Epigenetics equals chemical biology

Akane Kawamura[1,2]* & Ganesan[3]*

[1] Chemistry – School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK
[2] Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Oxford, OX1 3TA, UK
[3] School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

*Corresponding authors

One of the things we share in common is our job title of Professor of Chemical Biology. But what exactly is chemical biology? Although a precise definition is debatable, most will agree that it involves the application of chemical principles and experimental techniques to address questions pertaining to biology. The relatively new science of epigenetics is a good example of the power of this cross-disciplinary approach. The English physician William Harvey was the first to formally describe 'epigenesis' in his 1651 work '*Exercitationes de generatione animalium*' (Exercises on the Generation of Animals), although the concept dates back to the Greek philosopher Aristotle. Harvey made a distinction between *metamorphosis* (corresponding to an embryo arising in a fully formed state) and *epigenesis* (corresponding to an embryo undergoing gradual development) and even today it is worthwhile to read his description: 'Some, out of a material previously concocted, and that has already attained its bulk, receive their forms and transfigurations; and all their parts are fashioned simultaneously, each with its distinctive characteristic, by the process called metamorphosis, and in this way a perfect animal is at once born; on the other hand, there are some in which one part is made before another, and then from the same material, afterwards receive at once nutrition, bulk, and form: that is to say, they have some parts made before, some after others, and these are at the same time increased in size and altered in form. The structure of these animals commences from some one part as its nucleus and origin, by the instrumentality of which the rest of the limbs are joined on, and this we say takes place by the method of epigenesis, namely, by degrees, part after part; and this is, in preference to the other mode, generation properly so called.'

In the 20th century, these ideas were further refined by Conrad Waddington and eventually gave rise to our modern view of epigenetics as the study of heritable changes in a biological phenotype without an underlying change in genotype. While it is reasonable to postulate the existence of such phenomena, further progress beyond Aristotle's or Harvey's conjectures only became possible after a deeper knowledge of biology at the molecular level. The identification of the reversible modifications in chromatin and the methods for their detection, sequencing, quantification and imaging - these are all aspects that lie squarely within the remit of chemical biology and are discussed in contributions to this issue. Understanding how these structural alterations affect biomolecular form and function and thereby influence gene transcription is another topic embraced by chemical biologists and extensively chronicled in the accompanying reviews. Finally, the design and discovery of molecules that perturb epigenetic pathways is an enterprise of vigorous scientific activity undertaken by chemical biologists and medicinal chemists alike. This last topic is of tremendous value for both basic and translational epigenetics and has already impacted significantly

upon human healthcare. Eight drugs with an epigenetic target have successfully received regulatory approval for the treatment of hematological and breast cancer, while many other compounds are in various stages of clinical and preclinical development.

The issue of heritability or otherwise is often less critical for chemical biologists working in epigenetics. What we are interested in is the set of chemical alterations of nucleic acids and proteins, and how the resulting write-read-erase cycles modulate cellular states and transitions from one state to another. DNA methylation and histone acetylation, for example, are the two most widely studied epigenetic processes that result in transcriptional repression and activation respectively. However, in reality, non-heritable RNA is much more extensively modified than genotypic DNA, while reversible acetylation occurs in thousands of proteins in addition to histones. The underlying chemistry remains the same whether it takes place in chromatin or outside the nucleus and there is much to be gained by a holistic approach that focuses on pathways and their consequences instead of heritability. Meanwhile, in clinical therapy, patients treated with epigenetic drugs are unlikely to be worried about the possibility of transmitting the resulting epigenetic reprogramming to their future offspring. Although the current applications focus on life threatening diseases where such issues are less of a concern, there will come a time when we have to grapple with these and other philosophical questions and the ethical implications of epigenetic manipulation for society.

In this themed issue, we have gathered together a collection of reviews in which leading figures provide a timely review of the state of the art related to their area of expertise in epigenetic chemical biology. The authors have often made seminal contributions themselves and discuss the background and context of these discoveries as well as distil the most recent developments from around the world. In some cases, there is unavoidable overlap between one review and another. However, due to the difference in emphasis and viewpoints between authors, the material is never a mere duplication.

Sir Shankar Balasubramanian, a pioneer in the area of next generation sequencing and chemically modified DNA, and colleagues critically review our current understanding of the biological functions of modified DNA bases including recent discoveries such as the DNA methyltransferase isoform DNMT3c and the TET homolog CMD1. The major techniques available for modified base identification such as LC-MS, sequencing and editing are described. One of the most exciting applications of the technology lies in the potential for single cell DNA and RNA sequencing, as reviewed by Oppermann *et al*. The advances in this field are recounted, and the methods available are compared and contrasted. Data integration and comparison of datasets between experiments is an important consideration which is extensively discussed.

For epigenetic ‘writing’ to have a functional consequence, binding partners that ‘read’ these structural modifications are necessary. The extensive possibilities for domain crosstalk between protein reader domains are covered by Fujimori *et al*. The ZZ domains are emerging as important readers that modulate histone acetylation, while PHD domains are involved in the recognition of histone acetylation, crotonylation and methylation. Other topics that are described include the ubiquitin-dependent activation of the DOT1L and Set1 histone methyltransferases, the interplay between Tudor domains and Jumonji C demethylases and the targeting of PRC1 via its component protein EED. The review by Sbardella and Ciulli *et al.* summarises popular chemical biology techniques that can be used to investigate reader domains. The authors

discuss the ‘bump and hole’ studies developed by Ciulli as well as other approaches such as the use of synthetic peptide ligands, photolabeling, click reactions, fragment-based lead discovery and bivalent ligands.

Schofield *et al.* highlight one of the important links between energy metabolism and epigenetics. The enzyme isocitrate dehydrogenase (IDH) is frequently mutated in human cancers, resulting in imbalance between the metabolic pools of 2-oxoglutarate and D-2-hydroxyglutarate. These changes directly affect the epigenetic Jumonji C and TET enzymes that employ 2-oxoglutarate as a co-substrate. The cancer biology associated with elevated D-2-hydroxyglutarate is discussed, together with the progress in targeting IDH variants via medicinal chemistry.

Since its inception, the CRISPR/dCas9 technique has become the most widely used method for genome editing *in vitro*, in animal models and in proof-of-concept studies in humans. Rots *et al.* review the many recent applications of CRISPR/dCas9 to the locus-specific targeting of epigenetic enzymes. Examples involving local reprogramming of DNA and histone methylation and histone acetylation reveal the utility and potential of this approach compared to genome-wide editing. Further refinements involving conditional recruitment and small molecules are also discussed.

Several articles focus on the applications of epigenetic chemical biology to drug discovery. Jung *et al.* review the implementation of PROTACs (proteolysis targeting chimeras) to epignetic targets. Unlike traditional small molecule drugs that work by reversible or irreversible binding to their target, PROTACs are heterobifunctional molecules that bind to a protein of interest (POI) as well as a ubiquitin E3 ligase. The resulting ternary complex then promotes ubiquitination of the POI, leading to its selective degradation by the proteasome. Current approaches to PROTAC linker design, which is critical for the formation of a functional and catalytically competent ternary complex, are discussed followed by examples that target bromodomains, HDACs, sirtuins and PCAF/GCN5. PROTACs can be considered a subset of the more general concept of multitargeting agents. In their review, de Lera and Ganesan discuss the synthetic strategies towards epigenetic multitargeting and the five examples currently in clinical trials that combine HDAC or LSD1 inhibtion with additional mechanisms of action. Many preclinical examples have appeared within the last three years and these are reviewed together with future prospects for the field.

Mai *et al.* update their earlier 2017 review on the application of HDAC inhibitors to the treatment of parasitic disease. Recent developments involving *Plasmodium*, *Schistosoma*, *Trypanosoma* and *Leishmania* are discussed in detail. In addition to inhibitors of the zinc-dependent HDACs present in the parasite species, the coverage is expanded to include other epigenetic players such as HATs, sirtuins and bromodomains. In a wide-ranging contribution, Berdasco *et al.* discuss three important modalities of epigenetic therapy: multitargeting, synthetic lethality and combination therapy. The section on dual hybrids and multitargeting focuses on clinical applications and complements the more extensive reviews by Jung, de Lera and Ganesan mentioned above. This is followed by a discussion of synthetic lethality to address epigenetic loss-of-function mutations such as the use of the DOT1L methyltransferase inhibitor pinometostat in MLL-fusions and EZH2 inhibitors to target SWI/SNF mutations. There is an extensive discussion on the addition of epidrugs to chemotherapy to reduce tumor resistance, with a particular emphasis on newer biological immunotherapy agents such as PD1/PDL1 ligands and CART.

We very much hope that you will enjoy reading these articles as much as we did.

In addition, we would like to bring your attention to two other relevant articles that have recently appeared in *Current Opinion in Chemical Biology*:
In the Synthetic Biomolecules issue, Nakatsu, Hayashi and Okamoto review the methods for chemical synthesis of histone proteins, which is an important tool for the generation of histones with site-specific post-translational modifications.
(https://doi.org/10.1016/j.cbpa.2020.04.016)
In the Next Generation Therapeutics issue, Richart and Margueron review the therapeutic targeting of histone methyltransferases, which is particularly timely with this year's FDA approval of the methyltransferase inhibitor tazemetostat.
(https://doi.org/10.1016/j.cbpa.2019.11.009)