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Epigenetics and chromatin dynamics: a review and a paradigm for functional disorders

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Abstract

Background—Motility and functional gastrointestinal disorders have high prevalence in the community, cause significant morbidity and represent a major health care burden. Despite major advances in our understanding of the cellular and molecular basis of gastrointestinal neuromuscular functions, many of these diseases still defy mechanistic explanations. The biopsychosocial model underlying the current classification of functional gastrointestinal disorders recognizes and integrates the pathogenetic role of genetic, environmental and psychosocial factors but has not been associated with specific molecular mechanisms.

Purpose—Here we propose that this integrative function is encoded in the chromatin composed of the DNA and associated histone and nonhistone proteins and noncoding RNA. By establishing epigenetically heritable “molecular memories” of past stimuli including environmental challenges, the chromatin determines an individual’s responses to future insults and translates them into high-order outputs such as symptoms and illness behavior. Thus, surveying epigenetic signatures throughout the genome of affected cells in individual patients may make it possible to better understand and ultimately control the phenomena described by the biopsychosocial model. In this review we provide a high-level but comprehensive description of the concepts and mechanisms underlying epigenetics and chromatin dynamics, describe the mechanisms whereby the environment can alter the epigenome and identify aspects of functional gastrointestinal and motility disorders where epigenetic mechanisms are most likely to play important roles.

Keywords

Functional gastrointestinal disorders; nucleosome; DNA methylation; histone modifications; histone variants; chromatin remodeling; noncoding RNA; environmental epigenetics

The digestive tract is arguably the most complex organ system in our body consisting of cells that derive from all three germinal layers and possessing its own nervous and endocrine systems. Digestive diseases are correspondingly diverse in their etiology, pathogenesis and manifestations. Disorders such as gastroparesis, chronic idiopathic intestinal pseudo-obstruction, Hirschsprung’s disease and fecal incontinence originate from the gut’s neuromuscular compartment and involve smooth muscle cells, enteric neurons and mesenchymal cells such as interstitial cells of Cajal (ICC), and are thus termed motility disorders. Another, overlapping group of abnormalities such as functional dyspepsia,

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irritable bowel syndrome, gastroesophageal reflux and chronic constipation either reflect combinations of altered motility and secretion, visceral hypersensitivity and brain-gut dysregulation, or lack a well-defined pathology, and thus are better classified as functional gastrointestinal disorders.¹ Some of these syndromes have estimated prevalence rates reaching 30 percent of the U.S. population and others come with a major social burden or stigma.²

Over the past 150 years, research targeting fundamental mechanisms of gastrointestinal motility provided us with a census of key cell types and defined their role in motility. More recent investigations have uncovered the subcellular and molecular basis of these functions in health and disease³ and begun to analyze the contributions of the intestinal microbiome⁴ and genetic factors to disease manifestation.^{3,5-7} However, in the majority of patients no genetic association is obvious, and searching for “organic causes” in the traditional sense may be a futile effort.⁸

The current working model for the pathogenesis of functional gastrointestinal disorders is the biopsychosocial model,⁸ which stipulates a key role for a bidirectional interaction between psychosocial factors and physiological mechanisms occurring against a backdrop of genetic predisposition and early life experiences. While the biopsychosocial model has excellent clinical utility, it lacks a biochemically defined integrator that could combine genetic factors and cellular “memories” of past influences with precipitating changes in the external and/or internal environment to determine the individual’s responses at the cellular level and translate them into symptoms and illness behavior. Here we propose that epigenetic control of gene expression is ideally suited for this role, and surveying the associated molecular signatures throughout the genome of affected cells in individual patients would enable researchers to better understand and ultimately control the phenomena described by the biopsychosocial model. In this review we provide a high-level overview of the concepts and basic mechanisms underlying epigenetic control of gene expression, and describe pathways whereby environmental factors can cause heritable alterations in phenotype by modifying the epigenome. Finally, we identify aspects of functional gastrointestinal and motility disorders where epigenetic mechanisms are most likely to play important pathogenetic roles. Additional details and references are provided as Supporting Information.

The concept and scope of epigenetics

Although earlier studies had determined that naked DNA can carry and propagate genetic information, conclusive evidence that adult somatic nuclei contain *all* information required for embryonic development and function in the adult was first provided by experiments demonstrating that a nucleus taken from a somatic cell and introduced into an enucleated oocyte was competent to direct embryogenesis.⁹ A corollary to this concept is that the genome of differentiated cells is identical to that of the zygote from which it has developed.¹⁰ Despite this genetic homogeneity, cells of the same multicellular organism, including our more than 200 cell types, display an astonishing level of phenotypic heterogeneity, which is determined by the body of their transcribed genes.¹¹ The study of the mechanisms underlying phenotypic specification of diverse cells from the same zygotic genome during morphogenesis was first termed “epigenetics” by C. H. Waddington.¹²

Everyday experience indicates that cell identities, once established, are typically maintained for a lifetime and, in many cases, through a number of divisions. The idea that regulatory signals can be inherited in addition to genetic information has led to the current, most widely cited definition of epigenetics as “the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence” (Riggs et

al., 1996; quoted in ref.¹²). This concept and the underlying mechanisms provide an explanation for many—albeit not all—instances of non-Mendelian inheritance such as phenotypic differences between monozygotic twins. Furthermore, it also implies the retention of the normal or pathological phenotypes even in the absence of the signal responsible for their initiation. In this sense, epigenetic inheritance constitutes a form of “cellular memory”, a mechanistic basis for persistent, non-genetically determined biological response to developmental cues or changes in the external environment.

Demanding heritability as part of the definition of epigenetic signaling may, in a first approximation, limit the utility of epigenetics as a conceptual framework for physiologists, who often study nonproliferating, terminally differentiated cells. However, it appears likely that terminally differentiated cells such as neurons may have co-opted the same mechanisms for the long-term stabilization of their own transcriptome that in other cells maintain epigenetic states through cell divisions.¹³ Therefore, in the context of post-mitotic cells, the term “epigenetic” is best used mechanistically, i.e., in reference to the specific molecular mechanisms that establish and maintain, even after the disappearance of the initiating signal, phenotypic “variation [...] above and beyond changes in the DNA sequence”¹⁴ and have the *potential* of propagating the resultant changes through somatic divisions or via transgenerational inheritance.

Epigenetic research is mainly concerned with transcriptional regulation by *cis*-acting molecular signals, i.e., signals physically associated with the DNA and inherited with the chromatin during cell division. However, self-sustaining phenotypic traits can be transmitted, both horizontally and vertically, by mechanisms regulating transcription in *trans* (e.g., factors transmitted by partitioning of the cytoplasm)¹⁴ or by factors completely unrelated to transcriptional control (see Supplementary Note 1). However, in keeping with the approach followed by most authors, below we focus on epigenetic mechanisms concerned with establishing transcriptional patterns and propagating them through cell divisions.

Component molecular mechanisms underlying epigenetic control

The chromatin regulates access to the DNA template

Epigenetic control is concerned with indexing the genome according to the probability of use of the genetic material for mRNA and, ultimately, protein synthesis.¹⁵ The need for such a system arose with the evolution of eukaryotes, which must package an enlarged amount of genetic material into the confined space of the nucleus (Figure 1). Condensing and indexing the genome are functions of the chromatin, a nucleoprotein complex formed by the DNA with histone and nonhistone proteins. Thus, DNA-templated processes such as gene expression, DNA replication and repair are controlled, to a great extent, by the chromatin, which must assume an “open” configuration to provide access for the RNA polymerase II complex, the replication machinery and DNA repair systems to execute these functions.^{16,17} Conversely, chromatin compaction contributes to the establishment and maintenance of cellular phenotypes by repressing irrelevant gene expression, compensates for gene dosage inequalities by inactivating one of the X chromosomes in females of eutherian mammals, protects the integrity of the genome by silencing viral genomes and transposable elements, reduces transcriptional “noise” by repressing noncoding and repetitive sequences, protects chromosome ends (telomeres) and aids chromatid separation by providing attachment anchors in centromeres for kinetochore–microtubule assemblies during nuclear division.^{11,14,16,18,19} In interphase nuclei, decondensed, open or euchromatin (Figure 2) is ~10 times less dense than metaphase chromosomes and is more sensitive to deoxyribonuclease 1 (DNase1) treatment. Although it occupies most of the nuclear volume, it consists mainly of coding sequences that represent less than 2% of the genome. Sequences in the euchromatin are

either actively transcribed or inactive but poised for gene expression. In contrast, condensed or heterochromatin, which is localized along the nuclear envelope and in discrete clumps particularly at centromeres (Figure 2), is about as dense as metaphase chromosomes and consist of genomic sequences that are either permanently silenced (constitutive heterochromatin) or repressed in a cell-specific manner (facultative heterochromatin).¹⁵ Transposing expressed genes from euchromatin to or near centromeric or telomeric heterochromatin confers epigenetic repression to these genes leading to phenomena called position-effect variegation and telomere-position effects, respectively.¹⁵

Structure and organization of chromatin

The fundamental repeating unit of chromatin is the nucleosome, a nucleoprotein particle consisting of an octamer formed by two copies each of the core histones H2A, H2B, H3 and H4 around which the DNA double helix is wrapped²⁰ (Figure 1). Nucleosomes can be isolated by light treatment with micrococcal endonuclease, which cuts the intervening or linker DNA, leaving ~200 base pairs (bp) associated with the protein complex. Further digestion reduces the nucleosomal DNA to a length of 145–147 bp, which takes 1.67 left-handed turns around the histone core with entry and exit points located on the same side. The core histones form two types of subcomplexes: a relatively stable (H3-H4)₂ tetramer held together by interactions between H3 molecules and two H2A-H2B dimers (Figure 3). The (H3-H4)₂ tetramer accounts for the diameter of the core, forming a horseshoe-shaped structure that guides the central DNA gyre and which alone can organize DNA into particles. The two H2A-H2B dimers together form a second “horseshoe” that fits obliquely between the arms of the (H3-H4)₂ tetramer to complete a disc-shaped globular structure. Each heterodimer binds 2.5 helical turns of the DNA through ionic interactions between the basic histones and the negative charges of the phosphodiester backbones.²⁰ Through these interactions the octamer locks the DNA into a negatively supercoiled configuration.²¹ This arrangement alters the geometry of the double helix and, thereby, the accessibility of the sequences involved. Conversely, physical characteristics of specific DNA sequences can influence where and how nucleosomes are assembled (Supplementary Note 2). Each histone also has a highly conserved, flexible, N-terminal “tail” rich in amino acid residues that can be covalently modified (Figure 1).¹⁷ Histones H2A and H2B also have C-terminal tails containing modifiable residues. The DNA entering and exiting the nucleosome, as well as the central gyre can also be bound by the more variable linker (H1) histones (Figure 1). H1 histones facilitate the formation of higher-order chromatin structures and thus contribute to transcriptional regulation.²²

DNA more-or-less periodically wrapped around histone octamers can be recognized by electron microscopy as a ~10-nm, bead-on-a-string fiber²³ (Figure 1). Dynamic repositioning, disassembly and reformation of nucleosomes is required for the binding and progression of DNA and RNA polymerases.¹⁶ A more compact structure that can be seen in isolated interphase chromatin or *in vitro* is the 30-nm fiber (Figure 1). The most likely arrangement underlying this fiber is a solenoid formed by 6–8 nucleosomes per counterclockwise turn, which probably form a two-start helix.²⁴ This structure may represent an intermediate state between repressed and transcriptionally poised chromatin. Higher-level structures observed by light or electron microscopy can be formed by folded 30-nm fibers, which are then organized into 300–700-nm looped chromatin domains anchored to the nuclear periphery or other matrix such as lamins¹⁵ (Figures 1 and 2). The ultimate condensation that occurs during the formation of metaphase chromosomes during mitosis and meiosis facilitates the faithful segregation of the genetic material into daughter cells (Figure 1).

Histone modifications

A prominent feature of the core histones is the high number and diversity of covalently modified residues they can possess, predominantly, but not exclusively, in their “tails”, which are exposed on the surface of the nucleosomes and thus are more accessible for enzymes (Figure 1).¹⁷ The nomenclature for these modifications²⁵ identifies, in this order, the histone, the modified residue, the type of modification and the level of modification when more than one moiety can be accommodated. Thus, for example, H3R17me2a stands for Histone 3 dimethylated (me2) on aRginine 17 in an asymmetrical fashion, i.e., with both methyl groups occurring on the terminal amino group, leaving the terminal imino group unmodified. The IUPAC-IUBMB one-letter system is used for identifying the amino acid residues. With the aid of modification-specific antibodies and mass spectrometry, the following classes have been identified: acetylation of lysines (Kac), mono-, di- and trimethylation of lysines (Kme1, Kme2, Kme3, respectively), mono- and symmetrical or asymmetrical dimethylation of arginines (Rme1, Rme2s, Rme2a), phosphorylation of serines and threonines (Sph, Tph), monoubiquitylation of lysines (Kub1), SUMOylation (attachment of small ubiquitin-like modifier proteins) of lysines (Ksu), mono- and poly-ADP ribosylation of glutamic acid (Ear1, Earn),^{17,25} biotinylation of lysines (Kbio),²⁶ proline isomerization (Pcis/Ptrans) and deimination of arginines and methyl-arginines to citrulline.¹⁷ Recently, post-translational modification of histone serines and threonines by β -N-acetylglucosamine (*O*-GlcNAc) has been added to this list.²⁷ Most of these marks are dynamic and can be established and also removed by defined enzymes¹⁷ in response to environmental signals and intracellular pathways activated by them (Figure 4). The most dramatic way to remove modifications is to clip the N terminus of histone 3 by cathepsin L. To most modifications specific biological consequences have also been assigned,¹⁷ which depend not only on the type of modification that can be borne by a particular residue (e.g., the mutually exclusive acetylation or methylation of H3K9, which is activating and repressive, respectively), but also on the modified residue carrying the same modification (e.g., trimethylation of H3K4 and H3K27, which is activating and repressive, respectively), and the location of the modification in specific genomic regions such as the association of H3K9me3 with silenced promoters and actively transcribed coding regions. Modifications on particular histone tails can be highly abundant, making interactions and crosstalk between them likely.¹⁷ Together, these findings have led to the formulation of the “histone code” hypothesis stating that specific combinations of these marks can predict functional states of the chromatin.^{28,29}

Histone modifications exert their functions either by establishing global changes such as the formation of hetero- or euchromatin or by governing the DNA template-based processes of transcription, DNA repair and replication. Functional groups affixed to histones can trigger changes in higher-order chromatin structure by affecting histone-histone and histone-DNA contacts (*cis* effects; see Supplementary Note 3) or by recruiting or excluding nonhistone proteins (*trans* effects).³⁰ Recruitment of nonhistone proteins occurs via specific binding domains that recognize modifications in a particular context (Supplementary Note 4). Examples of specific functions of histone modifications are provided in Supplementary Note 5.

Histone variants

Histones also carry information relevant to DNA template-based tasks in their primary amino acid sequence. Structural differences between variant histones can affect their DNA binding and wrapping, post-translational modifications and ability to interact with other histones and regulatory complexes.^{21,31} The incorporation of histone variants into nucleosomes is carried about by histone chaperones in coordination with chromatin-remodeling complexes, which are examined below.

Histone variants can be grouped into canonical and noncanonical variants according to whether their expression is coupled to, or independent of, DNA replication: whereas canonical histones are concerned with DNA packaging and gene regulation, and thus are produced when new strands of DNA are synthesized, the noncanonical variants play roles in a wide variety of cellular processes including transcription and must be constitutively expressed.²¹ There are several genes for canonical histones, which either encode the same protein (e.g., H4) or differ only slightly. However, some of these differences may not be negligible. For example, canonical forms of H3, H3.1 and H3.2, only differ in a single amino acid residue, but are enriched in different silencing marks.³⁰ As a result, H3.1 may selectively mark silent or non-protein-coding loci and relegate them to constitutive heterochromatin, whereas H3.2 marks facultative heterochromatin.³⁰ Nevertheless, there are greater structural differences between canonical histones and their noncanonical counterparts (Supplementary Note 6).

Noncanonical H3 variants are the centromere-specific CenH3 (mammals: CENPA), which anchor kinetochore-microtubule assemblies of the mitotic spindle,²¹ and H3.3, which supports active transcription.³¹ Universally occurring histone H2A variants include H2A.Z, which supports transcription initiation in promoters of poised or actively transcribed genes but also accumulates in heterochromatin,^{31–33} and H2A.X, whose role is to support DNA repair at sites of DNA double strand breaks (S139ph-H2A.X or γ H2A.X).²¹ The animal-specific macroH2As inhibit transcription and contribute to the silencing of the inactive X chromosome of female mammals, whereas the mammal-specific H2A.Bbd (Barr body-deficient) variant is associated with active, accessible chromatin.²¹ The human sperm-specific TH2B may protect genes related to spermatogenesis from silencing by protamines, which mediate heavy condensation of sperm chromatin. (See Supplementary Note 7 for details.)

Chromatin-remodeling complexes

Access to DNA for initiation of template-based processes requires rearrangement of nucleosomes by chromatin-remodeling complexes (Figure 1).³⁴ These complexes utilize energy from ATP hydrolysis to disrupt histone-DNA contacts and slide nucleosomes along DNA, alter their spacing, evict entire nucleosomes or replace some of their core histones.³⁵

Chromatin-remodeling complexes contain ATPases homologous to ATP-binding helicases of the DEAD/H box-containing family.³⁵ These ATPases, which are encoded by 27 genes in humans, associate with several other proteins to form complexes that can be divided into four classes: the SWI/SNF (named after yeast mutants that cannot switch mating type or have a sucrose nonfermenting phenotype), ISWI (imitation switch), CHD (chromodomain helicase, DNA-binding) and INO80/SWR1 (Snf2-related CREBBP activator protein) families. Mammalian homologues of these complexes are known by several names;³⁶ here we follow the nomenclature used in ref.³⁵ The subunits that associate with the core ATPases include monomeric β -actin and other actin-related proteins.³⁵ The noncatalytic subunits may provide proximity for coupled reactions or opportunity for diversification of function through combinatorial assembly.

The currently favored “loop recapture” model of nucleosome remodeling proposes that the ATPase binds to the nucleosome at a weak DNA-histone contact and utilizes its 3' $\rightarrow$ 5' translocase activity to “pump” DNA into the nucleosome, thereby disrupting histone-DNA interactions and generating a loop of DNA. Propagation of this loop as a wave displaces DNA relative to the histones and enables nucleosome sliding, thus promoting the formation of open chromatin and transcriptional activation.^{34–36} Some complexes are also capable of ejection or exchange of octamers,³⁴ and SWR1 (human: SRCAP) may facilitate the selective removal of H2A-H2B and its exchange with H2A.Z-H2B. In contrast, the smaller ISWI

complexes make limited contacts with the nucleosome and linker DNA as dimers and facilitate the bidirectional and processive translocation of DNA.³⁵ By sampling linker DNA lengths ISWI complexes move nucleosomes to space them regularly, which promotes gene silencing.

Chromatin-remodeling complexes have diverse and sometimes surprising functions that include transcriptional repression, maintenance of pluripotency and tumor suppression (Supplementary Note 8).

Histone chaperones

Histone chaperones regulate nucleosome assembly and disassembly required for DNA-templated tasks in an ATP-independent fashion by preferentially binding either histone H3-H4 or H2A-H2B complexes and preventing their nonspecific interactions with DNA.³⁷ They represent structurally diverse groups of proteins (see ref.³⁷) and interact with numerous other chromatin-regulating factors. Important examples include SUPT6H (suppressor of Ty 6 homolog (*S. cerevisiae*); Spt6), which binds H3-H4 preferentially and stimulates transcription elongation³⁷ together with the H2A-H2B chaperone FACT (facilitates chromatin transcription) complex. FACT also contributes to DNA replication and repair.³⁸ CAF-1 (chromatin assembly factor 1) assembles (H3.1-H4)₂ tetramers from dimers and deposits them onto replicating DNA,³⁹ whereas HIRA (histone regulator A) performs the same function in non-replicating DNA using H3.3-H4 dimers.³⁷ Both CAF-1 and HIRA act synergistically with ASF1 (anti-silencing function 1), which mobilizes parental (H3-H4)₂ tetramers, splits them into dimers and, together with *de novo* synthesized histones, transfers them to CAF-1 and HIRA.³⁷ Nucleosome assembly is then completed by the addition of H2A-H2B dimers by FACT, nucleoplasmin and NAP-1 (nucleosome assembly protein 1). During disassembly, these steps are performed in reverse. NAP-1 also assists nucleosome sliding by removing H2A-H2B dimers and functions as a chaperone for linker histones.

DNA methylation and the role of CpG islands in the regulation of transcription

In vertebrates, plants and some invertebrates, postsynthetic modifications can occur not only on histone and nonhistone proteins but also on the DNA. The most common modified DNA base is 5-methylcytosine (5meC; Figure 1),⁴⁰ which can be further modified by successive oxidation steps to yield 5-hydroxymethylcytosine (5hmeC), formylcytosine (fC) and carboxylcytosine (caC).⁴¹ Modified C's are interpreted as C by enzyme complexes performing DNA-templated tasks but can influence the biological function of the genomic sequences in which they are contained.⁴⁰ The role of 5meC is to encode stable, heritable transcriptional silencing in *cis*,¹⁰ whereas 5hmeC appears to represent an intermediate toward loss of the 5meC mark and may have specific signaling functions as well.^{41,42} Most DNA methylation occurs in short palindromic CG sequences (CpG sites), although in embryonic stem (ES) cells, almost 25% of 5meC is associated with CHG or CHH sequences (where H is A, C or T), which disappear during differentiation.⁴³

In human somatic cells, 5meC represents approximately 1% of all DNA bases and is present in 70–80% of all CpG dinucleotides.¹⁰ However, CpG doublets are present at only ~20% of the frequency that would be expected from the proportion of C-G base pairs.⁴⁴ Excepted from this rule are the so-called CpG islands (CGIs): ~1-kb G-C-rich regions occurring at the 5' ends of many mammalian genes, which contain CpG at the expected frequency. Unlike the mostly methylated CpG sites scattered throughout the genome, CGIs are predominantly nonmethylated, regardless of their activity. The apparent depletion of CpG sites outside these islands is due to the mutagenic nature of 5meC: whereas both C and 5meC can undergo spontaneous deamination into U and T, respectively, the removal of T from T-G

mismatches is less efficient than the removal of U from U·G pairs, resulting in C·G to T·A point mutations and eventual global loss of CpG sites.

The palindromic CpG sites can be methylated on one or both strands of the DNA. Initial establishment of 5meCpG on unmodified DNA is rather slow and usually occurs at sites that are already repressed by other, less permanent mechanisms.¹⁰ *De novo* methylation is catalyzed by DNA methyltransferases DNMT3A and DNMT3B, which are excluded from promoters containing nucleosomes bearing activating marks and are preferentially recruited to heterochromatic regions. In contrast, methylation of the complementary strand at the same palindromic site is rapid and highly efficient. This maintenance methylation is due to DNMT1, which is preferentially recruited to hemimethylated CpGs by UHRF1 (ubiquitin-like with PHD and ring finger domains 1),^{10,41} and, to a lesser extent, to DNMT3A and DNMT3B.³³ As a result, most CpG sites are fully (symmetrically) methylated. The same mechanism is also responsible for the semiconservative copying of parental strand 5meCpG patterns onto the progeny strand at the time of DNA replication, ensuring their inheritance at mitosis or meiosis.

The different roles and kinetics of *de novo* and maintenance methylation steps, together with the longevity of 5meC marks make DNA methylation an ideal—and quintessential—mechanism mediating epigenetic memory: whereas slow establishment at genes that are already silent ensures that it locks in, rather than sets up, repressed transcriptional states, rapid reproduction on nascent DNA strands facilitates its propagation across many cell divisions and, in most cases, throughout an individual's lifetime¹⁰ and even transgenerationally.⁴⁵ These characteristics distinguish DNA methylation from the more dynamic histone modifications and turnover.^{17,46}

Most *de novo* DNA methylation occurs during early embryonic development to ensure genomic stability, to reduce transcriptional noise and to establish transcriptional programs for differentiation. Primordial germs cells undergo a second wave of DNA methylation to establish parent-specific genomic imprinting, i.e., the repression of transcription selectively from the paternal or maternal allele of certain genes (Supplementary Note 9). *De novo* promoter CGI methylation also occurs, albeit at a very slow rate, during aging, particularly in genes that are PcG targets in ES cells.⁴⁷ Permanent cell lines and cancer cells often gain CGI methylation as well.¹⁰ In fact, aberrant DNA methylation and other epigenetic changes may precede other transforming events such as mutations and genomic instability (Supplementary Note 10).^{33,48,49} Due to their early appearance, rarity in normal cells, and the robustness of their detectability in patient samples, hypermethylated CGIs have the promise to become useful as cancer biomarkers.⁵⁰ Paradoxically, CGI hypermethylation in cancer is accompanied by hypomethylation e.g. of juxtacentromeric and other repetitive sequences, which may lead to genomic instability through illegitimate recombination and cause transcriptional deregulation of neighboring genes.⁴⁸ Hypomethylation of genes with oncogenic potential has also been described in several cancers.^{48,49}

Transcriptional repression by DNA methylation is mediated by both exclusion and recruitment of various mediators.¹⁰ Proteins that are specifically recruited to 5meCpG are the methyl-CpG-binding proteins MeCP2, MBD1-4 and Kaiso,¹⁰ which exert their functions by anchoring co-repressor complexes such as SIN3A/histone deacetylase complex or the nucleosome remodeling/histone deacetylation complex NuRD to methylated DNA.¹¹ While these proteins perform redundant functions, *de novo* mutations in the X-linked *MECP2* gene underlie most cases of Rett syndrome, an extreme neurological syndrome affecting female heterozygotes.

CGIs are clearly related to transcription initiation: ~70% of annotated promoters contain a CGI and ~50% of CGIs coincide with promoters of annotated genes. Many of the remaining (“orphan”) CGIs may be tissue-specific, developmentally regulated⁵¹ or alternative promoters of known genes or noncoding RNAs (ncRNAs).⁴⁴ Intragenic CGIs may also represent cryptic promoters of alternative transcripts, whereas intergenic CGIs might function as boundaries or insulators of transcriptional domains. CGI promoters often lack core promoter elements and may permit initiation at several locations. CGIs are generally permissive for transcription initiation regardless of whether the genes they regulate are expressed or repressed as evidenced by the binding of RNA polymerase II at promoters of inactive genes.⁵² Consistent with this, CGIs often contain binding sites for certain ubiquitous transcription factors, they are relatively nucleosome-deficient, and their nucleosomes show high levels of activating histone marks.^{44,53} However, some CGIs become methylated and permanently silenced during normal development, particularly those that are already silent in ES cells.

DNA methylation, once established, is not readily removed except during early development. Yet, most CGIs remain unmethylated while the rest of the CpG sites become methylated. Since direct removal of the methyl group is unlikely because it is thermodynamically unfavorable,¹⁰ it has been speculated that ongoing DNA template-based processes may maintain the unmethylated state with relatively little energy.⁴⁴ However, the most important mechanisms may involve the recently discovered α -ketoglutarate- and Fe(II)-dependent dioxygenase TET1 (ten-eleven translocation 1), which is mainly expressed in ES cells, and the ubiquitous TET2 and TET3.⁴¹ Indeed, TET2 mutations may lead to CGI hypermethylation in cancer. These enzymes oxidize 5meC to 5hmeC and, eventually, caC, which are not recognized by 5meC-binding proteins. 5hmeC may not be maintained during DNA replication, thus leading to passive demethylation. In addition, 5hmeC may be an intermediate to active demethylation involving glycosylation or deamination into 5hmeU (which is then removed by base excision and repair) or by the formation and subsequent decarboxylation of caC.

Besides cancer and Rett syndrome, abnormal methylation has been shown to contribute to a variety of diseases and developmental syndromes including imprinting disorders, repeat-instability diseases, as well as DNMT- and chromatin-remodeling protein-related diseases.⁴⁸

Integrated view of epigenetic states and epigenomes

The epigenetic mechanisms described above do not function independently but, rather, cooperate to establish “epigenetic states” for specific genes.⁵⁴ Together, the chromatin states of all genes make up the individual cells’ epigenetic programs (epigenomes) that bring about cell type-specific functions from a single program that confers totipotency to the same genome in the zygote.¹⁵ This concept naturally raises the question whether an individual cell’s epigenome can be predicted by analyzing a finite number of features of the chromatin in a manner similar to how DNA sequencing is used to describe an individual’s (or a transformed cell’s) genotype. A popular formalization of this idea is that histone modifications occurring in a combinatorial manner may define and predict key epigenetic states and thus could constitute a “histone code” that extends the information potential of the genetic code.^{28,29} Furthermore, nonhistone proteins playing key roles in regulating chromatin are themselves targets of numerous, functionally important post-translational modifications that could be considered a “subcode” within the context of the primary histone code.^{55,56} However, the histone code hypothesis does not directly take into account the fact that, in addition to covalent modifications, histones also carry information in their primary sequence,²¹ position^{14,32} and even kinetics of turnover,⁴⁶ and this complexity is further

increased by the availability and combinatorial assembly of chromatin regulatory complexes⁵⁷ and recruitment of downstream regulators. Furthermore, post-translational histone modifications may not have the longevity required for long-term memory of transcriptional patterns.⁴⁶ To address this specific weakness, Allis and colleagues have proposed that their maintenance beyond a single cell cycle may instead depend on the genomic region-specific inheritance of histone variants, which preferentially bear certain marks (“histone barcode hypothesis”).³⁰ However, the histone code hypothesis has also been criticized for generally falling short of key criteria set forth for coding systems.^{58,59} While debate about the existence or utility of the histone code is still ongoing, recent studies involving integrative analysis of genome-wide distribution of histone modifications, chromatin-modifying and -remodeling proteins, DNaseI sensitivity, ncRNAs and transcriptional activity were able to identify a finite number of chromatin states that correlate with the behavior of the underlying genes, indicating that predictive, functional annotation of the chromatin may perhaps be possible.^{60–62} Key epigenetic features of genomic regions and their spatiotemporal organization are discussed further in Supplementary Note 11.

Dynamic control of epigenetic states

Epigenetic signatures are metastable

Epigenetic mechanisms appeared early in evolution to establish “molecular memories” of past stimuli including inductive cues for cell specialization, environmental challenges that require adaptive response patterns for survival, and harmful, stochastic events arising from within the cells such as transposon reactivation.¹⁴ While the resultant transcriptional patterns must be long-lived, lasting, in some cases, beyond the lifetime of the individual due to transgenerational inheritance,⁶³ they must also remain amenable to changes demanded by the organism’s internal and external environment. Plasticity is facilitated by both the constant establishment of new “memories” and inherent limitations to the maintenance of previously established epigenetic signatures. For example, epigenetic marks are not as faithfully reproduced during cell division as DNA sequences,¹⁵ and key determinants of epigenetic states such as nuclear architecture, histone levels and abundance of histone variants and modifications undergo major changes during natural aging.⁶⁴ Epigenetic inheritance through the germ line faces special obstacles including the erasure and reestablishment of parental imprints and other epigenetic marks in primordial germ cells and after fertilization (see Supplementary Note 9).⁶⁵ Thus, epigenetic memory is metastable rather than indefinite as it is maintained by dynamic equilibria set up by antagonistic mechanisms that establish/reestablish, reinforce and remove epigenetic signatures (Figure 4). We have previously discussed the key elements of epigenetic regulation. While a detailed review of the biochemical processes that contribute to dynamic establishment, maintenance and removal of epigenetic marks is beyond the scope of this paper, a high-level description of the specific mechanisms is provided in Supplementary Note 12.

Environmental control of epigenetic states

Homeostasis requires the constant adjustment of cellular behavior such as gene transcription in response to a dynamic environment. Adaptive responses become predictive if “remembered” and facilitate efficient responses to recurring stimuli. However, if adaptability does not keep up with the rate of change, conflict may arise between the actual and perceived environment. This concept underlies the “thrifty phenotype” hypothesis⁶⁶ formulated to explain how undernutrition during fetal and early postnatal life, which are characterized by high degree of epigenetic plasticity, may increase the individual’s risk of developing chronic disease such as metabolic syndrome, obesity, type 2 diabetes, hypertension and coronary heart disease later in life, when the environment no longer poses

nutritional limits, but biochemical adaptation is already limited.^{45,67,68} This concept also provides explanation for divergent disease phenotypes occurring on the same genetic basis e.g. in maternal twins experiencing different environments during development.

Although the epigenome never becomes static during an individual's life,⁶⁴ periods of especially high chromatin plasticity exist during development that are characterized by greater sensitivity to environmental inputs.⁶⁹ The first window is characterized by the erasure and reestablishment of genomic imprints in primordial germ cells following their migration into the gonadal ridge (around embryonic day 7.25 in the mouse).⁷⁰ The time lapse between demethylation and remethylation is sex-dependent: in males, remethylation occurs by embryonic day 14 but in females it is delayed until maturation in the adult during each estrous cycle.⁶⁹ The second window begins with a wave of global demethylation shortly after fertilization^{11,70,71} and ends with the remethylation of "junk" DNA and promoters of pluripotency and tissue-specific genes before the end of gastrulation.^{10,11,41,44} The third period of greater vulnerability is perinatal development¹³ when exposure to the environment increases dramatically. While epigenetic adaptability reduces with aging, certain systems remain especially sensitive to environmental changes even in the adult. For example, sustained neuronal activity and neurogenesis have been proposed to create vulnerability to epigenetic dysregulation by environmental toxicants and drugs in the adult brain,¹³ and the same may also hold true for the gut, which constantly experiences environmental insults including signals arising from the microbiome.⁴

Besides manifesting in the exposed individual, response patterns to environmental factors may be transmitted through the germ line if the gametes' epigenome is affected. Several epigenetic signals can be inherited transgenerationally¹⁴ including maternal transcription factors, ncRNAs, histone modifications and altered DNA methylation. To be considered transgenerationally inherited, a trait must be demonstrable in at least the F₃ generation when embryonic exposure is involved, since in this case not only the G₀ gestating female, but also the embryo (F₁) and its developing germ cells (F₂) are directly exposed to the environmental change. By the same token, after postnatal exposure, at least the F₂ generation must be assessed. Effects of several environmental factors including heavy metals, nutrients, chemical exposure and endocrine disruptors on disease susceptibility have been studied in the F₁₋₃ generations and beyond.⁴⁵ However, multigenerational effects can also be passed on behaviorally rather than biochemically. For example, rat pups that experience more maternal care in the form of licking and grooming and arched-back nursing display reduced fearfulness, blunted responses to stress as adults and display higher maternal behavior toward their pups.⁶⁷ The increased stress responses arising from low maternal nurturing were found to be due to reduced glucocorticoid hormone negative feedback resulting from increased DNA methylation and reduced H3K9ac and transcription factor binding in the glucocorticoid receptor promoter in the hippocampus.⁷²

Further examples of environmentally-induced epigenetic changes are provided in Supplementary Note 13. The potential role for an interaction between the environment and epigenetics in evolution is discussed in Supplementary Note 14.

Perspectives for functional and motility disorders

Several motility and functional gastrointestinal disorders with familial associations but lacking an identified genetic component⁷³ may involve altered transcriptional control and some also display a generational course consistent with epigenetic inheritance.

Congenital and pediatric gastrointestinal abnormalities without known genetic basis are often associated with delayed differentiation of ICC or abnormal expression of genes involved in neurotransmitter synthesis.⁷⁴ These include idiopathic gastric perforation,

meconium ileus and transient neonatal pseudo-obstruction, where ICC are underdeveloped, and infantile hypertrophic pyloric stenosis, where expression of neuronal nitric oxide synthase and peptidergic neurotransmitters is reduced. Infantile hypertrophic pyloric stenosis with its association with male sex and first born child and resolution over time is of particular interest as a potential epigenetically-driven disease. ICC and smooth muscle cells external to the myenteric region develop from common precursors expressing the stem cell factor receptor Kit in response to specific inductive cues from their tissue microenvironment and undergo a series of gene expression changes.⁷⁵ Differentiation of enteric neurons from neural crest precursors also involves major changes in gene expression patterns.⁷ Unraveling the epigenetic basis of these transitions may identify their role in delayed or disordered differentiation.

Motility disorders such as achalasia, gastroparesis, chronic idiopathic intestinal pseudo-obstruction and slow-transit constipation can also develop in adults from environmental, immune-related and infectious causes or without identifiable etiology.^{3,5,74,76} Loss of cell-specific gene expression, depletion of ICC and enteric neurons are frequently seen in these conditions and may reflect increased attrition due to cellular stress⁷⁷ or impaired differentiation from local stem/precursor cells.^{78–81} The upregulation of Kit expression in murine ICC stem cells during differentiation appears to involve epigenetic changes⁸² occurring under the influence of stem cell factor, whose expression in the microenvironment is in turn regulated by coordinated chromatin remodeling events directed by insulin-like growth factor-1.⁸³ Smooth muscle cells respond to partial obstruction with a transient hyperplasia followed by prolonged hypertrophy reflecting dynamic changes in transcription factor binding to the promoters of key smooth muscle genes,⁸⁴ which probably also involve chromatin remodeling. Epigenetic control of genes encoding neurotransmitters and their receptors defines the identities and functions of neurons⁸⁵ and may hold the key to understanding selective changes in neuron populations in a variety of conditions.³

Early life experiences including exposure to infections and highly stressful events play important roles in the pathogenesis of functional gastrointestinal disorders^{8,86} and are likely to involve heritable, chromatin-level changes in inflammatory/immune cells, the central nervous system and the gut neuromuscular apparatus. For example, nitric oxide released during acute inflammation has been found to exert an anti-inflammatory effect by increasing the expression of histone deacetylase 3, suppressing H4K12ac and thus excluding nuclear factor κ B from the promoter of the pro-inflammatory genes.⁸⁷ Resident intestinal macrophages are a key component of inflammatory processes but can also contribute to antioxidant defense.⁸⁸ Macrophage polarization toward pro-inflammatory vs. tolerant phenotypes strongly depends on the cytokine gene expression profile of these cells, which may reflect changes in histone acetylation.⁸⁹ In turn, inflammatory insults in rats have been shown to affect smooth muscle function later in life and several weeks after the subsidence of colonic inflammation indicating a likely epigenetic component.^{90,91} These changes may contribute to the pathogenesis of post-infectious irritable bowel syndrome and dyspepsia. Functional pain syndromes are also part of the functional gastrointestinal disorder spectrum⁸ and early postnatal exposure to esophageal acid has been shown to cause prolonged upregulation, particularly upon re-challenge in adulthood, of gene expression for *N*-methyl-D-aspartate receptor subunits and postsynaptic density protein 95 in the cingulate cortex, which is responsible for processing pain of esophageal origin.⁹² The underlying changes in chromatin remain to be studied. As discussed earlier, low maternal nurturing leads to increased stress responses later in life due to increased glucocorticoid receptor promoter DNA methylation in the hippocampus.⁷² Recently, similar changes were detected in peripheral blood T lymphocytes in patients with irritable bowel syndrome⁹³ and also in colon-innervating nociceptive dorsal root ganglion neurons of chronically stressed rats.⁹⁴ Downregulation of glucocorticoid receptor expression in the central amygdala may also

involve H3K9 deacetylation as shown in a rodent model of visceral hyperalgesia.⁹⁵ Nutrients and bacterial products are also likely to have major impact on gastrointestinal motor functions e.g. by modulating immune responses in the offspring after exposure during pregnancy and lactation⁹⁶ or by directly influencing chromatin-modifying enzymes.⁹⁷ Some of these products may even modulate hippocampal gene expression and behavior.⁹⁸ Since microbial populations differ considerably e.g. in patients with diarrhea- and constipation-predominant irritable bowel syndrome,⁹⁹ findings in animal models may also apply to patients.

In summary, motility and functional gastrointestinal disorders represent a fertile area for epigenetic research, which, in turn, may break existing barriers to understanding and controlling these common problems and mitigate their significant societal impact. The rapidly falling costs of next-generation sequencing methods is shifting focus from PCR-based detection of genomic sequences targeted by histones and nonhistone proteins to unbiased, genome-wide approaches but these methods demand strong bioinformatics support with all its associated costs. A potential problem that affects not only research but also interventions targeting epigenetic phenomena is the general lack of specificity of epigenetic drugs arising, to a great extent, from the promiscuous nature of their targets. While these caveats may temper enthusiasm toward epigenetic research, rapid advance in the field can be expected to solve many of these potential problems in the near future and open new opportunities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

4HB	4-helix bundle
5hmeC	5-hydroxymethylcytosine
5meC	5-methylcytosine
5meCpG	5-methyl-CG dinucleotide
ac	acetyl
ar	ADP-ribosyl
ASF1	anti-silencing function 1
Bbd	Barr body-deficient
bio	biotinyl
bp	base pair
caC	carboxylcytosine
CAF-1	chromatin assembly factor 1

CenH3	centromeric H3
CENPA	human CenH3
CGI	CpG island
CHD	chromodomain helicase, DNA-binding
CpG	CG dinucleotide
DNase1	deoxyribonuclease 1
DNMT	DNA methyltransferase
EC	euchromatin
ES cell	embryonic stem cell
FACT	facilitates-chromatin-transcription complex
fC	formylcytosine
HC	heterochromatin
HIRA	histone regulator A
hnRNA	heterogenous nuclear RNA
ICC	interstitial cell(s) of Cajal
ISWI	imitation switch
Kit	Kit oncogene
MBD	methyl-CpG binding domain protein
me	methyl
MeCP2	methyl CpG binding protein 2 (Rett syndrome)
NAP-1	nucleosome assembly protein 1
ncRNA	noncoding RNA
NuRD	nucleosome remodeling and histone deacetylation
O-GlcNAc	β -N-acetylglucosamine
PcG	polycomb group
ph or P	phospho-
Pol II	RNA polymerase II
RC	chromatin-remodeling complex
SIN3A	SIN3 transcription regulator homolog A (yeast)
SNF	sucrose nonfermenting
SRCAP	human SWR1
su	SUMOyl
SUMO	small ubiquitine-like modifier
SUPT6H	suppressor of Ty 6 (Spt6) homolog (<i>S. cerevisiae</i>)
SWI	mating type switch deficient
SWR1	Snf2-related CREBBP activator protein

TET	ten-eleven translocation
TH2B	testis-specific H2B
TrxG	trithorax group
ub	ubiquityl
UHRF1	ubiquitin-like with PHD and ring finger domains 1. The algorithm to decode abbreviated histone modifications is explained in detail in the text and is not repeated here

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Figure 1. Overview of chromatin organization and key elements of epigenetic control of transcription

A, Metaphase chromosome, the most compact form of chromatin. B and C, 300–700-nm fibers representing higher-order looping of 30-nm chromatin fibers. This level of organization is present in both metaphase and interphase chromatin. C, Chromatin loops attached to a proteinaceous scaffold. D, 30-nm fiber representing a two-start helix formed by 6–8 nucleosomes per left-handed turn. E, 11-nm, “bead-on-a-string” chromatin fiber consisting of nucleosomes and linker DNA. RC, chromatin-remodeling complex. A more detailed model of a nucleosome is shown in the center of the figure. Note histone tails exiting through channels formed by minor grooves of the looping DNA or on the surface of the nucleosome. The amino acid sequence of the N-terminal tail of histone H3 (blue) is shown on the left with some well-established histone modifications marked. Ac, acetylation; Me, methylation; P, phosphorylation. Under the model of the nucleosome is a naked DNA fiber containing two fully methylated CpG sites. The inset at the bottom illustrates the role of noncoding RNA (ncRNA) in establishing bivalent transcriptional states characteristic of some developmental regulator genes in embryonic stem cells:¹⁰⁰ Transcription initiation is

associated with the recruitment of trithorax group complexes (TrxG), which catalyze the activating histone modification H3K4me3 (green beads). Transcription of GC-rich, hairpin-forming ncRNA from CpG island-containing promoters may recruit polycomb group complexes (PcG), which catalyze the formation of the repressive histone mark H3K27me3 (purple bead). The conflicting marks establish a decision fork from which both further repression and activation can result depending on other *trans*-acting mediators. Pol II, RNA polymerase II; hnRNA, heterogenous nuclear RNA (mRNA precursor). Inset is adapted from ref.⁵² See main text for further details.

Figure 2. Euchromatin (EC) and heterochromatin (HC)

EC and HC localized along the nuclear envelope and in clumps within the nuclei of ICC (cells on the right) and a ganglion cell (left) in the myenteric region of the murine gastric antrum. Used with permission of the American Diabetes Association, from Ordog *et al.*, Remodeling of networks of interstitial cells of Cajal in a murine model of diabetic gastroparesis, *Diabetes*[®], Vol. 49, 2000; 1731–1739; permission conveyed through Copyright Clearance Center, Inc.

Figure 3. Crystal structure-based model of the canonical eukaryotic nucleosome

Left panel: View down the DNA superhelix axis. The histone octamer consists of an (H3-H4)₂ tetramer held together by a strong 4-helix bundle (4HB) between the two H3 molecules (purple) and two H2A-H2B dimers forming weak 4HBs between α helices of H2B (green) and H4 (blue). Brown color denotes histone H2A. Right panel: View perpendicular to the DNA superhelix axis. Note that the (H3-H4)₂ tetramer accounts for the diameter of the core particle by guiding the central DNA loop. The addition of the H2A-H2B dimers results in a left-handed helical ramp that locks the DNA into a negative supercoil. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cellular Biology (ref.²¹), copyright 2010.

Figure 4. Dynamic regulation of epigenetic states

Signals arising from the extracellular and intracellular milieu can dynamically change the epigenetic status of promoters. In response to signals increasing the nuclear levels of activating transcriptional factors (green arrows), continuous recruitment of the transcription initiation apparatus maintains high H3K4me3 levels (green beads) in the promoter regions by the continuous recruitment of TrxG complexes. At the same time, H3K27me3 (purple beads) levels may be reduced by inhibition of PcG-associated methyltransferase activity or histone demethylation and consequent eviction of PcG complexes. These changes result in shifting the balance of the bivalent chromatin state illustrated in the inset of Figure 1 to open chromatin and active transcription. In contrast, in the absence of activating transcription factors and/or in response to inhibitory signals (red arrows), PcG complexes may spread beyond the nucleation site to establish repression. The models of inactive and active genes are adapted from ref.⁵²