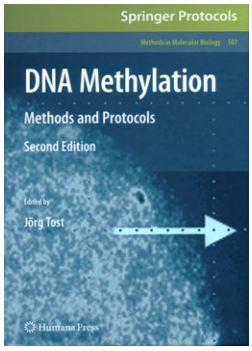
Getting to grips with DNA methylation



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DNA Methylation: Methods and Protocols (Methods in Molecular Biology Vol. 507, Second Edition)

Edited by Jörg Tost

Humana Press (2009) 330 pages
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In molecular terms, epigenetics is concerned with the study of chromatin organisation in the eukaryotic nucleus, with respect to the regulation of gene expression during the development of plants, fungi, insects and animals through embryogenesis to adulthood, and in disease states. A major intellectual foundation for the current intense study of epigenetic processes was the proposal in 1975 by Robin Holliday and John Pugh (Holiday and Pugh, 1975), and independently by Arthur Riggs (Riggs, 1975), that 5-methylcytosine (5mC) might have an important role in controlling gene activity. This expectation has since been shown in many organisms, and the molecular basis for the patterns of DNA methylation observed in different developmental or disease states is the focus of much deliberation. Questions revolve around, for example, the hierarchical interplay between DNA and histone-modifying activities, the contribution of non-coding RNAs, the generation of altered patterns of DNA methylation in cancer cells, the identification of DNA demethylases (clear in plants but more obscure in animals), and the role of the recently identified 5-hydroxymethylcytosine (5hmC) modification in animals (Kriaucionis and Heintz, 2009; Tahiliani et al., 2009).

Although epigenetics is much more than DNA methylation, it has been a popular initial entry point into the field owing to its relative ease of study and to the simple molecular models that explain its function (gene repression), as compared with the analysis of chromatin-based modifications. In vertebrates, DNA methylation occurs at the fifth position of cytosine in CpG dinucleotides in most tissues, but recently high levels of 5hmC have also been detected in embryonic stem (ES), Purkinje and granule cells (Kriaucionis and Heintz, 2009; Tahiliani et al., 2009). The functional significance of 5hmC is uncertain, but it may abrogate the biological functions of 5mC (Valinluck et al., 2004; Valinluck and Sowers, 2007). In general, the presence of DNA methylation (5mC) at regulatory sequences is associated with gene repression in vitro and in vivo.

DNA Methylation Protocols is purportedly a second edition but a comparison of the Table of Contents of each version highlights that there are few chapters or themes in common between the two books. This is partly a reflection of how much the field has advanced, but also a reflection of the reported presence of flawed techniques in edition 1 (see Smith, 2003). One other comment is that the protocols listed are grounded in the analysis of DNA methylation in vertebrates. Many screening and analytical protocols were developed in plants, insects and yeast. It seems a pity that no cross-referencing is presented in the second edition to emphasize common themes and differences among, and the advantages of, different model systems. The book's focus is firmly on enriching for or mapping 5mC itself, with few references made to the biology of the cytosine methyltransferases or to their functional roles in different developmental or disease settings.

This is an excellent set of protocols that are clearly laid out with a relatively common organization

With the above (and probably over-harsh) caveats in mind, this is an excellent set of protocols that are clearly laid out with a relatively common organization, which end with useful notes on background information and with trouble-shooting tips. It is divided into multiple sections on global, whole-genome, gene-specific and special application analysis. The techniques themselves can be divided further into how to enrich for methylated DNA for subsequent analysis or to directly analyse 5mC levels, globally and at specific locations. I first passed the book around the lab and asked: 'are the protocols easy to follow and similar to the methods we employ?' The answer was a resounding 'yes'. The standout chapters for us in the genome section were on methylated DNA immunoprecipitation (MeDIP), the MIRA (methylated CpG island recovery assay) technique for enriching for methylated DNA and the HELP assay. The HELP (*Hpa*II tiny fragment Enrichment by Ligation-mediated PCR) technique is able to detect cytosine methylation throughout the genome, including at every promoter region, thus allowing the quantification of locus-specific changes; for example, in cancer-derived DNAs. For screening, it is a bit daunting that up to eight MeDIPs assays are required to provide enough material for screening arrays and that the 5mC monoclonal antibody requires at least five co-methylated CpGs on the same strand, suggesting that the linear detection range of this antibody is limited for low-density methylated sequences. Nonetheless, the application of MeDIP has provided excellent initial 5mC profiling of mammalian genomes. Potentially, the MIRA technique provides a complementary approach that uses full-length MBD2 (methyl-CpG binding domain protein 2) and a co-factor [MBD3L1 (methyl-CpG binding domain protein 3-like 1)] to enrich for double-stranded methylated DNA that might have as few as two methyl groups. It should be noted that both of these techniques have been commercialized as kits, which might be useful to the novice user.

In the Introduction, it is suggested that array-based approaches may provide hypothesis-free driven research. I think this is an error, as array-based approaches are circumscribed by the quality and technical range of the array, with subsequent interpretation being highly dependent on the underlying hypothesis. This is clearly emphasized in the development and use of the HELP technique. Another chapter of note in this section is the use of the pharmaceutical inhibitor 5-aza-cytidine for cell line

demethylation and the subsequent screening of demethylated cells using expression arrays. The maintenance methyltransferase Dnmt1 becomes bound to DNA and is inactivated when 5-aza-cytidine is incorporated into CpG sites opposite a methylated CpG site on the template strand, leading to a rapid, passive loss of DNA methylation in cells and to the ectopic expression of genes that depend on Dnmt1 and DNA methylation for silencing.

Much of the gene-specific methylation analysis uses the bisulphite sequencing technique (originally developed by Frommer and colleagues (Frommer et al., 1992; Clark et al., 1994), which leads to the conversion of cytosine residues to uracil residues, but leaves 5-methylcytosine residues unaffected. This technique is comprehensively outlined in Chapter 14. A low-cost assay is also discussed in Chapter 20, which outlines methyl-sensitive restriction digestion in combination with real-time PCR for the quantification of methylation levels at specific sites. Recently, it has been noted that the potential presence of 5hmC in genomic DNA may challenge the interpretation of data using the bisulphite method, and this may need to be kept in mind in the future (Loenarz and Schofield, 2009). The last section contains protocols for analyzing methylated circulating DNA from patients, which might be useful for biomarker research and for subsequent whole genome analysis. Evidence of the speed of technological development is that there are no sections on how deep-sequencing protocols might be incorporated into the assays discussed. Overall, this is a practical book to have on the bench to determine genome and locus-specific DNA methylation levels. But the question remains, what does it all mean?

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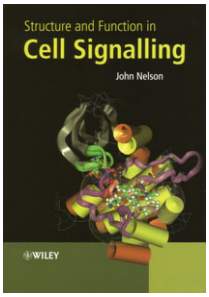
Helpful introduction to signal transduction



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Structure and Function in Cell Signalling

By John Nelson

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When studying cell signaling, it is easy to get overwhelmed with the complexity of each pathway and with the crosstalk between them. Teaching the basics of cell signaling therefore requires much simplification to make sense out of the chaos of signal transduction maps that, as John Nelson, the author of *Structure and Function in Cell Signalling*, points out, look like "inscrutable electronic circuits". Is it possible to write a book on signaling that embraces the complexity of the topic without overwhelming the reader? One might conclude that almost any effort would deliver something either too simplistic or destined to go quickly out of date. Not so. *Structure and Function in Cell Signalling* is not a collection of chapters written by experts in the field, and I think that this is a saving grace. Rather, this book is written from the perspective of a single author who is both a researcher and a teacher in the field. This gives it a cohesive storyline that links the topics together and provides the reader with much more continuity than is generally achieved in the usual 'edited by' collection of topics.

The history of how the cell signaling field developed is interspersed into the narrative of the book and provides a glimpse into some of the accomplishments of major figures in the early days of the field, such as Krebs, Fischer, Rodbell, Sutherland, Gilman and many others. The readers will enjoy hearing how the experiments of Krebs and Fischer on phosphorylase activation were temporarily stymied when they began to use centrifugation to clear debris instead of filtering cell homogenate through paper. This led them to discover that phosphorylase kinase, the first protein kinase to be identified, required calcium (which was contained in the paper filter), together with ATP, to convert inactive phosphorylase b to active phosphorylase a in the homogenate. The book provides further examples of fortuitous discoveries by keenly observant investigators, proving the point made by Louis Pasteur that "fortune favors the prepared mind" and encouraging students in the field to be alert and open to unexpected results.

The first chapter is devoted to basic concepts and to the historical progression that identified the major components of signaling systems, but the references to history continue throughout the book and provide interesting tidbits that ground our current terminology in the past. The multifunctional serine/threonine protein kinase GSK3, for example, has its name because it phosphorylates a certain residue known as site 3 on rabbit skeletal muscle glycogen synthase, although most students would now think of it primarily in a completely different context, namely as a regulator of β-catenin in the Wnt signaling pathway.

The book is divided into 10 chapters, ranging in subject from protein motifs, G proteins and G protein-coupled receptors, to growth factor and cell cycle signalling. Each chapter is broken up into sections that provide a clear outline of where the author is taking us. The second chapter starts by considering the fundamental properties of enzymes and receptors and then continues