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Biofluids, Cell Mechanics and Epigenetics: Flow-induced Epigenetic Mechanisms of Endothelial Gene Expression

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Abstract

Epigenetics is the regulation of gene expression (transcription) in response to changes in the cell environment through genomic modifications that largely involve the non-coding fraction of the human genome and that cannot be attributed to modification of the primary DNA sequence. Epigenetics is dominant in establishing cell fate and positioning during programmed embryonic development. However the same pathways are used by mature postnatal and adult mammalian cells during normal physiology and are implicated in disease mechanisms. Recent research demonstrates that blood flow and pressure are cell environments that can influence transcription via epigenetic pathways. The principal epigenetic pathways are chemical modification of cytosine residues of DNA (DNA methylation) and of the amino tails of histone proteins associated with DNA in nucleosomes. They also encompass the post-transcriptional degradation of mRNA transcripts by non-coding RNAs (ncRNA). In vascular endothelium, epigenetic pathways respond to temporal and spatial variations of flow and pressure, particularly hemodynamic disturbed blood flow, with important consequences for gene expression. The biofluid environment is linked by mechanotransduction and solute transport to cardiovascular cell phenotypes via signaling pathways and epigenetic regulation for which there is an adequate interdisciplinary infrastructure

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CONFLICT OF INTEREST

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with robust tools and methods available. Epigenetic mechanisms may be less familiar than acute genomic signaling to Investigators at the interface of biofluids, biomechanics and cardiovascular biology. Here we introduce a biofluids / cellular biomechanics readership to the principal epigenetic pathways and provide a contextual overview of endothelial epigenetic plasticity in the regulation of flow-responsive transcription.

Keywords

hemodynamics; epigenetics; endothelial phenotype; gene transcription; disturbed blood flow; mechanotransduction

INTRODUCTION

The mechanical properties of blood flow are ever-present at the luminal cell surface of the vascular endothelium. This biofluid interface plays a central role in the health and pathology of the endothelium through flow-driven cell deformation and mechanotransduction (Davies and Helmke 2010) that has been etensively studied by gene and protein expression, metabolism, and function (Davies 1995, 2009; Chiu et al 2011; Gimbrone and Garcia-Cardena 2016; Rizzo et al 1998) Transcription of the genomic (DNA) code to mRNA ("gene expression") in preparation for protein synthesis is well described to be mechano-sensitive (Garcia-Cardena et al 2001; Dekker et al 2002; Passerini et al 2004). Cis/trans mechanisms involving distal and proximal control elements and transcription factors, several of which are master regulators of endothelial phenotype, readily respond to hemodynamic patterns *in vivo* and recapitulated flow characteristics *in vitro.* However, although it was long recognized that these classical mechanisms are insufficient to completely account for the suppression and activation of genes, elucidation of the prominence in epigenetic transcriptional regulation of non-coding regions of the human genome has only emerged recently. In cardiovascular research, epigenetic mechanisms are now linked to hypercholesterolemia (Schiano et al 2015), hypertension (Friso et al 2015), exercise (Horsburgh et al 2015) and cigarette smoking (Joehanes et al 2016). It is estimated that the epigenomic code accounts for the regulation of 30% of mammalian proteins. In vascular research, mostly microRNA (miRNA) regulation of gene expression has been studied (Zhou et al 2011; Kumar et al 2014). Other epigenetic pathways, however, that regulate chromatin structure are now identified with flow-mediated transcription. Here we introduce epigenetic mechanisms to a readership that may be less familiar with the emerging roles of biofluids and biomechanics in epigenetics, particularly in cardiovascular research, and outline the influence of cell epigenetic status including its plasticity in the regulation of endothelial transcription in flow-sensitive environments (Jiang et al 2014; Davies et al 2014; Dunn et al 2014).

EPIGENETIC MECHANISMS AND BIOFLUID MECHANICS

Figure 1 *outlines the packaging of DNA into nucleosomes that organize and condense to form the chromosomes in the cell nucleus.* In genetic regulation the fixed DNA code contains all of the sequence information and cis/trans elements required to facilitate gene expression. However, a powerful overlay (or *epi*-layer) of control is provided by flexible

epigenetic regulation of gene expression in which chemical modification of DNA and its associated histone proteins occurs *without a change to the DNA sequence.* The epigenetic tags result in conformational changes of DNA and histones that regulate gene expression. In some cases epigenetic modifications are fixed, stable and inherited, but in other instances they are dynamic and change in response to environmental stimuli. It is increasingly apparent that physical and biomechanical forces, including hemodynamic flow characteristics, are environmental stimuli that induce epigenetic regulation of gene transcription.

Main pathways

Gene expression can be turned on or off by three types of epigenetic pathways that operate to promote or inhibit mRNA synthesis or selectively degrade mRNA once it is made (Jaenisch and Bird 2003; Matouk and Marsden 2014; Turgeon et al 2014). Each epigenetic pathway is distinct but interacts with the others ensuring integration into a complex hierarchy of gene regulation:

a. *DNA methylation:* methylation of cytosine residues in DNA, often at or in proximity to a gene promoter,

b. *Histone code:* posttranslational modifications to histone amino-terminal tails, particularly acetylation that alters the compaction of DNA coiled around histones,

c. *Non-coding RNAs:* post-transcriptional targeting of mRNA by short and long ncRNAs (microRNAs and lncRNAs respectively) that facilitate transcript degradation. Since ncRNAs make up >97% of the transcriptome, there is vast potential for dynamic epigenomic* regulation of gene expression, including through biomechanical stimulation of the epigenomic code.

Figure 2 summarizes the overlayering of biomechanics-induced epigenetic changes onto genetic regulation of gene expression. Acute transcriptional responses to biomechanics (e.g. early response genes) are regulated by the activation of existing cis and trans factors. However a sustained stimulus alters the epigenetic equilibrium, changes the conformation of DNA and histones, and regulates access of transcription factors and other cis/trans regulators to their cognate DNA binding sites. The resulting regulation may be permissive (gene expression) or inhibitive (gene silencing).

Figure 3a outlines the transcription of DNA to mRNA after which the transcript is normally further processed by the cell; regulation of this step is where epigenetic pathways converge.

1. DNA methylation

DNA methylation adds a methyl group to the 5-carbon of cytosine residues (5-mC) in DNA, usually at cytosine-phosphate–guanine dinucleotides (CpG) sites. Figure 3b outlines methylation of a limited number of upstream CpG sites in the proximal promoter and/or

*Epigenomics: The global analysis of epigenetic changes across the entire genome

distal DNA regions resulting in suppression of gene transcription. The decoration of DNA by methyl groups changes its conformation and mechanical properties (Severin et al 2011) altering interaction with the transcriptional machinery of the cell. CpG sites tend to be more prevalent near gene promoters upstream of exon coding regions. Unmethylated CpG permits gene transcription; conversely, methylation of cytosines results in gene silencing.

Central regulators of DNA methylation

CpG sites are methylated by DNA methyltransferase (DNMT) enzymes. Three active mammalian methyltransferases DNMT1, 3A and 3B promote methylation which is reversed by DNA demethylation through the TET (tet methylcytosine dioxygenase) gene pathway. Flow-induced changes in DNMT1 and 3A have recently been reported (Davies et al 2014; Dunn et al 2014; Jiang et al 2014, 2015b; Zhou et al 2011). DNMT1 is primarily a maintenance enzyme to ensure fidelity of methylation patterns during somatic cell division whereas DNMT3A and 3B regulate *de novo* DNA methylation. While the methylation or non-methylated state of each cytosine/CpG site is a binary event, the overall status of a cytosine DNA region is a composite methylation score of multiple cells. Evaluation of the contribution that methylation of each cytosine (or cluster of CpGs) exerts on gene transcription can be assessed experimentally, e.g. for KLF4 (Jiang et al 2014), or surveyed on a chromosome-wide basis (Weber et al 2005, Jiang et al 2015a). Within disturbed flow regions *in vivo*, an inverse relationship between hypermethylation and mRNA expression generally exists when the CpG sites are upstream of the promoter but not when intragenic cytosines are methylated (Jiang et al 2015a).

Flow-mediated DNA methylation

Disturbed vs undisturbed flow regions *in vivo* predict atherosusceptibility or atheroprotection, respectively (Davies et al 2009; Chiu and Chien 2011). In the context of epigenetics, differential DNA methylation regions (DMR) map to vascular sites of disturbed and undisturbed flow (Jiang et al 2015a). These differences can be recapitulated *in vitro*(Jiang et al 2014) and, importantly, can be induced by surgical manipulation of flow *in vivo* (Dunn et al 2014). Complete methylated DNA immunoprecipitation sequencing (MeDIP-seq) of *in vivo* arterial endothelium associated with disturbed (*vs* undisturbed) flow in pigs (Jiang et al 2015a) identified differentially methylated DNA enrichment in exons and 5'UTR sequences of annotated genes, approx 60 of which are linked to cardiovascular disease. *Thus dynamic DNA methylation appears to be a genome-wide epigenetic mechanism of transcriptional control related to the local site-specific biomechanical stresses of flow.* Furthermore, disturbed flow promoted DNA hypermethylation that resulted in transcriptional suppression in human endothelial cells in culture (Davies et al 2014; Dunn et al 2014; Jiang et al 2014, 2015a). Collectively these studies in pigs and mice and cultured cells identified endothelial genes and their promoters as DMR-sensitive epigenomic targets associated with site-specific disturbed flow.

2. Histone remodeling

In the cell nucleus chromatin DNA is wrapped around proteins called histones to form a composite unit, the nucleosome (Figure 4a), in which ~146 bp of DNA is wrapped around

each histone octameric core (Kornberg and Lorch 1999). Whereas DNA methylation proceeds equally effectively in DNA that is wound around histones (Figure 4b) or in the linear arrangements of DNA between nucleosomes (*cf.* Figure 3b). The histone amino terminal domains extend from the core as 'tails' that can be modified by lysine acetylation and methylation, serine phosphorylation and lysine ubiquitination and sumoylation (Figure 4c). Modification of amino acid residues of histone tails profoundly changes the biophysical association between DNA and histone protein influencing its compaction state. The degree of compaction or relaxation of DNA wound around histones is instrumental in allowing or preventing access to the DNA by transcription factors, repressors, activators and other cofactors of cis/trans transcriptional regulation.

The most dynamic (and reversible) regulation of histone-wound DNA relaxation is lysine acetylation that allows access of transcriptional regulators to cis-DNA binding domains (Figure 4d) presumably by neutralizing the strong positive charge of lysine. In contrast deacetylated histone tightly condenses the chromatin creating a barrier to small molecule regulators and preventing DNA transcription (Shahbazian and Grunstein 2007).

Central regulators of histone acetylation

Histone acetylases (HAT) and deacetylases (HDAC) catalyze the net acetylation state usually in conjunction with multi-protein complexes (Figure 4d).

The other dominant histone tail modification is the methylation of lysine and arginine, which is regulated by histone-N-methyltransferases. Combinations of particular methylated histone tail residues and their degree of methylation (histone "marks") can promote or prevent transcription by histone-DNA compaction. Histone methylation may be independent of DNA methylation at the promoter region (Roh et al 2008). The net effect of these dynamic histone modifications, all of which are posttranslational, is to control accessibility to the nucleosomal DNA by other regulatory proteins, cofactors and transcriptional inhibitors.

Flow-mediated histone remodeling

The molecular basis for epigenetic histone modification by shear stress in endothelial cells was established in cultured human umbilical vein endothelial cells (Illi et al 2003); activation of histone H3 phosphorylation and H3/H4 acetylation by flow (*vs* no-flow) was increased by an HDAC inhibitor. However, to recapitulate *in vivo* flow characteristics (where flow is rarely stationary) comparisons of pulsatile disturbed *vs* undisturbed flow are necessary. Disturbed flow increased HDAC3 expression leading to protection against apoptosis by forming a complex with the serine/threonine protein kinase Akt, thereby promoting cell survival (Zampetaki et a 2010). A more comprehensive survey of HDACs in disturbed flow demonstrated upregulated expression and nuclear accumulation of HDAC1/2/3 and HDAC5/7 whereas undisturbed flow promoted nuclear export of HDACs 5 and 7 (Lee et al 2012). The protective antioxidant transcription factor NF-E2–related factor 2 (Nrf2) was deacetylated by association with HDAC1/2/3 in disturbed flow (Fledderus et al 2008), and a NAD-dependent HDAC, Sirtuin 1 (SIRT1), that protects against oxidative stress prevalent in disturbed flow regions *in vivo* was shown to associate with endothelial NOS (NOS3), promoting its availability (Chen et al 2010).

3. MicroRNA and lncRNA -associated gene regulation

RNA derived from non-coding sequences in the transcriptome (ncRNAs) can regulate gene expression by targeting newly synthesized mRNA for degradation. Short 20–26 nucleotide microRNAs (miRNA) and long non-coding RNAs (lncRNA; >200 nucleotides) are prominent examples.

Micro-RNAs (miRNAs)

miRNAs, are highly-conserved small RNAs that regulate post-transcriptional gene expression by binding to target mRNA, promoting mRNA degradation and/or inhibiting translation of the protein-coding genes (Ambros 2004) as outlined in Figure 4e[i]. Pairing between cognate mRNA 3' UTR and miRNA 5' regions is centered on seed nucleotides (2–7nt) that primarily determine miRNA target selection (Bartel 2009). Individual miRNAs fine-tune the synthesis of many genes. Given the widespread scope but modest repression of transcriptomes by individual miRNAs, net cell phenotypes likely result from the coordinated actions on multiple targets by single miRNAs or integrated regulation of key pathways by multiple miRNAs (Liang 2009). Consequently there are many interactions between miRNAs and epigenetic DNA methylation and chromatin remodeling (Chuang and Jones 2007). For example, miRNAs can be involved in establishing DNA methylation by targeting methyltransferases DNMT1, 3a, and 3b (Rajewsky 2006). In turn, DNMT inhibitors such as 5-azacytosine that inhibit DNA methylation can affect miRNA expression, e.g. upregulation of miR127 (Saito et al 2006). Similar reciprocities were reported with respect to chromatin structure through miRNA regulation of key histone modifiers such as HDAC4; at the same time 4-phenylbutyric acid, an HDAC inhibitor, upregulated some miRNAs. Collectively, these miRNA epigenetic interactions represent complex dynamic regulation at multiple levels. It is likely that many of the flow-sensitive miRNAs identified to date contribute to epigenetic chromatin structure-function via mechanotransduction-specific DNA methylation and histone modifications.

Flow-mediated miRNAs

MicroRNAs have been extensively studied in the endothelium especially in inflammation, atherogenesis and angiogenesis and have been shown to play important roles in flow-induced endothelial cell responses both *in vivo* and *in vitro* (Fang et al 2010, 2012; Zhou et al 2011; Kumar et al 2014). The co-localization of disturbed flow and endothelial activation by the pro-inflammatory transcription factor NFkB is highly relevant to the identification of miRNAs −10a, −92a, −146, −155 and −181b as negative regulators of the NFkB signaling pathway (Fang et al 2010; Cheng et al 2014). Although miRNAs exist as a diverse interactive network, the loss/gain of function of only a single miRNA can lead to quite focused physiological effects *in vitro* and *in vivo* (Daniel et al 2014) suggesting a narrower therapeutic potential than might be expected.

Long noncoding RNAs (lncRNAs)

There is currently much interest in epigenetic regulation by a diverse population of lncRNAs of which there are several thousand in the mammalian genome. It is estimated that each annotated gene is overlapped by multiple lncRNA isoforms. There is no common

mechanism of action and they can both promote and suppress transcription by interactions with DNA, mRNA and histones (Figure 4e[ii]). lncRNA 'archetypes' include cis and trans targeting, enhancement, decoy, scaffold, conformational and coactivation/corepressor mechanisms (Lee 2012). The association of lncRNA with cardiovascular diseases (Uchida and Dimmeler 2015) attests to widespread involvement in heart and blood vessel pathophysiology at multiple levels. In an endothelial study, *in vitro* silencing of the lncRNA MALAT1 promoted change from a proliferative to a migratory endothelial phenotype while MALAT1 deletion or inhibition *in vivo* reduced vascular growth (Michalik et al 2014). The role of lncRNAs in endothelial inflammatory pathways is currently unknown despite a strong impetus to further identify the lncRNAs involved in controlling the vascular inflammatory response (Kumar et al 2014).

lncRNAs in Flow

In contrast to miRNAs, lncRNAs are only recently under investigation in flow mechanotransduction and without publications to date. An abstract (Hartung et al 2014) reported that simple laminar flow *vs* no-flow in vitro) regulates metalloprotease AMZ2 expression via 'lncRNA2' binding to the repressive chromatin mark H3K27me3 (methylation of lysine 27 within histone H3).

Despite current challenges in functional interpretation confounded by isoforms and multiple interactions, intense interest in lncRNA will likely yield important principles about their role in endothelial cell regulation.

DISCUSSION

Variation in genomic DNA sequence alone incompletely explains risk factor associations with cardiovascular diseases, suggesting a significant role for epigenetics (Webster et al 2013); tying into this concept is hemodynamics as a risk factor for atherosusceptibility (Davies et al 2013; Gimbrone and Garcia-Cardena 2016). Epigenetic sensitivity of cells to the biomechanical environment is intrinsic to an understanding of mechanotransduction mechanisms.

Flow studies suggest a mechano-sensitive physiological regulatory role for DNA methylation in addition to its well-studied functions in development and differentiation, many of which are permanent. However, it is currently unknown if the DNA methylation status of most endothelial genes is fixed *in vivo* during development or whether the flow responses are readily reversible and/or inheritable. For example, while a methylome 'snapshot' of pig endothelial HOX genes in disturbed flow regions revealed most to be differentially methylated compared to undisturbed flow (Jiang et al 2015a), only HoxA5 changed its methylation score *in vivo* in mice following the surgical induction of disturbed flow in a region of previously undisturbed flow (Dunn et al, 2014). Therefore it is conceivable that only a subset of genes demonstrate epigenetic plasticity to hemodynamics, an important consideration that is addressable by systematic combined MeDIP-sequencing/RNA-sequencing of flow-exposed cells in vitro and further manipulation of flow in vivo. To date, only a small number of gene subsets responsive to hemodynamic characteristics have been identified from *in vivo* manipulations and MeDIP studies of the methylome. In contrast

to DNA methylation, most histone modifications are rapid and reversible, particularly histone acetylation and methylation which elicit dynamic epigenetic regulation; determining the time constants under flow conditions of chromatin state change (through histone marks) is an attractive quest complementary to DNA methylation. Endothelial flow studies of posttranscriptional regulation of mRNA by ncRNAs, the third arm of epigenetic regulation, are most active for small miRNAs with rapidly increasing interest in lncRNA as more tools become available. Each of these mechanisms is likely involved in the short and long term maintenance and adaptability of endothelial phenotypes in the cardiovascular system with important epigenetic implications for the initiation and development of disease through strong biophysical and biomechanical regulatory input.

DNA methylation, chromatin remodeling by histone modification and ncRNA transcriptional regulation are driven by enzymatic activation (DNMTs, HDACs, HATs) that in turn are themselves subject to complex regulation that appear to be directly influenced by flow/biomechanics. A major challenge is to define the mechanisms of flow specificity of such fundamental widespread regulators in the cell. Measurement of the effects of specific molecular knockdown are useful but not definitive and potential therapeutic inhibitors of, for example, DNMTs do not discriminate between DNMT1 and 3A both of which have been demonstrated to be inducers of flow-responsive DNA methylation (Zhou et al 2011; Jiang et al 2014; Dunn et al 2015). As in flow-mediated endothelial mechanotransduction, there are epigenetic flow responses that converge with, as well as parallel, classic pathways of cell metabolism or occupy critical positions as pathway nodes. Since they are integrated into overall cell regulation, their relative importance will emerge from hierarchical analyses under normal and stressed conditions. In addition to Hox genes for example, inflammatory pathway genes, Kruppel-like Factors (KLFs), anti-oxidant transcription factor (Nrf2) and ER-stress responders are of topical interest.

The ability of the endothelium to respond to disturbed flow by sensing different hemodynamic forces may reside in subcellular spatio-temporal mechanotransduction criteria transmitted throughout the cell by the cytoskeleton and converted to chemical/metabolic responses (mechanotransduction) at multiple decentralized subcellular locations (Davies 2009; Davies and Helmke 2010; Figure 5) including transmission of stress to the nucleus (Maniotis et al 1997). Changes in the epigenome could be directly attributable to strain-induced nuclear structural changes and/or be secondary to mechanotransduction at other subcellular locations (Dolmetsch et al 2001) including the viscoelastic cytoskeleton itself (Janmey 1998). While flow–induced stresses are directly transmitted to the cytoskeleton and distributed widely throughout the cell (Davies and Helmke 2010), the measurements are microscale; the conversion from stress/strain to cell chemistry remains unknown and may occur at the nanoscale as conformational changes in single proteins; for example nucleic acid polymers can be considered as prestressed structures (Ingber 1998).

An important additional consideration is that convective transport in disturbed flow is significantly changed. Site-specific flow separation in arteries creates transients vortices of average lower flow velocity/shear stress within which solute and particle retention times are significantly longer than in undisturbed flow. The artery wall is constantly producing reactive oxidant species (ROS) such as free radicals and other potent intermediary

metabolites. Active peptides and hormones generated from precursors in the blood are rapidly metabolized at the vessel surface. This may be why disturbed flow ROS is higher in flow separated regions than in nearby undisturbed flow regions (Civelek et al 2011) creating an unbalanced local environment that may influence epigenetic pathways. Oxidative damage is known to target assembly complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands (O'Hagan et al 2011). Since the NFkB pathway is also more active in disturbed flow (Passerini et al 2004) and Rel/p65 is able to recruit DNMTs to specific genome loci (Liu et al 2012), these pathways may influence DNMT enrichment and DNA methylation.

FUTURE PERSPECTIVE

The epigenomic code therefore has the potential to figure prominently in disease susceptibility and pathogenesis arising from environmental influences independent of, or in concert with, mutations and single nucleotide polymorphisms (SNP) linked to multifactorial diseases, including hypertension and cardiovascular disease. The role of disturbed flow as a hemodynamic regulator of epigenomic DNA methylation-mediated and histone-mediated gene expression may play a prominent role in endothelial vascular function and dysfunction. Nevertheless, the cellular mechanisms that link hemodynamics and biomechanical stress to the epigenomic code remain relatively unexplored.

A mature infrastructure now exists to support such studies. Lowering of barriers between traditional disciplines has led to collaborative and integrated research in well established interdisciplinary departments, institutes and centers that also provide formal training at the graduate student and postdoctoral levels. In addition to bioengineering departments, most mechanical and chemical engineering and materials science departments are heavily involved in biology and biomedical projects through collaborative or integrated (Center) mechanisms. There is complementary integration of engineering and physical sciences into academic medicine where the practice and teaching of rigorous quantitation alongside descriptive biomedicine has been significantly enhanced and where engineering approaches are embedded in research. Challenges that arise in epigenetic research are addressable because of resources and tools that were previously unavailable. These include affordable next generation sequencing facilities, sophisticated high resolution MRI applied to 4D flow as well as to tissue structure, the computing power for bioinformatics analyses of sequencing datasets and for complex computational modeling of dynamic systems, and great precision in gene editing of cells both in vitro and in vivo. The formation of national and international epigenomic consortia such as the ENCODE (ENCyclopedia Of DNA Elements) Project (Abbott 2010; National Human Genome Research Institute et al 2004) facilitate academic interactions.

Given the associative relationships between risk factors and cardiovascular disease, the interplay of mechanotransduction and epigenomic regulation of vascular phenotypes - with the potential for novel therapeutic interventions - warrants further investigation.

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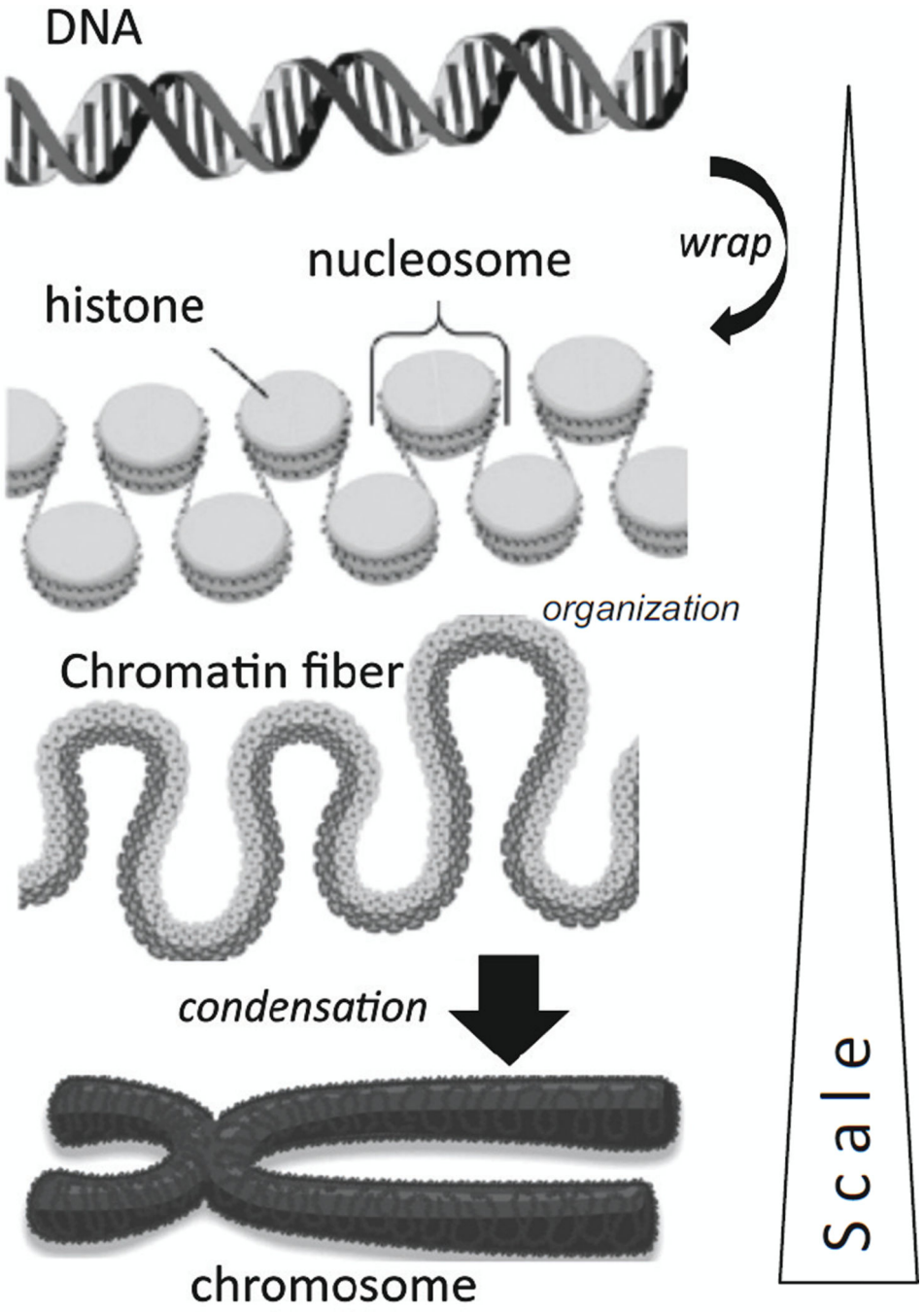

Figure 1.
In the nucleus, DNA wraps around histone proteins to form linked nucleosomes that organize into chromatin fibers that condense to form a chromosome.

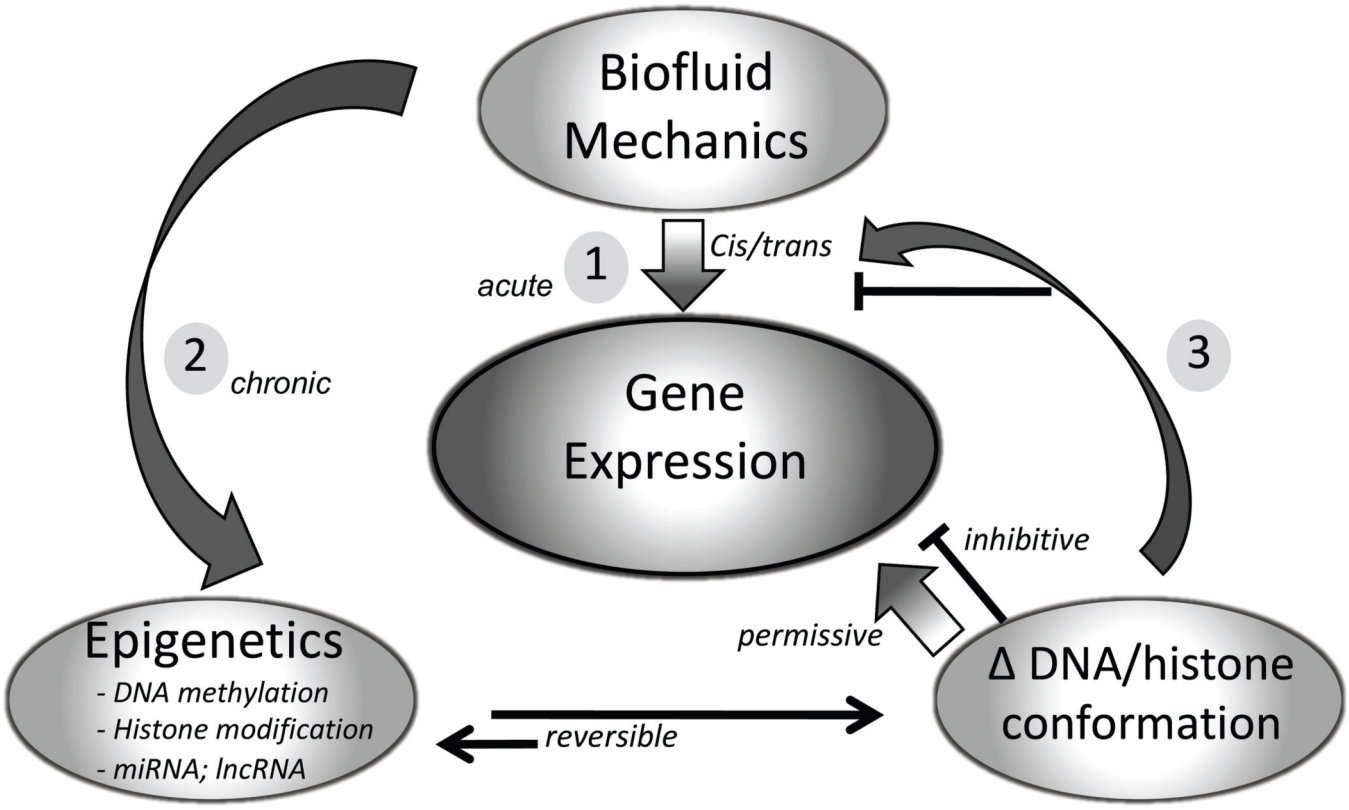

Figure 2.

Summary of mechano-regulation of gene expression by epigenetic modification of DNA/histone biophysical conformation. Regulation of transcription occurs by cis/trans mechanisms at any existing state of epigenetic status (1). However, mechanotransduction can also induce changes in epigenetic status (2) for selective genes – usually over longer periods (24h+ for flow). The resulting DNA/histone state directly silences/permits transcription or indirectly regulates upstream molecules (3) involved in cis/trans gene expression. The net effect is epigenetic oversight of transcription spread throughout the genome.

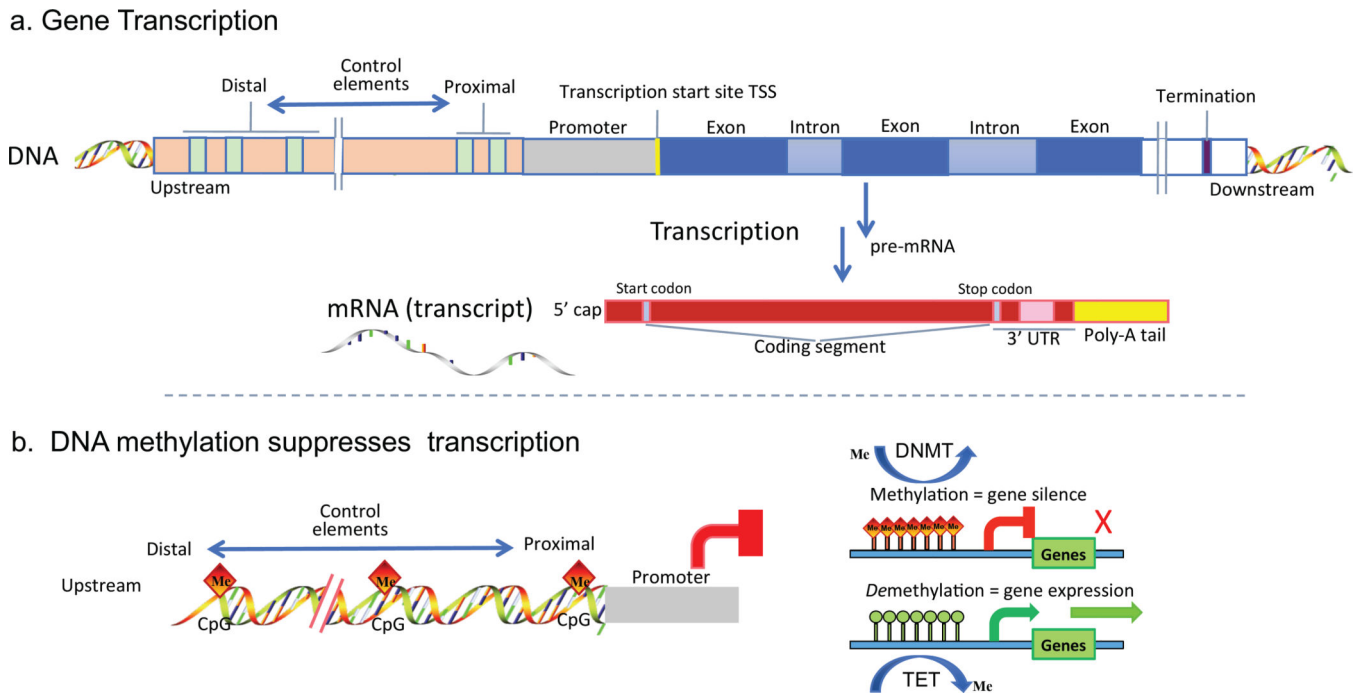

Figure 3.

a. Gene transcription. Outline of mRNA synthesis (transcription) from a typical DNA region illustrating the coding region (blue), upstream promoter region (green) and proximal and distal cis/trans control elements. Single strand mRNA is copied from the coding region, the introns are removed from pre-mRNA and the exon information with start and stop codons are capped and tailed to form a discrete transcript (mature mRNA). **b**. DNA methylation. Methylation of cytosines of cytosine-guanosine dinucleotides (CpG) at or proximal to a promoter in DNA block transcription. The methylated DNA interacts differently with the transcript machinery of the cell. DNMT enzymes coordinate the methylation step in concert with binding protein complexes (not shown). For some genes, silencing by DNA methylation may be reversible (demethylation) by enhanced TET enzyme activity.

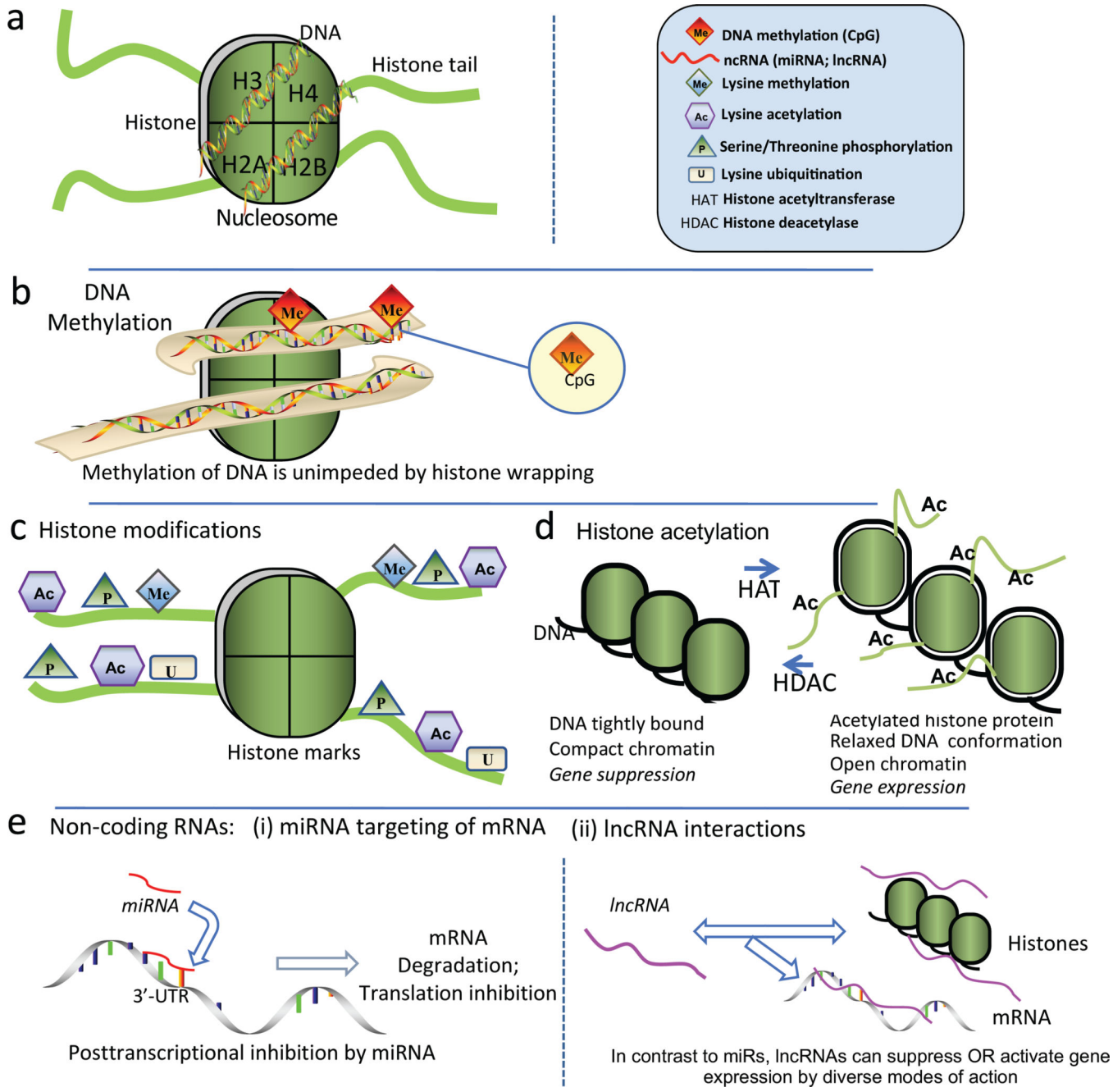

Figure 4.

a. Nucleosome structural elements. DNA is wrapped around each histone octomer protein that has basic amino acid-rich tail domains. Each core contains two copies of the histones H2A, H2B, H3 and H4, the amino terminal domains of which extend from the core as basic amino acid-rich 'tails' that can be modified, principally by acetylation and methylation (see Figure 4c) **b**. Proximal promoter DNA methylation at CpG islands results in gene silencing by transcriptional repression (*cf.* Fig 4b). **c**. Posttranslational 'marks' on N-terminal histone tails constitute a histone code; they include lysine acetylation and methylation, serine phosphorylation and lysine ubiquitination and sumoylation that remodel chromatin by

changing histone protein-DNA association to influence transcriptional regulation. **d**. Chromatin remodeling by HATs and HDACs. Histone acetylation relaxes tightly wound compact DNA to enable access of transcriptional regulators. HDACs deacetylate to restore compact chromatin and prevent transcription. **e**. Posttranscriptional RNA-based mechanisms include short (microRNAs) and long noncoding RNAs (lncRNAs). **e[i]**.miRNAs target the 3'-untranslated region of mRNA promoting transcript degradation and also translation destabilization. **e[ii]**. lncRNA facilitate changes of histone methylation through complex mechanisms that can result in enhanced or repressed gene expression depending upon the histone mark. Modified from Jiang et al (2015b).

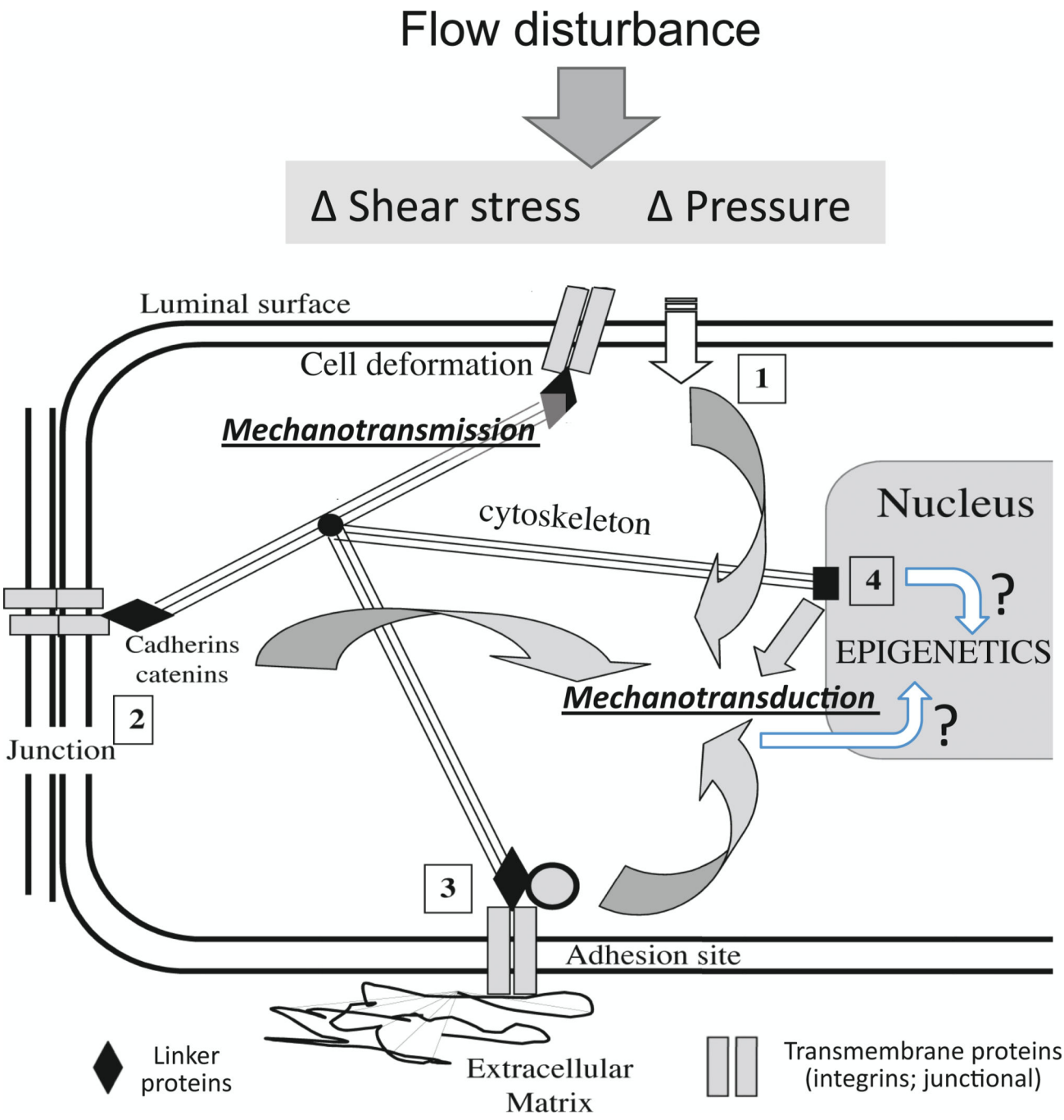

Figure 5.

In a decentralized model of flow-induced endothelial mechanotransduction (Davies 1995), the cytoskeleton plays a central role in mechanotransmission of tensional changes throughout the cell that distributes forces to multiple subcellular locations. Mechanical forces at the luminal surface (1), cell junctions (2), adhesion sites (3) and nuclear structures (4) can elicit mechanotransduction responses. The mechanotransduction sites are not mutually exclusive and are biochemically interconnected.

Epigenetic status (which is entirely nuclear) is influenced by two broad mechanisms (marked by ? in the figure): - (i) by the local deformation of nuclear structure (Maniotis et al 1997) or (ii) by downstream second messengers in extranuclear mechanotransduction pathways that enter the nuclear space (Dolmetsch et al 2001). It is notable that cell fate decisions in differentiating stem cells can be manipulated by extracellular substrates and/or stiffness changes that alter nuclear mechanics (Buxboim et al 2010). The powerful dependence of cell fate decisions upon DNA methylation suggests a strong link regulation of transcription by nuclear mechanics through epigenetic pathways. Alternatively cytoplasmic second messengers from plasma membrane receptors, junctional, cytosketal and adhesion site mechanotransduction are free to move into the nucleus to regulate transcription by activating DNMTs, HATs, HDACs and other epigenetic enzymes. Modified from Davies (2009).