade, a handful of so-called small molecule 'epidrugs' that inhibit specific epigenetic pathways were approved by the FDA for the treatment of haematological cancers. Epigenetic therapy is in fact 50 years old since the earliest clinical trials of 5-azacytidine began in 1967, long before its mechanism of action as a DNA methyltransferase inhibitor was discovered.

Further progress will require a molecular toolbox of high affinity small molecules that serve as probes of epigenetic pathways in cells and *in vivo*, aid in their target validation and provide leads for optimization to clinical candidates. In this venture, selectivity is of paramount importance to enable the dissection of function between specific isoforms of epigenetic protein families. From a therapeutic angle, it is likely that an isoform-selective epidrug will be useful in maintaining patient benefit while reducing the side effects observed with a pan-inhibitor. The discovery of such selective compounds was a major focus at our meetings, and a review of cofactor targeting for epigenetic enzymes is provided by Ganesan [13]. In histones, the most well studied post-translational modification is acetylation, which is removed by the zinc-containing histone deacetylases (HDACs). Ganesan [14] reports hydroxamic acid-containing HDAC inhibitors that are primarily selective for HDAC6. Hydroxamic acids are widely found in natural products and synthetic compounds as a metal-binding unit, and a biosynthetic example is described by Challis [15]. Complementary to the HDACs, the sirtuins perform an identical deacylation reaction using NAD^+ as cofactor rather than zinc. Both Jung [16] and Yoshida [17] report the discovery of selective sirtuin2 inhibitors. Besides acylation, methylation is the other major histone post-translational modification investigated for drug discovery. Inhibitors of both the lysine and arginine methyltransferases (KMTs and PRMTs) are in clinical trials, as are inhibitors of the reversing lysine demethylases (KDMs), and the race is on for the first drug to receive approval. The KMT field is reviewed by Copeland [18] who led Epizyme's pioneering efforts in this area, and new examples of EZH2 inhibitors are reported by the Mai group [19]. The related PRMTs, for which inhibitor development is less advanced than with KMTs, are the subject of the paper by Arimondo [20], while Schofield [21] reports research on the Jumonji C demethylases.

5. Epigenetic chemical biology

As evidenced by this themed collection, epigenetic chemical biology has already delivered much and promises to impact our lives even more in the future. A cross-disciplinary approach that integrates chemistry, biology and medicine is necessary for success and we hope these articles will inspire others to work in this direction. It would be impudent for a chemist like myself to attempt a definition of epigenetics. Nevertheless, I can offer an *operational* definition of chemical epigenetics that I trust will meet with approval by others working in this exciting field:

Chemical epigenetics is the study of the structural modifications of nucleic acids and histone proteins and how these influence gene expression.

Data accessibility. This article has no additional data.

Competing interests. I declare I have no competing interests.

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