The Company of
Biologists

COMMENTARY

Remodeling the epigenome and (epi)cytoskeleton: a new paradigm for co-regulation by methylation

Cheryl Walker[1],* and Warren Burggren[2]

ABSTRACT

The epigenome determines heritable patterns of gene expression in the absence of changes in DNA sequence. The result is programming of different cellular-, tissue- and organ-specific phenotypes from a single organismic genome. Epigenetic marks that comprise the epigenome (e.g. methylation) are placed upon or removed from chromatin (histones and DNA) to direct the activity of effectors that regulate gene expression and chromatin structure. Recently, the cytoskeleton has been identified as a second target for the cell's epigenetic machinery. Several epigenetic 'readers, writers and erasers' that remodel chromatin have been discovered to also remodel the cytoskeleton, regulating structure and function of microtubules and actin filaments. This points to an emerging paradigm for dual-function remodelers with 'chromatocytoskeletal' activity that can integrate cytoplasmic and nuclear functions. For example, the SET domain-containing 2 methyltransferase (SETD2) has chromatocytoskeletal activity, methylating both histones and microtubules. The SETD2 methyl mark on chromatin is required for efficient DNA repair, and its microtubule methyl mark is required for proper chromosome segregation during mitosis. This unexpected convergence of SETD2 activity on histones and microtubules to maintain genomic stability suggests the intriguing possibility of an expanded role in the cell for chromatocytoskeletal proteins that read, write and erase methyl marks on the cytoskeleton as well as chromatin. Coordinated use of methyl marks to remodel both the epigenome and the (epi)cytoskeleton opens the possibility for integrated regulation (which we refer to as 'epiregulation') of other higher-level functions, such as muscle contraction or learning and memory, and could even have evolutionary implications.

KEY WORDS: Actin, Epigenetics, Epiregulation, DMNT, SETD2, Tubulin

Introduction
Epigenetics and the 'epigenome'

The term 'epigenetics' was coined in 1942 by Conrad Waddington to encompass biological concepts and processes that involved genetics and inheritance, but which extended beyond what was then regarded as a shadowy, spectral world of phenotype controlled by unknown external factors (Waddington, 1942). In the modern era of molecular biology, epigenetics is understood as determining heritable patterns of gene expression in the absence of changes in DNA sequence, allowing different cellular-, tissue- and organ-specific phenotypes to be specified by a single organismic genome.

[1]Center for Precision Environmental Health, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. [2]Department of Biological Sciences, University of North Texas, 1155 Union Circle #305220, Denton, TX 76203, USA.

*Author for correspondence (cheryl.walker@bcm.edu)

C.W., 0000-0001-5787-6423

Borrowing from the Greek word 'epi' (which translates to 'above'), epigenetics has now become a central pillar upon which rests modern genetics, cell biology, development and even evolution – for an entry into the voluminous literature see: Biswas and Rao (2018); Burggren (2016, 2020); Burggren and Crews (2014); Crews et al. (2014); Gonzalez-Recio et al. (2015); Jablonka (2017); Janssen et al. (2017); Lowdon et al. (2016); Perez and Lehner (2019); Sarkies (2019); Seymour and Becker (2017); Skinner (2015); Vidalis et al. (2016); Wang et al. (2017); Whipple and Holeski (2016); Zhong (2016). (Note: the reader should be advised that numerous variants on the definitions of the terms 'epigenetics', and especially the associated 'epigenetic inheritance', are to be found in that literature. Fortunately for the authors, sorting out this semantic quagmire is beyond the scope of this Commentary.)

Central to the concept of epigenetics is the 'epigenome', defined as the pattern of small chemical modifications made to both the histones and DNA that make up chromatin. Strahl and Allis first proposed the concept that these modifications on histones comprise a 'histone code' (see Glossary) that directs gene transcription and creates a 'language' that broadly regulates transcription and other aspects of chromatin biology (Strahl and Allis, 2000). We now appreciate the epigenome as the primary mechanism for 'operationalizing' the genome. It is of vital importance to within-life development, as well as healthy maturation and aging; if perturbed, it can impact subsequent generations through transgenerational, 'non-genetic' inheritance (Booth and Brunet, 2016; Stricker et al., 2017). Table 1 presents a non-exclusive list of the best understood chromatin marks and some of their actions. From a mechanistic perspective, a suite of proteins often termed 'readers', 'writers' and 'erasers' are responsible for programming (and reprogramming) the epigenome (Fig. 1). Recent reviews of these proteins and their actions have been provided by Biswas and Rao (2018), Trevino et al. (2015) and Yang et al. (2016), among others.

Methylation of the epigenome

Although more than a dozen different post-translational modifications (PTMs) have been identified on chromatin, methylation appears to be the epigenetic mark (see Glossary) that is primarily responsible for the heritable component of the epigenome, i.e. once methyl marks are installed, patterns of methylation on histones and DNA are faithfully copied in daughter cells after each cell division. Methylation on both DNA and chromatin is read, written and erased by effector proteins, methyltransferases and demethylases (see Glossary), respectively, and this methylation is the focus for this Commentary. Most effectors that 'read' methyl marks do so using methyl-binding domains (see Glossary) that recognize and bind methyl marks on chromatin (Javaid and Choi, 2017). Epigenetic methyl 'writers' are histone and DNA methyltransferases that add methyl groups to histones (e.g. the histone H3 lysine 36 trimethyl mark, H3K36me3)

Glossary

Basal bodies
A mitotic organizing center of the cell at the base of the primary cilium, formed by the centrosome when cells are not in mitosis

Chromatocytoskeletal
Activity of a dual-function epigenetic regulator acting on both chromatin and the cytoskeleton

CpG islands
Regions of DNA ≥200 bp in length with a high frequency of CpG sites

Demethylase
Enzyme that removes methyl groups from target proteins such as DNA or histones

Domain
Modular component of a protein with a specialized function in recognizing or modifying target proteins

Epigenetic machinery
Proteins that add, recognize and/or remove epigenetic modifications of chromatin in a coordinated and circumscribed way

Epigenetic marks
Small chemical modifications to the histones and DNA that affect the function of the genome in a hereditary manner

Epimutations
Heritable changes to the epigenome that can result in abnormal transcriptional repression of active genes and/or abnormal activation of usually repressed genes

Epiregulation
Regulation of structure and function of the genome and cytoskeleton, including but not limited to the reading, writing and erasure of methyl marks, by the epigenetic machinery

Histone code
Combinatorial post-translational modifications that direct gene transcription and/or chromatin conformation

Methyltransferases
Enzymes that add methyl groups to lysine, arginine and histidine (or other) amino acid residues of target proteins

Midbody
The collapsed central spindle microtubules that continue to join daughter cells until cleavage during cytokinesis

Polycomb repressive complex 2 (PRC2)
A multi-component methyltransferase enzyme complex containing the histone methyltransferase EZH2 that writes the H3K27me3 mark on chromatin

Tubulin code
Post-translational modifications that direct tubulin tertiary and quaternary structure and function

and DNA (e.g. methylation of the 5′ position of cytosine, 5 mC). Methyl erasers are demethylases such as the Jumonji proteins that remove methyl marks from histones, and the Tet enzymes that remove methyl groups from 5 mC.

Highly specific, methyl 'readers' can distinguish between different epigenetic marks, and even between the same type of modification located on different residues of DNA or histones, using domains that bind to specific modifications on chromatin. The presence of these domains allows the protein readers to match a surface groove or cavity to a specific epigenetic mark, not unlike the classic 'lock and key' mechanism described for enzyme–substrate interaction. For example, even though they are both trimethylated, readers can differentiate between trimethylated lysine 27 and trimethylated lysine 4 on histone H3 (the H3K27me3 and H3K4me3 marks, respectively), with H3K27me3 read as a repressive mark and H3K4me3 read as an active mark for gene expression (Brykczynska et al., 2010). Readers can even differentiate between different modifications on the same residue. For example, histone H3 lysine 27 can be acetylated or trimethylated, with H3K27ac read as an active mark, and

H3K27me3 read as a repressive mark for transcription (Pasini et al., 2010). Frequently, reading domains are also utilized by enzymes that function as epigenetic writers or erasers. This allows one epigenetic mark to direct the addition or removal of another mark on chromatin. For example, histone methyl marks can direct the activity of DNA methyltransferases, which read these marks to methylate CpG sites in DNA to silence specific loci (Morselli et al., 2015; Ooi et al., 2007) or mark intergenic regions (Weinberg et al., 2019). Importantly, this close coordination of reading, writing and erasing actions by specialized proteins results in what are termed 'read-write' or 'read-erase' mechanisms, an important component of what is often referred to as the epigenetic machinery (see Glossary).

As mentioned above, methyl marks on chromatin can provide either positive or negative regulation of gene expression. DNA methylation is particularly important when it occurs on cytosine bases adjacent to guanine bases, which – when clustered – are commonly referred to as CpG islands (see Glossary). Methylation of these islands can effectively silence gene expression, as can repressive histone methyl marks such as H3K27me3 (Vaissiere et al., 2008). Other histone marks can promote gene expression; for example, H3K4me3 on chromatin is associated with active gene promoters (Shilatifard, 2012). Epigenetic methyl marks can also act at a distance; for example, monomethylation of histone H3 at lysine 4 (H3K4me1) is associated with enhancer regions of transcriptionally active domains of chromatin, and the repressive histone H3 lysine 9 trimethyl mark (H3K9me3) causes heterochromatic silencing of long stretches of chromatin associated with X inactivation (Becker et al., 2016).

'Moonlighting' by the epigenetic machinery

As a result of several decades of epigenetics research, and more recent initiatives such as the Epigenome Roadmap Project (http://www.roadmapepigenomics.org), we now understand at the molecular and biochemical level how the epigenetic machinery remodels chromatin in the nucleus. We have also learned that these functions are highly conserved from yeast to humans. Consequently, we now have available genome-wide, nucleotide-level detail on epigenomes from many different tissues and cell types. While epigenetic research continues at a robust pace, we are now poised to look outside the nucleus and ask a new question: is the activity of this epigenetic machinery restricted to remodeling chromatin in the nucleus, or might this apparatus also remodel extra-nuclear targets in the cell? Below, we lay out the evidence that the cytoskeleton has emerged as a second, important target for epigenetic readers, writers and erasers. In this Commentary, we consider how methyl marks may function to remodel, regulate and integrate the structure and function of the epigenome and cytoskeleton.

The ever-expanding reach of regulation by methylation

The knowledge of how epigenetic marks regulate gene expression at the molecular and biochemical level is rapidly changing our views on many aspects of biology and medicine. The additional realization that an epigenetic machinery exists with the ability to remodel histones and DNA to regulate chromatin structure and gene expression has provided a major expansion of our understanding of genetics, inheritance, disease pathogenesis (for example, in the fields of cancer and autism) and even evolution (see below). Yet, from this fundamental understanding of the workings of the epigenetic machinery, another key question has emerged: is the coordinated programming of epigenetic marks and the associated remodeling limited to chromatin, or could the epigenetic machinery

Table 1. A selection of the most common epigenetic marks and their possible effects

General target	Marker	Position	Effects
Chromatin	Mono-, di- or tri-methyl groups (-CH$_3$)	The 5th carbon atom of cytosine ring of DNA DNA methylation occurs at single or highly enriched CpG-dense sites throughout the genome Occurs on lysine and arginine residues of histones	When present in CpG-dense 'islands' near promoters, methyl groups inhibit transcription, reducing or altogether silencing gene expression When present on histones, methyl groups can be associated with either activation or repression of gene expression depending on the specific lysine/arginine residue, and type of mark (mono-, di- or tri-methylation)
	Acetyl group (-CH$_3$CO)	NH$_3^+$ group of lysine in histone tails of nucleosome of packaged dsDNA	Acetylation causes DNA to assume a less condensed structure, facilitating enhanced gene transcription
	Ubiquitin, a 76 amino acid (~8.6 kDa) protein	Lysine residues of histone proteins of nucleosome of packaged dsDNA	Ubiquitination causes DNA to assume a less-condensed structure, facilitating enhanced gene transcription Influences the activity of other chromatin remodelers with read-write activity that 'read' this mark to add another
	Phosphate group (PO$_4^{3-}$)	Tyrosine residues of histone proteins	Phosphorylation causes DNA to assume a less-condensed structure, facilitating enhanced gene transcription Influences the activity of other chromatin remodelers with read-write activity that 'read' this mark to add another
Cytoskeleton	Mono-, di- or tri-methyl groups (-CH$_3$)	Added to lysine 40 (K40) and histidine 73 (H73) residues of tubulin and actin, respectively	K40 methylation is associated with dynamic microtubules of the mitotic spindle H73 methylation regulates actin polymerization
	Acetyl group (-CH$_3$CO)	Added to lysine 40 of tubulin	Associated with microtubule stability

remodel other structures, and thus regulate other aspects of cell biology, perhaps in coordination with its activity on chromatin? Indeed, as reviewed below, we know that several of the enzymes involved in modifying the epigenome can 'moonlight' by catalyzing reactions beyond the epigenome. This raises a second question: which is the 'main' and which is the 'secondary' function for a machinery known for its activity on chromatin that is also acting on the cytoskeleton?

Tubulin post-translational modifications and the 'tubulin code'

Following on the heels of the histone code hypothesis, cell biologists have proposed a similar 'tubulin code' (see Glossary) for regulation of microtubule structure and function (Barisic and Maiato, 2016; Gadadhar et al., 2017; Janke, 2014; Verhey and Gaertig, 2007). Microtubules are key elements of the cytoskeleton that consist of α- and β-tubulin heterodimers. Polymers of these heterodimers form tubular structures – microtubules – that provide

structure and shape to the cell, and are involved in mitosis, motility and intracellular transport (Matamoros and Baas, 2016; Wloga et al., 2017). The tubulin code hypothesis states that tubulin isotype expression and PTMs form a code used by the cell to encrypt the structural, spatial, temporal and functional information that specifies microtubule biology.

One example of a tubulin code modification of microtubules linked to cytoskeletal biology is acetylation, which occurs on lysine 40 of α-tubulin (αTubK40ac) (Sadoul and Khochbin, 2016). αTubK40ac marks microtubules of the mitotic spindle, as well as the extraordinarily stable microtubules of the axoneme of cilia. The αTubK40ac mark provides structural constraints that contribute to microtubule stability (Eshun-Wilson et al., 2019), but whether this mark is 'read' by other effectors is not clear. For example, microtubules marked by αTubK40ac are preferred tracks for transport by kinesin-1 motors (Cai et al., 2009; Reed et al., 2006), although kinesin-1 does not directly read this mark (Kaul et al., 2014; Walter et al., 2012). The αTubK40ac mark is written by alpha
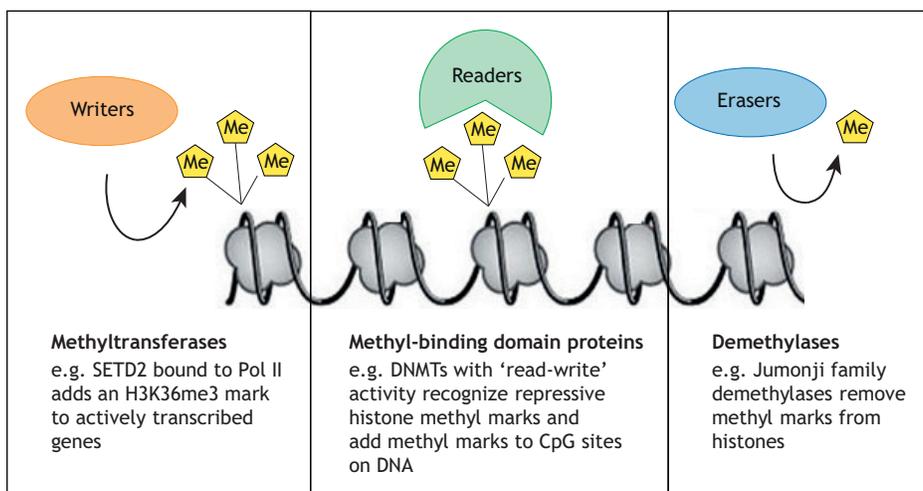

Fig. 1. Writers, readers and erasers of epigenetic markers. As an example, the figure shows information on proteins that can write, read and erase methyl groups (Me). Methyl groups on histones can be sensed (read) by methyl-binding domain proteins (e.g. DNA methyltransferases, DMNTs) acting as readers (green). New methyl groups can be added to chromatin by methyltransferases (e.g. SETD2) acting as writers (orange). Pol II, RNA polymerase II. Alternatively, existing methyl groups can be removed from chromatin by demethylases (e.g. Jumonji demethylases) acting as erasers (blue).

Writers

Methyltransferases
e.g. SETD2 bound to Pol II adds an H3K36me3 mark to actively transcribed genes

Readers

Methyl-binding domain proteins
e.g. DNMTs with 'read-write' activity recognize repressive histone methyl marks and add methyl marks to CpG sites on DNA

Erasers

Demethylases
e.g. Jumonji family demethylases remove methyl marks from histones

tubulin acetyltransferase (αTAT) and erased by the deacetylases sirtuin 2 (SIRT2) and histone deacetylase 6 (HDAC6), which – although a member of the histone deacetylase family – does not have significant activity on histones (Howes et al., 2014; Li et al., 2013; Magiera et al., 2018). Thus, although acetylation is a component of both the histone and tubulin codes, there are distinct acetylase/deacetylase enzymes that write and erase this mark on chromatin and microtubules; consequently, until recently, there has been little reason to believe that the histone and tubulin codes were anything other than conceptually linked.

The view that the histone and tubulin codes were distinct was recently revised when one of the key chromatin methyl writers, the histone methyltransferase SETD2, was discovered to also methylate microtubules (Park et al., 2016). This demonstrated for the first time a shared mark (methylation) and a shared writer (SETD2) for that mark on chromatin and the cytoskeleton. SETD2 writes the H3K36me3 mark on chromatin. This writing activity is mediated by an interaction between SETD2 and RNA polymerase II (Pol II), which facilitates SETD2 methylation of histones in conjunction with Pol II transcription of active genes (Hacker et al., 2016; McDaniel and Strahl, 2017). SETD2 also adds a trimethyl mark to lysine 40 of α-tubulin (αTubK40me3) on microtubules in mitotic cells (Park et al., 2016). This newly discovered PTM is located on spindle microtubules, and is required for normal chromosome segregation and cytokinesis (Chiang et al., 2018; Park et al., 2016).

The discovery that SETD2 methylates microtubules as well as chromatin not only adds a new mark to the tubulin code, but now directly links the histone and tubulin codes in an exciting new, functional way. In addition to marking sites of active gene transcription, the H3K36me3 SETD2 mark on chromatin also plays a role in DNA repair, facilitating non-homologous end joining (Fnu et al., 2011), DNA double strand break repair (Carvalho et al., 2014; Li and Wang, 2017), mismatch repair (Li, 2013) and homologous recombination repair (Heyer et al., 2006; Pfister et al., 2014). Thus, the discovery that the αTubK40me3 SETD2 mark on microtubules is required for proper chromosome segregation meant that SETD2 and its methyl mark function on both histones and spindle microtubules to maintain genomic stability (McDaniel and Strahl, 2017; Wagner and Carpenter, 2012), an unexpected convergence of function in two distinct cellular compartments (chromatin and the cytoskeleton).

Methylation of the cytoskeleton: chromatocytoskeletal activity of the epigenetic machinery

The quite unexpected convergence of SETD2 action on both histones and microtubules suggests the intriguing possibility that other components of the epigenetic machinery may act on both chromatin and the cytoskeleton to coordinately regulate, and integrate the functions of, these important elements of the cell. Supporting this concept, many cytoskeletal proteins contain conserved recognition sequences for histone methyltransferases, and several have been shown to be methylated on lysine, arginine or histidine residues (Iwabata et al., 2005). One unbiased proteomic screen for lysine-methylated proteins in the cytoplasm of migrating neural crest cells identified 182 methylated proteins, including cytoskeletal proteins actin, tubulin, myosin, spectrin, filamin and tropomyosin (Vermillion et al., 2014). A more in-depth analysis of one of these, the actin elongation promoting factor EF1a1, identified five sites for lysine methylation that were involved in neural crest migration (Vermillion et al., 2014). Other mass spectrometry screens have identified these and other cytoskeletal proteins as targets for methylation (see Phosphosite.org for an extensive database). Unfortunately, we do not yet know how many of these numerous cytoskeletal methylation events are catalyzed by the same methyltransferases that also modify chromatin structure and function.

In addition to α-tubulin methylation by SETD2, other examples exist of remodelers with chromatocytoskeletal activities (see Glossary). Although methylation is a newly discovered post-translational modification of microtubules, methylation of the actin cytoskeleton (actin and associated proteins) has been previously reported [for reviews, see Song and Brady (2015); Terman and Kashina (2013); Wloga et al. (2017)]. The best-known methyl mark on actin is histidine 73 methylation (actin-H73me), with methylation at this site promoting actin polymerization. Recently, the methyltransferase responsible for this mark was identified as SETD3 (Dai et al., 2019; Guo et al., 2019; Kwiatkowski et al., 2018; Wilkinson et al., 2019). However, whether SETD3 acts as a writer on chromatin is controversial (Eom et al., 2011; Wilkinson et al., 2019).

Another writer acting on the actin cytoskeleton is EZH2, the histone methyltransferase of the polycomb repressive complex 2 (PRC2) (see Glossary). This methyltransferase is responsible for the H3K27me3 mark on chromatin, a repressive mark that inhibits gene expression. EZH2 binds to α-actin (Chen et al., 2017; Su et al., 2005), and localizes to sites of membrane ruffling, where actin cytoskeletal reorganization occurs (Su et al., 2005). EZH2 is associated with increased stability of actin filaments, although the site and mechanism of methylation have not been identified (Chen et al., 2017).

In addition to EZH2, chromatocytoskeletal activity has also been reported for WDR5, a scaffolding protein that participates in read-write functions on chromatin (Guarnaccia and Tansey, 2018). As a methyl reader, WDR5 binds to di-methylated arginine (H3R2me2) residues on histone tails, a mark of genes poised for active transcription (Migliori et al., 2012). As a writer, WDR5 is a component of the COMPASS complex, which writes the histone H3 lysine 4 mono-, di- and tri-methyl marks that are associated with actively transcribed genes (Bochynska et al., 2018). WDR5 has a non-chromatin role on the actin cytoskeleton, binding to basal bodies (see Glossary) and acting as an organizing center to stabilize and maintain filamentous (F)-actin lattice architecture in multi-ciliated cells such as mucociliary epithelial cells (Kulkarni et al., 2018). WDR5 also localizes to microtubules of the mitotic spindle and midbody (see Glossary), where it promotes abscission (cleavage) to maintain genomic stability (Ali et al., 2017; Bailey et al., 2015). Unfortunately, it is unknown whether these non-chromatin functions of WDR5 involve methylation of the actin cytoskeleton.

Cytoskeleton-associated proteins can also be the targets of methylation. The actin-binding protein talin, which plays a role in cell migration, is a target for EZH2 methylation (Gunawan et al., 2015). Methylation disrupts the interaction of talin with actin and promotes the turnover of adhesion structures. Arginine methylation by protein arginine methyltransferase 2 (PRMT2) of the actin nucleator Cobl promotes actin binding and dendritogenesis, a major cytoskeletal process shaping neuronal cells (Hou et al., 2018). PRMT2 can methylate histones (Lakowski and Frankel, 2009) and is responsible for asymmetrical dimethylation of histone H3 at arginine 8 (H3R9me2a) (Dong et al., 2018). Finally, the cytoskeleton-associated protein tau, which plays a causal role in fibril development in Alzheimer's disease, is also lysine methylated (Kontaxi et al., 2017), although the responsible 'writer' is unknown. Interestingly, this PTM changes qualitatively with aging and disease

Journal of Experimental Biology

pathogenesis (Funk et al., 2014; Huseby et al., 2019), suggesting that methylation may play a role in these processes.

'Epiregulation' – from the genome to the cytoskeleton and beyond?

Structure is an important commonality between chromatin and the cytoskeleton. The cytoskeleton provides structural integrity for the cell, and the structure of the cytoskeleton itself determines much of its function (Bodakuntla et al., 2019; Fletcher and Mullins, 2010). This is also the case for chromatin, where epigenetic marks direct chromatin structure (looping, condensation, etc.), which in turn influences function, i.e. transcription. For example, acetylation and active histone methyl marks promote an open chromatin conformation permissive for gene expression, whereas repressive histone methyl marks and other repressive epigenetic marks such as DNA methylation cause a closed chromatin conformation that represses gene expression.

As our appreciation of the importance of the epigenetic machinery beyond its traditional chromatin activity expands, and its remodeling functions are extended to the cytoskeleton, the term 'epigenetic' becomes unduly limiting and even inaccurate in conceptualizing and describing how this machinery functions in a broader context. Provocatively, Fletcher and Mullins in their review of a decade ago speculated 'long-lived cytoskeletal structures can function as a cellular "memory" that integrates past interactions with the mechanical microenvironment and influences future cellular behavior' and provide 'epigenetic determinants of cell shape, function and fate' (Fletcher and Mullins, 2010).

Given the increasingly far reach beyond the genome of marks 'traditionally' recognized as only altering chromatin function and structure, we suggest that the terminology needs to keep pace. Consequently, here we employ the new term 'epiregulation' (see Glossary) as more accurate than 'epigenetic regulation' when discussing the expanding functions of dual-function readers, writers and erasers that determine not only chromatin, but also cytoskeleton structure and function. The identification of their chromatocytoskeletal activity expands what was formerly thought to be a purely epigenetic machinery to cellular epiregulators that act on and coordinate the structure and function of chromatin and the cytoskeleton.

The expanding role of methylation in modifying the structure of cytoskeletal proteins leads to another intriguing question: could epiregulation be acting beyond the cell to coordinate tissue, organ and organismal level functions? Actin, as an expanded target of epiregulation, is a nearly ubiquitous protein that exists as monomeric globular (G) protein or polymerized filamentous (F) structures in the cell. Frequent state changes occur between G- and F-actin (Copeland, 2019; Le Floc'h and Huse, 2015; Li et al., 2018; Yamashiro and Watanabe, 2019). These state changes are involved in functions as diverse as cell motility, cell-to-cell communication and the regulation of cell shape (Dominguez and Holmes, 2011; Pollard, 2016; Rottner et al., 2017). At the tissue level, actin plays an important role in muscle contraction. Together with its partner molecule myosin, actin collectively makes up ~90% of the proteins within myocytes, forming the thin (actin) and thick (myosin) filaments of the myofibril, the basic unit of muscle contraction. If epiregulation of actin and/or myosin via methyl (or other) marks directs changes in polymerization, tertiary or quaternary structure of the actin cytoskeleton, could epiregulation then actually play a role in modulating muscle contraction?

Expanding on this concept of epiregulation, both microtubules and actin are important components of the neuronal cytoskeleton, where they play key roles in neuron migration, process extension and synaptogenesis. If epiregulators act on microtubules and/or actin of neurons, could epiregulation direct the cytoskeletal remodeling required for these activities? If so, epiregulation could play an as yet undescribed role in brain development and learning and memory. We postulate that epiregulation of methyl (and other) marks on these and other cellular targets might indeed allow chromatocytoskeletal epiregulators to direct somatic tissue-level activity.

Phenotypic plasticity, epiregulation and evolution

Major unanswered questions in epigenetics and evolution include: how has the epigenetic programming itself evolved? How does natural selection act on the epigenome? How do epigenetic alterations ('epimutations'; see Glossary) become permanently fixed into the epigenome? How is evolution of the epigenome over time coordinated with evolution of its cognate genome? The epigenetic programming (orchestrated changes in DNA and histone methylation) that directs patterns of gene expression to specify tissues (such as epithelia and muscle) and organs (such as liver and kidney) during differentiation is not specified in the genetic sequence of the genome it controls. How, then, does this epigenetic programming evolve?

Traditionally, evolution of the epigenome is understood in two principal ways: (1) via the appearance of epimutations (environmentally induced or spontaneous) and (2) via epigenome-directed mutations in DNA. First appreciated in plants (Furrow, 2014; Rhounim et al., 1992; van der Graaf et al., 2015), but now widely understood to be associated with all epigenomes, epimutations are heritable changes to the epigenome, e.g. DNA or histone methyl marks that can result in changes in gene expression and phenotype upon which natural selection can act (Sarkies, 2019). This model treats epimutations similarly to DNA mutations: epimutations lead to altered gene/protein expression resulting in phenotypic changes that can be acted on by natural selection (Cropley et al., 2012). Satisfying the heredity component of this model requires that epimutations are transmissible to subsequent generations (Burggren, 2016). Similar to DNA mutations, for an epimutation to be inherited by subsequent generations, based on known biology it must be transmitted via the germ cell(s) of the founding generation.

The second way in which epigenetics and evolution interact emerges from the long-appreciated fact that sites for DNA methylation are hypermutable [reviewed in Jones et al. (1992); Storz et al. (2019)]. Methylated cytosines are sensitive to deamination, which results in $5\,mC{\rightarrow}T$ mutations. This results, for example, in rates of point mutations of a gene being higher when the CpG islands next to that gene are methylated (Xia et al., 2012), as are meiotic crossover rates in plants (Melamed-Bessudo and Levy, 2012). In this scenario, the epigenome contributes to evolution secondary to facilitating changes in the genome, with DNA methylation acting as an underlying driver for new DNA mutations and phenotypes upon which natural selection could act (Rošić et al., 2018).

Despite knowing the general outline of these relationships for over a quarter of a century (Holliday and Grigg, 1993; Jackson-Grusby and Jaenisch, 1996), evolutionary biologists and epigeneticists are still struggling to incorporate mechanisms of epigenetic inheritance into the nexus of genetics, phenotypic plasticity, natural selection and evolution. The discovery that the components of the epigenetic machinery discussed here (with the potential for more yet to be discovered) are dual-function proteins with chromatocytoskeletal activity, opens exciting new windows

into understanding phenotypic plasticity, evolutionary biology and epigenetics. The potential for epiregulation to coordinate cytoskeletal biology at the cellular and organismal level (such as in muscle contraction and neuronal function) with heritable changes in the epigenome could have profound implications for evolutionary theory. Both mutations and epimutations can be heritable and acted upon by natural selection in fundamentally similar ways. However, coordinated regulation of the cytoskeleton and epigenome by epiregulation may provide heretofore unappreciated ways for natural selection to drive evolution (Fig. 2).

DNA alterations are, of course, the stuff of natural selection and Darwinian evolution, but would differ from epiregulation-driven evolution in several fundamental ways, as shown in Table 2 and Fig. 2. Importantly, an environmental exposure that alters the chromatocytoskeletal activity of an epiregulator to cause a change in phenotype (one mechanism for phenotypic plasticity), could also exert selective pressure upon that epiphenotype, and cause changes in the epigenome that could drive the emergence of hereditary

epimutations. Therefore, in contrast to genetic (classical) evolution where DNA alteration necessarily precedes the emergence of a phenotype upon which natural selection can act, changes in epiregulator activity could produce an epiphenotype upon which natural selection could act that precedes the emergence of a hereditary epimutation. In this way, a changing environment could exert directionality by changing chromatocytoskeletal activity to produce an advantageous phenotype while concomitantly changing the epigenome to drive the emergence of new epimutations that encode that phenotype. We would propose the term 'epigenetic entrainment' for this process. The literature is replete with examples of environmental exposures that modulate the activity of epiregulators, including availability of dietary nutrients required for 1-carbon metabolites needed as methyl donors, changes in oxygen levels that affect the activity of demethylases, and environmental exposures that change cell signaling pathways and the activity of kinases that phosphorylate epiregulators to increase or decrease their activity (for a review, see Walker, 2016).
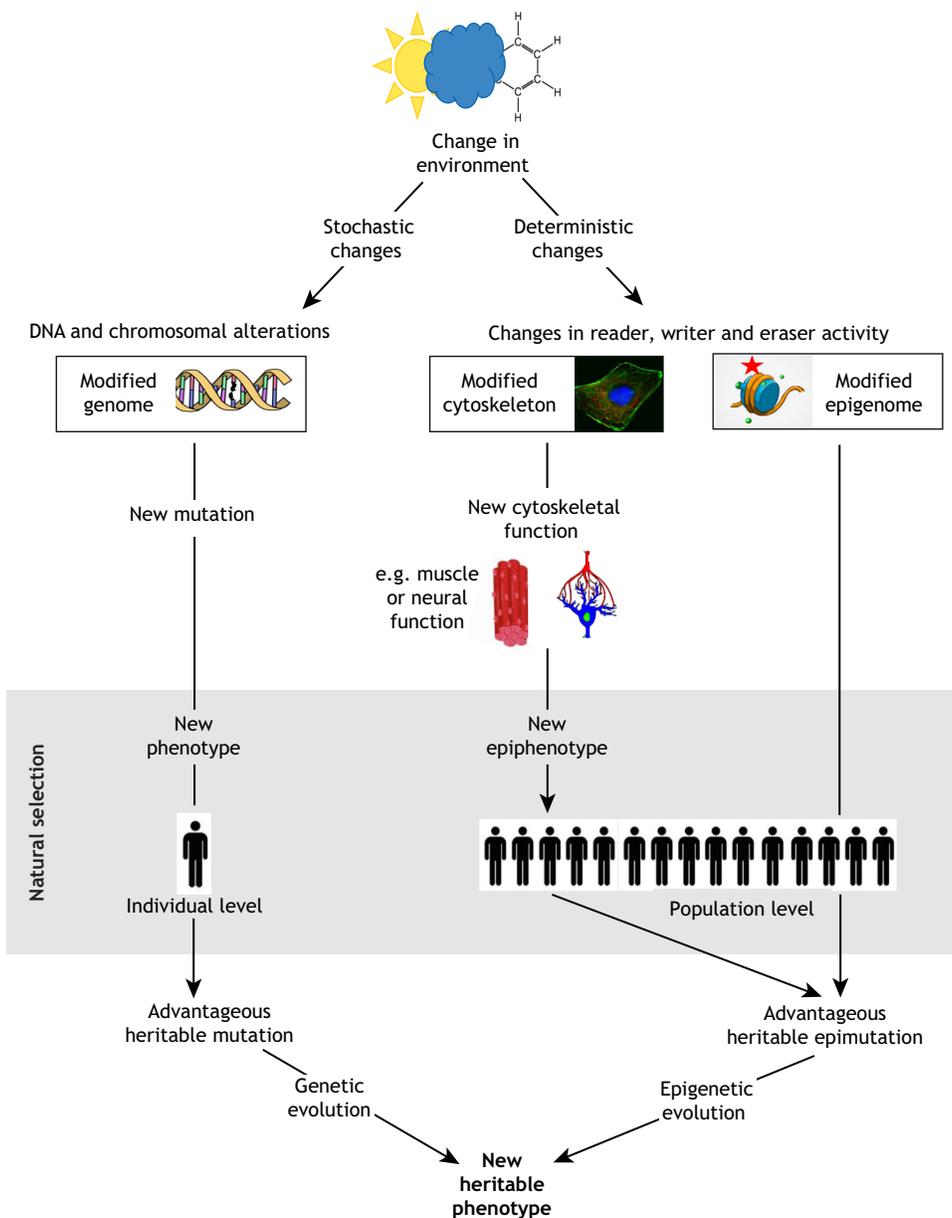

Fig. 2. A comparison of genetic and epigenetic drivers for evolution. Genetic evolution is driven by stochastic DNA alterations. In contrast, evolution driven by epiregulator activity would be deterministic: environmental perturbation of reading, writing or erasing activity of epiregulators would cause specific and predictable changes in the epigenome and epicytoskeleton. For example, decreased oxygen levels inhibit erasers that remove methyl marks, increasing epigenetic (and, we predict, epicytoskeletal) methylation. Increased cytoskeletal methylation could then produce new phenotypes upon which natural selection could act, and perturbations of the epigenome to drive emergence of hereditary epimutations. Thus, the environment determines the activity of epiregulators, and changes in epiregulator activity determine the nature of the modifications that occur to the epigenome and epicytoskeleton. A second distinction is that whereas stochastic DNA alterations occur at the level of the individual, an environmental perturbation that affects chromatocytoskeletal activity has the potential to affect the epicytoskeleton and epigenome of all individuals within an exposed population. Thirdly, perturbation of chromatocytoskeletal activity would be predicted to have an immediate and direct impact on the epicytoskeleton, potentially giving rise to phenotypes upon which natural selection could immediately act. Finally, in contrast to a new phenotype arising from an irreversible DNA alteration, a new epicytoskeleton-dependent phenotype caused by altered activity of an epiregulator would be reversible until fixed in the population by either a hereditary mutation or epimutation.

Table 2. A comparison of impacts in a changing environment resulting from mutation by DNA alteration and by epiregulation

Characteristics	DNA alteration as driver for evolution	Epiregulation as driver for evolution
Target	Genome	Epigenome and epicytoskeleton, with potential coordination between the two
Distribution and rate of spread within species	Unique events in single individuals Slow spread of adaptive genotype by natural selection	Population-wide response to a changing environment/ perturbation Can produce rapid changes in the epigenome and epicytoskeleton
Predictability of alteration	Stochastic, although mutations are not entirely random, occurring more often as errors during DNA repair	Deterministic – an environmental perturbation of a given reader, writer or eraser acting on the epigenome will have a predictable impact on its epiregulation
Reversibility	Essentially irreversible, very low probability of second mutation reversing mutated genome to original state	Relatively plastic and reversible, able to act in both forward and reverse directions through addition and removal of marks on the cytoskeleton and epigenome
Persistence	Permanent, within individual's lifetime and potentially across generations to offspring Expansion into the population occurs on an evolutionary time scale Can persist in the absence of selective pressure	Epiphenotype dependent on continued epiregulator activity until fixed in epigenome Changes maintained within or across generations as long as the environmental conditions persist and/or until fixed as hereditary epimutations
Effect on fitness	Advantageous, neutral or deleterious	Advantageous, neutral or deleterious
Natural selection	Occurrence of hereditary mutation producing new phenotype precedes natural selection Selective pressure does not directly cause mutation or phenotype upon which it acts	Selective pressure can act on new epiphenotype prior to emergence of hereditary epimutation Selective pressure can drive changes in epiregulation to cause emergence of new epiphenotype

What might be the consequences for evolution if environmental changes modulate the activity of chromatocytoskeletal epiregulators? If environmentally induced changes in epiregulator activity result in advantageous changes in cytoskeletal function, for example altered muscular or neural function, then natural selection for that epiphenotype could occur as long as the epiregulator activity responsible persisted. Selective pressure for this altered epiregulator activity could also drive changes in the epigenome, with the potential to become fixed over time as hereditary epimutations. If so, this would predict that over evolutionary time, coordinated functions for marks on the epigenome and epicytoskeleton would arise, such as a role for methyl marks made by SETD2 on both chromatin and the cytoskeleton participating in maintenance of genomic stability.

Equally importantly, regulation of the chromatocytoskeletal activity of epiregulators in response to changes in the environment would be predicted to occur on a population-wide level (Fig. 2). A graphic example of epigenetically driven population-wide modulation of phenotype conversion involves the locust *Schistocerca gregaria*. This locust converts from its typical solitarious form to a gregarious form – famed for the swarms of locusts that plague Africa and other regions. The morphological, physiological and behavioral transition from solitarious to gregarious form begins within hours of the appropriate environmental cues, and is thought to result from epigenetic changes that simultaneously affect as many as 80 million individuals (Burggren, 2017; Ernst et al., 2015). Fascinatingly, these changes are also accompanied by changes in learning and memory (Simoes et al., 2013). Could these arise by concurrent coupling of epigenetic and epicytoskeletal changes?

Finally, this hypothesis of coordinated regulation between genome and cytoskeleton could be examined experimentally via several approaches, such as altering the activity of a specific writer or eraser and determining whether coordinate changes occur in their cognate methyl marks on both chromatin and the cytoskeleton. Similarly, it would be of interest to determine whether the 'rules' governing the histone code also apply to the tubulin (or actin) code. For example, are the same methyltransferase/demethylase pairs that add/remove a specific histone methyl mark on chromatin also involved in addition and removal of that cognate methyl mark on microtubules of the cytoskeleton?

Conclusions and perspectives

Molecular biologists have learned much about the epigenetic machinery, and the epigenome itself is now well defined for numerous cell types, tissues and even disease states. Emerging data are showing that these readers, writers and erasers have chromatocytoskeletal activity, thereby contributing to remodeling functions in the cytoplasm as well as the nucleus. This opens up the exciting possibility that this machinery may not only regulate the structure of chromatin and the cytoskeleton, but also serve to integrate their functions. In light of this possibility, we suggest that the term epiregulation – that is, coordinated regulation of the epigenome and cytoskeleton to integrate key biological processes using a shared machinery – may better capture how this machinery functions in a broader context.

We predict that with the expansion of the concept of epiregulation will come a new perspective to our understanding of both normal and pathophysiological processes. For example, epiregulation may help us understand how cells coordinate epigenetic differentiation programs with acquisition of specialized cytoskeletal structures. Such coordination could affect brain development by coordinating patterns of gene expression with remodeling of the neuronal cytoskeleton. This, in turn, could modify such basic neural functions as learning, memory and behavior in a wide variety of animals, either as adaptations or maladaptations. Similarly, in the study of human diseases such as cancer and autism spectrum disorder (ASD), where mutations in components of the epigenetic machinery (including SETD2) frequently occur, we need to look beyond changes in gene expression caused by these defects to examine their impact on the cytoskeleton. In creating a deeper understanding of cancer this could mean, for example, determining how mutations in chromatocytoskeletal remodelers cause cytoskeletal defects contributing to cell migration and metastasis, and in ASD, how mutations in chromatocytoskeletal remodelers impact neurodevelopment, learning and memory.

We hope and anticipate that physiologists, morphologists, ecologists, evolutionary biologists and others working on molecular and cellular regulation may find useful ways to explore epiregulation as a new paradigm for interpreting their data. Thus, moving both conceptually and pragmatically beyond 'epigenetic regulation' to 'epiregulation' may lead to experimental paradigms

that supplement searches for modified gene expression as a result of addition or removal of epigenetic markers with expanded investigations of methylation beyond the genome itself. Such experiments would establish a new frontier in our investigation of evolution of the epigenome specifically and regulatory mechanisms more broadly, perhaps extending to evolutionary biology itself.

References

Ali, A., Veeranki, S. N., Chinchole, A. and Tyagi, S. (2017). MLL/WDR5 complex regulates Kif2A localization to ensure chromosome congression and proper spindle assembly during mitosis. *Dev. Cell* **41**, 605-622.e7. doi:10.1016/j.devcel.2017.05.023

Bailey, J. K., Fields, A. T., Cheng, K., Lee, A., Wagenaar, E., Lagrois, R., Schmidt, B., Xia, B. and Ma, D. (2015). WD repeat-containing protein 5 (WDR5) localizes to the midbody and regulates abscission. *J. Biol. Chem.* **290**, 8987-9001. doi:10.1074/jbc.M114.623611

Barisic, M. and Maiato, H. (2016). The tubulin code: a navigation system for chromosomes during mitosis. *Trends Cell Biol.* **26**, 766-775. doi:10.1016/j.tcb.2016.06.001

Becker, J. S., Nicetto, D. and Zaret, K. S. (2016). H3K9me3-dependent heterochromatin: barrier to cell fate changes. *Trends Genet.* **32**, 29-41. doi:10.1016/j.tig.2015.11.001

Biswas, S. and Rao, C. M. (2018). Epigenetic tools (the writers, the readers and the erasers) and their implications in cancer therapy. *Eur. J. Pharmacol.* **837**, 8-24. doi:10.1016/j.ejphar.2018.08.021

Bochynska, A., Luscher-Firzlaff, J. and Luscher, B. (2018). Modes of interaction of KMT2 histone H3 lysine 4 methyltransferase/COMPASS complexes with chromatin. *Cells* **7**, E17. doi:10.3390/cells7030017

Bodakuntla, S., Jijumon, A. S., Villablanca, C., Gonzalez-Billault, C. and Janke, C. (2019). Microtubule-associated proteins: structuring the cytoskeleton. *Trends Cell Biol.* **29**, 804-819. doi:10.1016/j.tcb.2019.07.004

Booth, L. N. and Brunet, A. (2016). The aging epigenome. *Mol. Cell* **62**, 728-744. doi:10.1016/j.molcel.2016.05.013

Brykczynska, U., Hisano, M., Erkek, S., Ramos, L., Oakeley, E. J., Roloff, T. C., Beisel, C., Schübeler, D., Stadler, M. B. and Peters, A. H. (2010). Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat. Struct. Mol. Biol.* **17**, 679-687. doi:10.1038/nsmb.1821

Burggren, W. W. (2016). Epigenetic inheritance and its role in evolutionary biology: re-evaluation and new perspectives. *Biology* **5**, 24. doi:10.3390/biology5020024

Burggren, W. W. (2017). Epigenetics in insects: mechanisms, phenotypes and ecological and evolutionary implications. In *Epigenetics: How the Environment Can Have Impact on Genes and Regulate Phenotypes*, Vol. 53 (ed. H. Verlinden), pp. 1-30. Elsevier.

Burggren, W. W. (2020). Phenotypic switching resulting from developmental plasticity: fixed or reversible? *Front. Physiol.* **10**. doi:10.3389/fphys.2019.01634

Burggren, W. W. and Crews, D. (2014). Epigenetics in comparative biology: why we should pay attention. *Integr. Comp. Biol.* **54**, 7-20. doi:10.1093/icb/icu013

Cai, D., McEwen, D. P., Martens, J. R., Meyhofer, E. and Verhey, K. J. (2009). Single molecule imaging reveals differences in microtubule track selection between Kinesin motors. *PLoS Biol.* **7**, e1000216. doi:10.1371/journal.pbio.1000216

Carvalho, S., Vitor, A. C., Sridhara, S. C., Martins, F. B., Raposo, A. C., Desterro, J. M., Ferreira, J. and de Almeida, S. F. (2014). SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint. *Elife* **3**, e02482. doi:10.7554/eLife.02482

Chen, R., Kong, P., Zhang, F., Shu, Y.-N., Nie, X., Dong, L.-H., Lin, Y.-L., Xie, X.-L., Zhao, L.-L., Zhang, X.-J. et al. (2017). EZH2-mediated alpha-actin methylation needs lncRNA TUG1, and promotes the cortex cytoskeleton formation in VSMCs. *Gene* **616**, 52-57. doi:10.1016/j.gene.2017.03.028

Chiang, Y. C., Park, I. Y., Terzo, E. A., Tripathi, D. N., Mason, F. M., Fahey, C. C., Karki, M., Shuster, C. B., Sohn, B. H., Chowdhury, P. et al. (2018). SETD2 haploinsufficiency for microtubule methylation is an early driver of genomic instability in renal cell carcinoma. *Cancer Res.* **78**, 3135-3146. doi:10.1158/0008-5472.CAN-17-3460

Copeland, J. (2019). Formins, golgi, and the centriole. *Results Probl. Cell Differ.* **67**, 27-48. doi:10.1007/978-3-030-23173-6_3

Crews, D., Gillette, R., Miller-Crews, I., Gore, A. C. Skinner, M. K. (2014). Nature, nurture and epigenetics. *Mol. Cell. Endocrinol.* **398**, 42-52. doi:10.1016/j.mce.2014.07.013

Cropley, J. E., Dang, T. H. Y., Martin, D. I. K. and Suter, C. M. (2012). The penetrance of an epigenetic trait in mice is progressively yet reversibly increased by selection and environment. *Proc. Biol. Sci.* **279**, 2347-2353. doi:10.1098/rspb.2011.2646

Dai, S., Horton, J. R., Woodcock, C. B., Wilkinson, A. W., Zhang, X., Gozani, O. and Cheng, X. (2019). Structural basis for the target specificity of actin histidine methyltransferase SETD3. *Nat. Commun.* **10**, 3541. doi:10.1038/s41467-019-11554-6

Dominguez, R. and Holmes, K. C. (2011). Actin structure and function. *Annu. Rev. Biophys.* **40**, 169-186. doi:10.1146/annurev-biophys-042910-155359

Dong, F., Li, Q., Yang, C., Huo, D., Wang, X., Ai, C., Kong, Y., Sun, X., Wang, W., Zhou, Y. et al. (2018). PRMT2 links histone H3R8 asymmetric dimethylation to oncogenic activation and tumorigenesis of glioblastoma. *Nat. Commun.* **9**, 4552. doi:10.1038/s41467-018-06968-7

Eom, G. H., Kim, K.-B., Kim, J. H., Kim, J.-Y., Kim, J.-R., Kee, H. J., Kim, D. W., Choe, N., Park, H.-J., Son, H.-J. et al. (2011). Histone methyltransferase SETD3 regulates muscle differentiation. *J. Biol. Chem.* **286**, 34733-34742. doi:10.1074/jbc.M110.203307

Ernst, U. R., Van Hiel, M. B., Depuydt, G., Boerjan, B., De Loof, A. and Schoofs, L. (2015). Epigenetics and locust life phase transitions. *J. Exp. Biol.* **218**, 88-99. doi:10.1242/jeb.107078

Eshun-Wilson, L., Zhang, R., Portran, D., Nachury, M. V., Toso, D. B., Löhr, T., Vendruscolo, M., Bonomi, M., Fraser, J. S. and Nogales, E. (2019). Effects of alpha-tubulin acetylation on microtubule structure and stability. *Proc. Natl. Acad. Sci. USA* **116**, 10366-10371. doi:10.1073/pnas.1900441116

Fletcher, D. A. and Mullins, R. D. (2010). Cell mechanics and the cytoskeleton. *Nature* **463**, 485-492. doi:10.1038/nature08908

Fnu, S., Williamson, E. A., De Haro, L. P., Brenneman, M., Wray, J., Shaheen, M., Radhakrishnan, K., Lee, S.-H., Nickoloff, J. A. and Hromas, R. (2011). Methylation of histone H3 lysine 36 enhances DNA repair by nonhomologous end-joining. *Proc. Natl. Acad. Sci. USA* **108**, 540-545. doi:10.1073/pnas.1013571108

Funk, K. E., Thomas, S. N., Schafer, K. N., Cooper, G. L., Liao, Z., Clark, D. J., Yang, A. J. and Kuret, J. (2014). Lysine methylation is an endogenous post-translational modification of tau protein in human brain and a modulator of aggregation propensity. *Biochem. J.* **462**, 77-88. doi:10.1042/BJ20140372

Furrow, R. E. (2014). Epigenetic inheritance, epimutation, and the response to selection. *PLoS ONE* **9**, e101559. doi:10.1371/journal.pone.0101559

Gadadhar, S., Bodakuntla, S., Natarajan, K. and Janke, C. (2017). The tubulin code at a glance. *J. Cell Sci.* **130**, 1347-1353. doi:10.1242/jcs.199471

Gonzalez-Recio, O., Toro, M. A. and Bach, A. (2015). Past, present, and future of epigenetics applied to livestock breeding. *Front. Genet.* **6**, 305. doi:10.3389/fgene.2015.00305

Guarnaccia, A. D. and Tansey, W. P. (2018). Moonlighting with WDR5: a cellular multitasker. *J. Clin. Med.* **7**, E21. doi:10.3390/jcm7020021

Gunawan, M., Venkatesan, N., Loh, J. T., Wong, J. F., Berger, H., Neo, W. H., Li, L. Y., La Win, M. K., Yau, Y. H., Guo, T. et al. (2015). The methyltransferase Ezh2 controls cell adhesion and migration through direct methylation of the extranuclear regulatory protein talin. *Nat. Immunol.* **16**, 505-516. doi:10.1038/ni.3125

Guo, Q., Liao, S., Kwiatkowski, S., Tomaka, W., Yu, H., Wu, G., Tu, X., Min, J., Drozak, J. and Xu, C. (2019). Structural insights into SETD3-mediated histidine methylation on beta-actin. *Elife* **8**, e43676 doi:10.7554/eLife.43676.031

Hacker, K. E., Fahey, C. C., Shinsky, S. A., Chiang, Y. J., DiFiore, J. V., Jha, D. K., Vo, A. H., Shavit, J. A., Davis, I. J., Strahl, B. D. et al. (2016). Structure/function analysis of recurrent mutations in SETD2 protein reveals a critical and conserved role for a SET domain residue in maintaining protein stability and histone H3 Lys-36 trimethylation. *J. Biol. Chem.* **291**, 21283-21295. doi:10.1074/jbc.M116.739375

Heyer, W. D., Li, X., Rolfsmeier, M. and Zhang, X. P. (2006). Rad54: the Swiss Army knife of homologous recombination? *Nucleic Acids Res.* **34**, 4115-4125. doi:10.1093/nar/gkl481

Holliday, R. and Grigg, G. W. (1993). DNA methylation and mutation. *Mutat. Res.* **285**, 61-67. doi:10.1016/0027-5107(93)90052-H

Hou, W., Nemitz, S., Schopper, S., Nielsen, M. L., Kessels, M. M. and Qualmann, B. (2018). Arginine methylation by PRMT2 controls the functions of the actin nucleator Cobl. *Dev. Cell* **45**, 262-275.e8. doi:10.1016/j.devcel.2018.03.007

Howes, S. C., Alushin, G. M., Shida, T., Nachury, M. V. and Nogales, E. (2014). Effects of tubulin acetylation and tubulin acetyltransferase binding on microtubule structure. *Mol. Biol. Cell* **25**, 257-266. doi:10.1091/mbc.e13-07-0387

Huseby, C. J., Hoffman, C. N., Cooper, G. L., Cocuron, J. C., Alonso, A. P., Thomas, S. N., Yang, A. J. and Kuret, J. (2019). Quantification of tau protein lysine methylation in aging and Alzheimer's disease. *J. Alzheimer's Dis.* **71**, 979-991. doi:10.3233/JAD-190604

Iwabata, H., Yoshida, M. and Komatsu, Y. (2005). Proteomic analysis of organ-specific post-translational lysine-acetylation and -methylation in mice by use of anti-acetyllysine and -methyllysine mouse monoclonal antibodies. *Proteomics* **5**, 4653-4664. doi:10.1002/pmic.200500042

Jablonka, E. (2017). The evolutionary implications of epigenetic inheritance. *Interface Focus* **7**, 20160135. doi:10.1098/rsfs.2016.0135

Jackson-Grusby, L. and Jaenisch, R. (1996). Experimental manipulation of genomic methylation. Semin. Cancer Biol. 7, 261-268. doi:10.1006/scbi.1996.0034

Janke, C. (2014). The tubulin code: molecular components, readout mechanisms, and functions. J. Cell Biol. 206, 461-472. doi:10.1083/jcb.201406055

Janssen, K. A., Sidoli, S. and Garcia, B. A. (2017). Recent achievements in characterizing the histone code and approaches to integrating epigenomics and systems biology. Methods Enzymol. 586, 359-378. doi:10.1016/bs.mie.2016.10.021

Javaid, N. and Choi, S. (2017). Acetylation- and methylation-related epigenetic proteins in the context of their targets. Genes (Basel) 8, E196. doi:10.3390/genes8080196

Jones, P. A., Rideout, W. M., , III, Shen, J. C., Spruck, C.-H. and Tsai, Y. C. (1992). Methylation, mutation and cancer. BioEssays 14, 33-36. doi:10.1002/bies.950140107

Kaul, N., Soppina, V. and Verhey, K. J. (2014). Effects of α-tubulin K40 acetylation and detyrosination on kinesin-1 motility in a purified system. Biophys. J. 106, 2636-2643. doi:10.1016/j.bpj.2014.05.008

Kontaxi, C., Piccardo, P. and Gill, A. C. (2017). Lysine-directed post-translational modifications of tau protein in Alzheimer's disease and related tauopathies. Front. Mol. Biosci. 4, 56. doi:10.3389/fmolb.2017.00056

Kulkarni, S. S., Griffin, J. N., Date, P. P., Liem, K. F., , Jr and Khokha, M. K. (2018). WDR5 stabilizes actin architecture to promote multiciliated cell formation. Dev. Cell 46, 595-610.e3. doi:10.1016/j.devcel.2018.08.009

Kwiatkowski, S., Seliga, A. K., Vertommen, D., Terreri, M., Ishikawa, T., Grabowska, I., Tiebe, M., Teleman, A. A., Jagielski, A. K., Veiga-da-Cunha, M. et al. (2018). SETD3 protein is the actin-specific histidine N-methyltransferase. Elife 7, e37921. doi:10.7554/eLife.37921

Lakowski, T. M. and Frankel, A. (2009). Kinetic analysis of human protein arginine N-methyltransferase 2: formation of monomethyl- and asymmetric dimethyl-arginine residues on histone H4. Biochem. J. 421, 253-261. doi:10.1042/BJ20090268

Le Floc'h, A. and Huse, M. (2015). Molecular mechanisms and functional implications of polarized actin remodeling at the T cell immunological synapse. Cell. Mol. Life Sci. 72, 537-556. doi:10.1007/s00018-014-1760-7

Li, G. M. (2013). Decoding the histone code: Role of H3K36me3 in mismatch repair and implications for cancer susceptibility and therapy. Cancer Res. 73, 6379-6383. doi:10.1158/0008-5472.CAN-13-1870

Li, L. and Wang, Y. (2017). Cross-talk between the H3K36me3 and H4K16ac histone epigenetic marks in DNA double-strand break repair. J. Biol. Chem. 292, 11951-11959. doi:10.1074/jbc.M117.788224

Li, Y., Shin, D. and Kwon, S. H. (2013). Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes. FEBS J. 280, 775-793. doi:10.1111/febs.12079

Li, P., Bademosi, A. T., Luo, J. and Meunier, F. A. (2018). Actin remodeling in regulated exocytosis: toward a mesoscopic view. Trends Cell Biol. 28, 685-697. doi:10.1016/j.tcb.2018.04.004

Lowdon, R. F., Jang, H. S. and Wang, T. (2016). Evolution of epigenetic regulation in vertebrate genomes. Trends Genet. 32, 269-283. doi:10.1016/j.tig.2016.03.001

Magiera, M. M., Singh, P., Gadadhar, S. and Janke, C. (2018). tubulin posttranslational modifications and emerging links to human disease. Cell 173, 1323-1327. doi:10.1016/j.cell.2018.05.018

Matamoros, A. J. and Baas, P. W. (2016). Microtubules in health and degenerative disease of the nervous system. Brain Res. Bull. 126, 217-225. doi:10.1016/j.brainresbull.2016.06.016

McDaniel, S. L. and Strahl, B. D. (2017). Shaping the cellular landscape with Set2/SETD2 methylation. Cell. Mol. Life Sci. 74, 3317-3334. doi:10.1007/s00018-017-2517-x

Melamed-Bessudo, C. and Levy, A. A. (2012). Deficiency in DNA methylation increases meiotic crossover rates in euchromatic but not in heterochromatic regions in Arabidopsis. Proc. Natl. Acad. Sci. USA 109, E981-E988. doi:10.1073/pnas.1120742109

Migliori, V., Muller, J., Phalke, S., Low, D., Bezzi, M., Mok, W. C., Sahu, S. K., Gunaratne, J., Capasso, P., Bassi, C. et al. (2012). Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Nat. Struct. Mol. Biol. 19, 136-144. doi:10.1038/nsmb.2209

Morselli, M., Pastor, W. A., Montanini, B., Nee, K., Ferrari, R., Fu, K., Bonora, G., Rubbi, L., Clark, A. T., Ottonello, S. et al. (2015). In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. Elife 4, e06205. doi:10.7554/eLife.06205

Ooi, S. K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z., Erdjument-Bromage, H., Tempst, P., Lin, S. P., Allis, C. D. et al. (2007). DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 448, 714-717. doi:10.1038/nature05987

Park, I. Y., Powell, R. T., Tripathi, D. N., Dere, R., Ho, T. H., Blasius, T. L., Chiang, Y. C., Davis, I. J., Fahey, C. C., Hacker, K. E. et al. (2016). Dual chromatin and cytoskeletal remodeling by SETD2. Cell 166, 950-962. doi:10.1016/j.cell.2016.07.005

Pasini, D., Malatesta, M., Jung, H. R., Walfridsson, J., Willer, A., Olsson, L., Skotte, J., Wutz, A., Porse, B., Jensen, O. N. et al. (2010). Characterization of an antagonistic switch between histone H3 lysine 27 methylation and acetylation in the transcriptional regulation of Polycomb group target genes. Nucleic Acids Res. 38, 4958-4969. doi:10.1093/nar/gkq244

Perez, M. F. and Lehner, B. (2019). Intergenerational and transgenerational epigenetic inheritance in animals. Nat. Cell Biol. 21, 143-151. doi:10.1038/s41556-018-0242-9

Pfister, S. X., Ahrabi, S., Zalmas, L. P., Sarkar, S., Aymard, F., Bachrati, C. Z., Helleday, T., Legube, G., La Thangue, N. B., Porter, A. C. et al. (2014). SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability. Cell Reports 7, 2006-2018. doi:10.1016/j.celrep.2014.05.026

Pollard, T. D. (2016). Actin and actin-binding proteins. Cold Spring Harbor Perspect. Biol. 8, a018226. doi:10.1101/cshperspect.a018226

Reed, N. A., Cai, D., Blasius, T. L., Jih, G. T., Meyhofer, E., Gaertig, J. and Verhey, K. J. (2006). Microtubule acetylation promotes kinesin-1 binding and transport. Curr. Biol. 16, 2166-2172. doi:10.1016/j.cub.2006.09.014

Rhounim, L., Rossignol, J. L. and Faugeron, G. (1992). Epimutation of repeated genes in Ascobolus immersus. EMBO J. 11, 4451-4457. doi:10.1002/j.1460-2075.1992.tb05546.x

Rošić, S., Amouroux, R., Requena, C. E., Gomes, A., Emperle, M., Beltran, T., Rane, J. K., Linnett, S., Selkirk, M. E., Schiffer, P. H. et al. (2018). Evolutionary analysis indicates that DNA alkylation damage is a byproduct of cytosine DNA methyltransferase activity. Nat. Genet. 50, 452-459. doi:10.1038/s41588-018-0061-8

Rottner, K., Faix, J., Bogdan, S., Linder, S. and Kerkhoff, E. (2017). Actin assembly mechanisms at a glance. J. Cell Sci. 130, 3427-3435. doi:10.1242/jcs.206433

Sadoul, K. and Khochbin, S. (2016). The growing landscape of tubulin acetylation: lysine 40 and many more. Biochem. J. 473, 1859-1868. doi:10.1042/BCJ20160172

Sarkies, P. (2019). Molecular mechanisms of epigenetic inheritance: possible evolutionary implications. Semin. Cell Dev. Biol. 97, 106-115. doi:10.1016/j.semcdb.2019.06.005

Seymour, D. K. and Becker, C. (2017). The causes and consequences of DNA methylome variation in plants. Curr. Opin. Plant Biol. 36, 56-63. doi:10.1016/j.pbi.2017.01.005

Shilatifard, A. (2012). The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu. Rev. Biochem. 81, 65-95. doi:10.1146/annurev-biochem-051710-134100

Simoes, P. M., Niven, J. E. and Ott, S. R. (2013). Phenotypic transformation affects associative learning in the desert locust. Curr. Biol. 23, 2407-2412. doi:10.1016/j.cub.2013.10.016

Skinner, M. K. (2015). Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-Lamarckian concept that facilitates neo-Darwinian evolution. Genome Biol. Evol. 7, 1296-1302. doi:10.1093/gbe/evv073

Song, Y. and Brady, S. T. (2015). Post-translational modifications of tubulin: pathways to functional diversity of microtubules. Trends Cell Biol. 25, 125-136. doi:10.1016/j.tcb.2014.10.004

Storz, J. F., Natarajan, C., Signore, A. V., Witt, C. C., McCandlish, D. M. and Stoltzfus, A. (2019). The role of mutation bias in adaptive molecular evolution: insights from convergent changes in protein function. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374, 20180238. doi:10.1098/rstb.2018.0238

Strahl, B. D. and Allis, C. D. (2000). The language of covalent histone modifications. Nature 403, 41-45. doi:10.1038/47412

Stricker, S. H., Köferle, A. and Beck, S. (2017). From profiles to function in epigenomics. Nat. Rev. Genet. 18, 51-66. doi:10.1038/nrg.2016.138

Su, I.-H., Dobenecker, M. W., Dickinson, E., Oser, M., Basavaraj, A., Marqueron, R., Viale, A., Reinberg, D., Wulfing, C. and Tarakhovsky, A. (2005). Polycomb group protein ezh2 controls actin polymerization and cell signaling. Cell 121, 425-436. doi:10.1016/j.cell.2005.02.029

Terman, J. R. and Kashina, A. (2013). Post-translational modification and regulation of actin. Curr. Opin. Cell Biol. 25, 30-38. doi:10.1016/j.ceb.2012.10.009

Trevino, L. S., Wang, Q. and Walker, C. L. (2015). Phosphorylation of epigenetic 'readers, writers and erasers': implications for developmental reprogramming and the epigenetic basis for health and disease. Prog. Biophys. Mol. Biol. 118, 8-13. doi:10.1016/j.pbiomolbio.2015.02.013

Vaissiere, T., Sawan, C. and Herceg, Z. (2008). Epigenetic interplay between histone modifications and DNA methylation in gene silencing. Mutat. Res. 659, 40-48. doi:10.1016/j.mrrev.2008.02.004

van der Graaf, A., Wardenaar, R., Neumann, D. A., Taudt, A., Shaw, R. G., Jansen, R. C., Schmitz, R. J., Colome-Tatche, M. and Johannes, F. (2015). Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. Proc. Natl. Acad. Sci. USA 112, 6676-6681. doi:10.1073/pnas.1424254112

Verhey, K. J. and Gaertig, J. (2007). The tubulin code. Cell Cycle 6, 2152-2160. doi:10.4161/cc.6.17.4633

Vermillion, K. L., Lidberg, K. A. and Gammill, L. S. (2014). Cytoplasmic protein methylation is essential for neural crest migration. J. Cell Biol. 204, 95-109. doi:10.1083/jcb.201306071

Vidalis, A., Živković, D., Wardenaar, R., Roquis, D., Tellier, A. and Johannes, F. (2016). Methylome evolution in plants. Genome Biol. 17, 264. doi:10.1186/s13059-016-1127-5

Waddington, C. H. (1942). The epigenotype. *Endeavour* **1**, 18-20.

Wagner, E. J. and Carpenter, P. B. (2012). Understanding the language of Lys36 methylation at histone H3. *Nat. Rev. Mol. Cell Biol.* **13**, 115-126. doi:10.1038/nrm3274

Walker, C. L. (2016). Minireview: epigenomic plasticity and vulnerability to EDC exposures. *Mol. Endocrinol.* **30**, 848-855. doi:10.1210/me.2016-1086

Walter, W. J., Beranek, V., Fischermeier, E. and Diez, S. (2012). Tubulin acetylation alone does not affect kinesin-1 velocity and run length in vitro. *PLoS ONE* **7**, e42218. doi:10.1371/journal.pone.0042218

Wang, Y., Liu, H. and Sun, Z. (2017). Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans. *Biol. Rev. Camb. Philos. Soc.* **92**, 2084-2111. doi:10.1111/brv.12322

Weinberg, D. N., Papillon-Cavanagh, S., Chen, H., Yue, Y., Chen, X., Rajagopalan, K. N., Horth, C., McGuire, J. T., Xu, X., Nikbakht, H. et al. (2019). The histone mark H3K36me2 recruits DNMT3A and shapes the intergenic DNA methylation landscape. *Nature* **573**, 281-286. doi:10.1038/s41586-019-1534-3

Whipple, A. V. and Holeski, L. M. (2016). Epigenetic inheritance across the landscape. *Front. Genet.* **7**, 189. doi:10.3389/fgene.2016.00189

Wilkinson, A. W., Diep, J., Dai, S., Liu, S., Ooi, Y. S., Song, D., Li, T. M., Horton, J. R., Zhang, X., Liu, C. et al. (2019). SETD3 is an actin histidine methyltransferase that prevents primary dystocia. *Nature* **565**, 372-376. doi:10.1038/s41586-018-0821-8

Wloga, D., Joachimiak, E. and Fabczak, H. (2017). Tubulin post-translational modifications and microtubule dynamics. *Int. J. Mol. Sci.* **18**, E2207. doi:10.3390/ijms18102207

Xia, J., Han, L. and Zhao, Z. (2012). Investigating the relationship of DNA methylation with mutation rate and allele frequency in the human genome. *BMC Genomics* **13** Suppl. 8, S7. doi:10.1186/1471-2164-13-S8-S7

Yamashiro, S. and Watanabe, N. (2019). Quantitative high-precision imaging of myosin-dependent filamentous actin dynamics. *J. Muscle Res. Cell Motil.* **41**, 163-173. doi:10.1007/s10974-019-09541-x

Yang, A. Y., Kim, H., Li, W. and Kong, A. N. (2016). Natural compound-derived epigenetic regulators targeting epigenetic readers, writers and erasers. *Curr. Top. Med. Chem.* **16**, 697-713. doi:10.2174/1568026615666150826114359

Zhong, X. (2016). Comparative epigenomics: a powerful tool to understand the evolution of DNA methylation. *New Phytol.* **210**, 76-80. doi:10.1111/nph.13540