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INVITED REVIEW

Epigenetic regulation and mechanobiology

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Abstract Mechanical force regulates a variety of cellular functions through inducing modulations in nuclear chromatin structures and epigenetic landscapes (outside in). The epigenetic modifications, in turn, regulate gene expressions and affect phenotypic outcomes, including cytoskeletal organization and cell–cell/cell–ECM interactions (inside out). While there have been significant advances in the understanding of mechanotransduction in the nucleus, there is still a lack of knowledge on the potential mechanisms through which mechanical cues affect epigenetic and chromatin regulations to determine genetic outcomes. This review firstly focuses on the current understanding of epigenetic regulations and then summarizes how mechanotransduction and epigenetic modification couple together to regulate molecular and cellular functions, eventually causing functional phenotype changes *e.g.*, diseases. Lastly, we introduce related technologies for mechanistic studies, particularly fluorescence resonance energy transfer (FRET) biosensors for the visualization of dynamic epigenetic regulations in single living cells, as well as the applications of FRET biosensors to visualize mechanotransduction events occurring in the nucleus. These studies could provide new insights into epigenetics in regulating the physiological and pathological processes in living cells under different mechanical environments.

Keywords Epigenetic modification, Mechanobiology, Live cell imaging, Fluorescence resonance energy transfer (FRET)

INTRODUCTION

Epigenetics, changing gene expression without altering the DNA sequence, plays an essential role in governing genomic regulation and ultimately cell fates (Li *et al*. 2012). It has been well documented that external mechanical force can be transmitted from the cell membrane through the cytoplasm to the nucleus, which can induce modulations in nuclear chromatin structures and epigenetic landscapes to impact gene transcription (Irianto *et al*. 2016; Kelkhoff *et al*. 2016; Peng *et al*. 2016; Wang *et al*. 2016). However, it is still largely unclear how epigenetics is regulated spatiotemporally in relation to genomics. Due to cell–cell heterogeneity, conventional ChIP-Seq technology is insufficient for measuring the dynamic nature of epigenetic signals crucial for cellular function in single cells (Li *et al*. 2012). Genetically encoded biosensors based on fluorescent proteins (FPs) and fluorescence resonance energy transfer (FRET) could be powerful tools for single live cell imaging to elucidate the

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mechano-chemical mechanisms underlying the adaptive epigenetic response and cell fate to understand nuclear mechanotransduction.

EPIGENETIC REGULATION AND MECHANOBIOLOGY IN THE NUCLEUS

Chromatin

Chromatins are highly ordered nuclear structures that contain DNA, histones, and other chromosomal proteins. The fundamental unit of chromatin is the nucleosome, which is wrapped by 147 base pairs of genomic DNAs. The nucleosome consists of two copies of the four core histones (H): H2A, H2B, H3, and H4, which are tied together by the linker histone H1. Each histone within the nucleosome contains a flexible N-terminus tail (Black *et al*. 2012; Kornberg 1974; Kornberg and Thomas 1974).

Epigenetic regulation of the chromatin is often achieved through the modulation of nucleosomes. It is the ensemble of mechanisms of concurrent chromatin modification that modulates global manners without affecting the DNA sequence itself. Such epigenetic regulations mainly include DNA methylation (Laird 2003), histone post-translational modifications (PTMs) (Jenuwein 2001), chromatin remodeling (Zhang and Reinberg 2001), and non-coding RNAs (ncRNAs); all these regulations exert their various functions in a coordinated manner (Allis and Jenuwein 2016). DNA methylation occurs on nucleotide cytosine next to guanine (CpG) to regulate the transition of chromatin states. During DNA methylation, DNA methyltransferases (DNMTs) aid the covalent addition of a methyl group from S-adenosylmethionine to the 5′ position of cytosine (Yu *et al*. 2011). DNA methylation is known to affect the formation of constitutive heterochromatin. Histone PTMs are also important for chromatin geometry and gene expression (Inaba *et al*. 2015). Histone tails can combine and undergo various PTMs, allowing regulatory proteins to access the genome dynamically (Önder *et al*. 2015). In addition to histone PTMs, chromatin remodeling can be achieved by ATP-dependent chromatin-remodeling complexes, during which ATP hydrolysis is used to alter the histone–DNA interactions. (Lusser and Kadonaga 2003). On the other hand, nucleosome positioning also contributes to epigenetic regulations. It has been found that nucleosomes assemble in heterogeneous groups of varying sizes interspersed with nucleosome-depleted regions. RNA polymerase II preferentially associates with smaller clutches, while linker histone H1 and heterochromatin are more enriched in larger ones, suggesting that transcription can be activated more preferentially in smaller clutches (Ricci *et al*. 2015). This review mainly focuses on histone post-translational modifications.

Chromatin and histone PTMs

The first histone modification reported was histone acetylation in the early 1960s (Allfrey *et al*. 1964). Since then, more than 100 different histone PTMs have been identified and categorized (Abranches *et al*. 2013; Cota *et al*. 2013; Goss 2014; Kouzarides 2007; Luger *et al*. 1997; Tan *et al*. 2011) (Table 1). Currently, six major PTMs are known to occur on histone tails in cells: acetylation, methylation, phosphorylation, adenosine diphosphate (ADP)-ribosylation, SUMOylation, and ubiquitylation, each with their own distinct functions and regulatory mechanisms.

Histone PTMs are important for the activation state of both promoters and enhancers. Histones are primarily modified on the lysine and arginine residues on histone tails of the H3 and H4 subunits. Histone PTMs have been shown to play central roles in regulating gene expression by modulating chromatin assembly and by coordinating the recruitment of specific readers or other chromatin-modifying or chromatin-remodeling enzymes (Strahl and Allis 2000). Distinct histone modifications are dynamically catalyzed or erased by specific enzymes in response to environmental challenges, while new types of biologically important PTMs, their modifiers, and their readers are continuing to be identified (Andrews *et al*. 2016).

Histone acetylation is the first reported PTM to be associated with gene activation and has since been identified as the most frequent PTM (Allfrey *et al*. 1964). Histone acetylation and deacetylation are observed on conserved lysines of the N-terminal tails of all four core histones and are conducted by histone acetyltransferases (HATs) and histone deacetylase (HDACs), respectively. The HATs catalyze the acetylation of specific lysines within histones by transferring an acetyl moiety from the cofactor acetyl-CoA to the lysine, which neutralizes the lysine's positive charge and potentially weakens the interactions between histones and DNA for chromatin remodeling, a process important to gene expression (Fig. 1). HDACs, on the other hand, catalyze the reverse reaction and are also generally associated with gene repression (Bannister and Kouzarides 2011; Lee and Grant 2019). Histone acetylation has a significant effect on gene expressions. Researchers recently discovered that some cancer cells with increased acetylation levels on H3K18 and H3K27 share the characteristics of truncated acetyltransferase genes

Table 1 Classes of post-translational histone modification

Histone modification	Residues modified	Putative function
Acetylation	Lysine, serine, threonine	Transcription, repair, replication, condensation
Methylation	Lysine, arginine	Transcription, repair
Phosphorylation	Serine, threonine	Transcription, repair, condensation
Ubiquitination	Lysine	Transcription, repair
SUMOylation	Lysine	Transcription
ADP ribosylation	Glutamic acid	Transcription

A selection of the common varieties of histone PTMs including the residues modified and putative roles for the modifications. This list is not comprehensive (Abranches *et al.* 2013; Goss 2014; Kouzarides 2007; Tan *et al.* 2011)

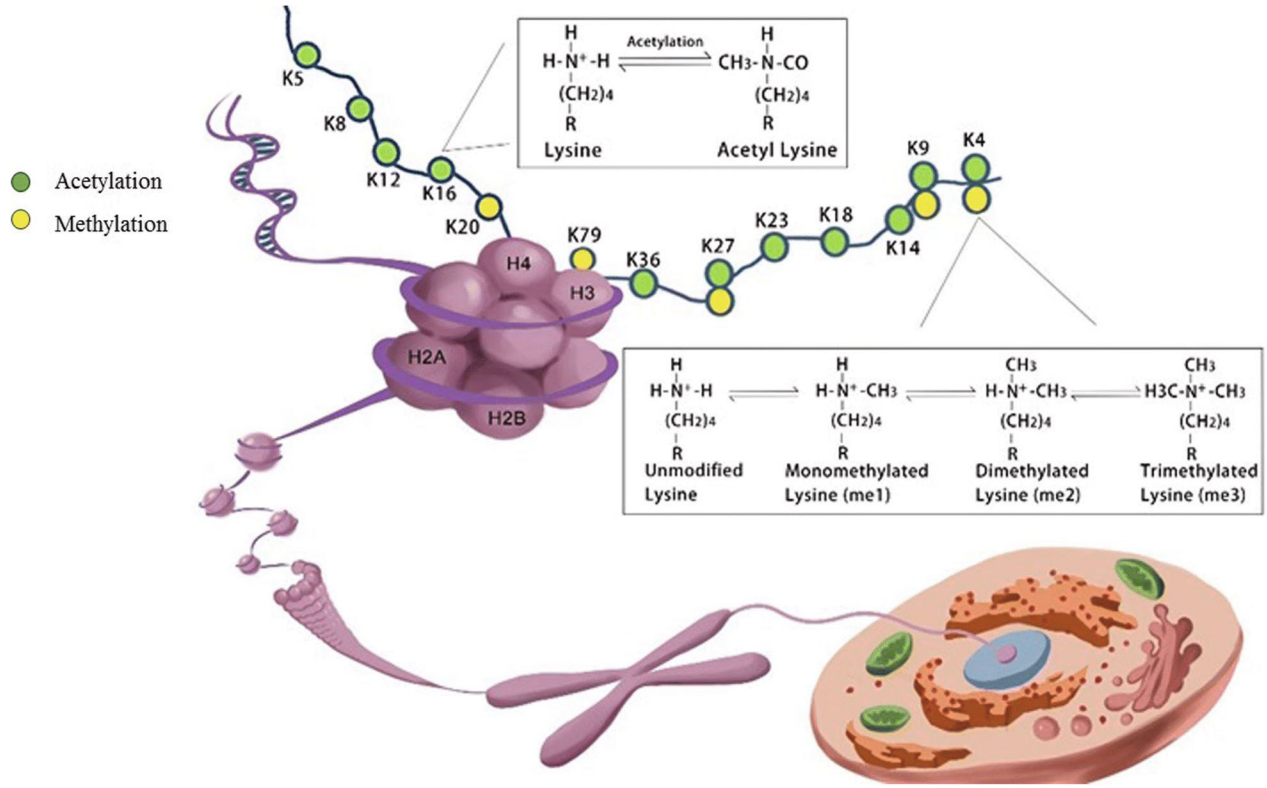

Fig. 1 Schematic representation of a nucleosome. A nucleosome functions as the fundamental packing unit of chromatin. Different PTMs (mainly acetylation and methylation) at core histones and the processes of these modifications are also shown (He *et al.* 2018)

EP300 and CREBBP, suggesting a functional consequence of malfunction in epigenetic factors (Ghandi *et al.* 2019).

Histone methylation mainly occurs in lysines and arginines. Lysine residues can be mono-, di-, or trimethylated, whereas arginines can be mono-methylated or symmetrically or asymmetrically di-methylated (Fig. 1) (Han *et al.* 2019). The different levels of methylation greatly increase the complexity of histone modification and regulation of gene expressions (Bannister and Kouzarides 2005, 2011; Bedford and Clarke 2009; Ng *et al.* 2009; Xhemalce *et al.* 2011; Han *et al.* 2019). Histone lysine methylations have been found on a range of lysine residues in various histones, including K4, K9, K27, K36, and K79 residues in histone H3, K20 in histone H4, K59 in the globular domain of histone H4 (Dillon *et al.* 2005), and K26 in histone H1B (Cai *et al.* 2010). The methylation level is controlled by enzymes called histone methyltransferases (HMTs) and histone demethylases (HDMs) that possess strong substrate specificity. Histone methylation does not alter the charge of histone proteins, unlike the case with histone acetylation and phosphorylation. Transcriptional activities can be regulated by the methylation of histone tails

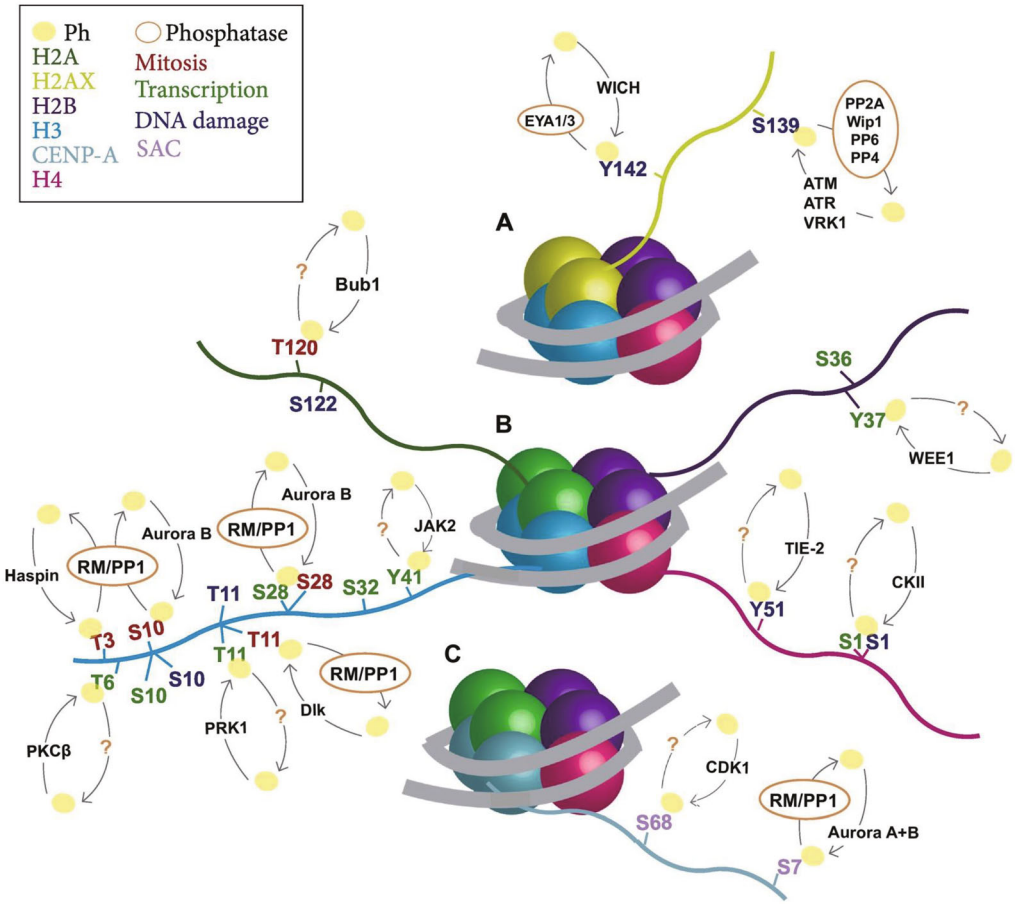

Fig. 2 Histone phosphorylation regulation model. The main phosphorylated residues of histones are shown, with the corresponding kinases and phosphatases. Residues in *red* indicate phosphorylation during mitosis; in *green* during DNA transcription; in *blue* during DNA damage; and in *light purple* during spindle assembly checkpoint (SAC). Histone H2AX is colored in *light green* (**A**), H2A in *dark green* (**B**), H2B in *purple*, H3 in *blue*, H4 in *pink*, and CENP-A in *light blue* (**C**). Phosphatases involved are highlighted in *orange*. RM: Repo-man; PP1, PP2A, PP5, and PP6: protein phosphatase 1, 2A, 5, 6, respectively. ATM: ataxia-telangiectasia mutated; ATR: ataxia-telangiectasia and Rad3-related protein; VRK: vaccinia-related protein kinases; JAK: Janus kinase; PKC: protein kinase C; Dlk: death-associated protein (DAP)-like kinase; PRK: phosphoribulokinase; CKII: casein kinase II (Gil and Vagnarelli 2019)

which function as a recognition motif to recruit effector proteins to local chromatin regions (Cloos *et al*. 2008). Thus, histone lysine methylation can be associated with either activation or repression of transcription, depending on the functions of the effectors.

Histone phosphorylation is another highly dynamic mark that takes place on serines, threonines, and tyrosines, predominantly in the N-terminal histone tails (Xhemalce *et al*. 2011). The phosphorylation level is controlled by kinases and phosphatases that add and remove the phosphate group, respectively (Oki *et al*. 2007). Histone kinases catalyze the addition of a phosphate group from ATP to the hydroxyl group of the target amino acid side chain, which increases the negative charge of histone to affect the chromatin structure (Fig. 2). The specific histone phosphorylation modifications are regulated during different stages of the cell cycle, involving different sets of kinases and phosphatases. For instance, the proteins phosphatase 1 (PP1) can rapidly neutralize the action of Aurora B kinase, which causes a large-scale genome-wide phosphorylation events at H3S10ph and H3S28ph during mitosis (Goto *et al*. 2002; Sugiyama *et al*. 2002). However, it is still unclear how the kinases are accurately recruited to the phosphorylation sites on histones at chromatins. Even less is known regarding the roles of histone phosphatases, although it is clear that a high level of phosphatase activity exists in the nucleus to direct a rapid turnover of histone dephosphorylations (Bannister and Kouzarides 2011).

Among the different HMTs, SUV39H1 is the first identified histone lysine methyltransferase (HKMT)

which targets H3K9 for tri-methylation (Bannister and Kouzarides 2005). Many pieces of evidence suggest a direct role of histone H3K9 methylation as a histone marker, which is positively correlated with DNA methylation and participated in repressive heterochromatin formation in tumorigenesis. In the cell cycle, H3K9me3 is also important for HP1 recruitment to regulate gene expression, chromatin packaging, and heterochromatin formation (Dormann *et al*. 2006). However, the dynamics of H3K9me3 remains controversial, mainly due to the lack of the appropriate tool for H3K9me3 detection in living cells.

The interactions between different histone chemical modifications show that the same modification of different histone residues can modify the occurrence of other modifications. This process is also regulated by other histone residues. On the other hand, different modifications of the same amino acid residue on histones may be synergistic or antagonistic at the same time. The interaction between different modifications of the same histone is called *cis*-effect, and the interaction between modifications of different histones is called *trans*-effect.

Epigenetic regulations in relation to the cell cycle

The cell cycle consists of a chain of inter-connected events, during which DNA is accurately duplicated, and chromosomes are segregated into two genetically identical daughter cells. These events outline the two major phases of the cell cycle: the synthetic phase (S-phase), where DNA replication takes place, and the mitotic phase (M-phase), characterized by chromosomal segregation followed by cellular division. These phases are separated by two gap phases, G1- and G2-phase, respectively, during which cellular growth and checkpoint controls occur. The process of a cell cycle is controlled by chromatin modifiers. Locally, chromatin modifiers control the expression of individual genes; globally, they control chromatin condensation and chromosome segregation (Fig. 3). It is reported that the histone tail modifications on H3 and H4 are required for the normal progression of the cell cycle (Morgan *et al*. 1991). Indeed, many histone modifications are of fundamental importance across cell types (Ernst *et al*. 2011).

The S-phase is a crucial stage of the cell cycle where histones and DNA modifications must be deposited correctly to the newly synthesized chromatin. Global methylation levels of the histone H3 and histone H4 tails have been detected to increase in late G1- and S-phase (Bonenfant *et al*. 2007). H3K79 di-methylation by KMT4/Dot1 is required for efficient entry into the S-phase, which is an example of transcriptional control of cell cycle genes (Schulze *et al*. 2009). The overexpression of KDM4A, an H3K9me3 demethylase, results in a faster progression through S-phase, possibly due to the better chromatin accessibility increase in replication forks and alteration of replication timing at heterochromatin regions (Black *et al*. 2012). Conversely, the loss of KDM4A in MDA-MB-231 breast cancer cells leads to a G1/S arrest and decreased proliferation rates (Black *et al*. 2010). Chromatin microenvironment can also affect the cell cycle indirectly by changing the replication timing. For example, KMT6/EZH2, by regulating epigenetic H3K27me3 levels, can target transcriptional regulations of cell cycle proteins Cyclin D1, E1, and A2 (Black and Whetstine 2011; Bracken *et al*. 2003). H3K9Ac, which peaks during G1-phase, has been reported to remain high during S-phase together with H4K16Ac (McManus and Hendzel 2006; Rice 2002). In contrast, H4K20 and H3K79 methylations were not detected on newly deposited histones until later time points in the cell cycle (Scharf *et al*. 2009).

The G2-phase of the cell cycle is a short phase marked by significant protein synthesis. Histone PTMs often maintain across the G2-phase and the following M-phase. During mitosis, cell growth is halted, and cellular energy is focused on the division into two daughter cells. A large number of transcription factors are displaced from the chromatin and general transcription is also suppressed (Egli *et al*. 2008; Gottesfeld and Forbes 1997). Histone modifications are important for chromatin condensation and chromosomal segregation during this phase in several cell types (Liu *et al*. 2017; Stephens *et al*. 2018). Histone phosphorylation is upregulated during M-phase and linked to chromatin condensation, such as H3T3p, H3T11p, H3.3S31p, H2AS1p, and H4S1p (Bonenfant *et al*. 2007; Kang *et al*. 2007). Transcription is believed to be turned off during mitosis, which is in accordance with observed global deacetylation of histones. A dramatic decrease in acetylation has been reported on histones H3K9, H3K18, H3K23, H4K5, H4K8, H4K12, and H4K16 during this stage (Bonenfant *et al*. 2007; McManus and Hendzel 2006; Rice 2002). Lysine deacetylation has also been observed on histone H2A and H2B, in particular, H2AK5, H2BK12, H2BK15, and H2BK20. In addition to its role in transcriptional repression, lysine deacetylation is thought to ensure a correct packaging of nucleosomes into metaphase chromosomes (Bonenfant *et al*. 2007). This explanation is further supported by the observed destabilization of the chromatin fiber and the decondensation of the chromatin upon the induction of multiple events of acetylation on the histone tails (Kruhlak *et al*. 2001). Shogren-Knaak *et al*. showed that H4K16Ac,

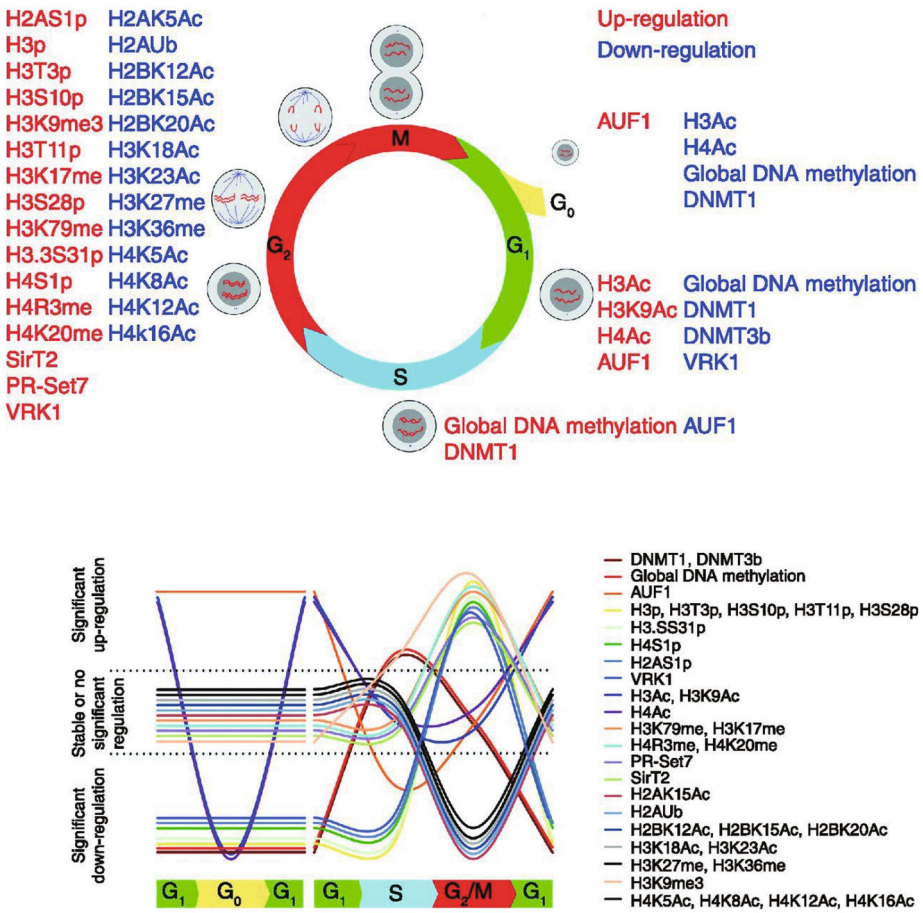

Fig. 3 Histone dynamics across the cell cycle. Histone marks and chromatin-associated proteins are summarized by up- (*red*) and down- (*blue*) regulation during each phase of the cell cycle. The time courses of these histone marks within the cell cycle are also represented in the graph (Kheir and Lund 2010)

in particular, is responsible for chromatin decondensation (Shogren-Knaak 2006). In addition, SirT2, an HDAC specific for H4K16, has been shown to be highly expressed and associated with condensed chromatin exclusively during mitosis (Vaquero 2006).

The low levels of transcriptional activity during the mitotic phase is also evident from the histone methylation patterns. Lys20 methylation on histone H4 is generally increased, and its high level is correlated with the deacetylation of H4K5, H4K8, and H4K12 and, in particular, H4K16 observed during M-phase (Bonenfant *et al*. 2007; Rice 2002). Other notable methylation dynamics during G2/M-phase occur on H3K17, H3K79, and H4R3 (Bonenfant *et al*. 2007; Pesavento *et al*. 2008; Rice 2002). H3K9me3 was identified as a marker of heterochromatin and epigenetic silencing regions, which typically are located close to the centromeres (Schotta *et al*. 2004; Stewart *et al*. 2005). Accordingly, H3K9me3 is dynamically regulated in a cell cycle-dependent manner (Duan *et al*. 2008; McManus *et al*. 2006; Park *et al*. 2011). H3K9me3 is increased rapidly in G2 to reach a maximum, followed by a quick decline (McManus *et al*. 2006). It is also proposed that an increase in H3K9me3 at the late G2-phase and early mitosis may be needed to stabilize the pericentric heterochromatin so that the centromeres and kinetochores have a rigid structure required for proper tension transmission to the inner centromere (Heit *et al*. 2009). However, this hypothesized scenario is in contrast to the results of the genome-wise dynamic change of H3K9me3 in the overall cell cycle (Black *et al*. 2012). Another recent study further revealed an association between chromatin and a lysine demethylase KDM4C during mitosis, which is accompanied by a decrease in the mitotic levels of H3K9me3 (Kupershmit *et al*. 2014). A most recent study further proved that the histone methylations and their combinations could serve as codes to determine the overall gene expressions and phenotypic outcomes. In particular, the coordinated regulation of H3S10p and H3K9me3 may facilitate the timely dissolution of

heterochromatin-like structures and allow accessibility by chromatin-remodeling complex for an efficient chromatin reorganization during mitosis (Peng *et al*. 2018).

EPIGENETIC REGULATION IN MECHANOBIOLOGY

Cells in our body are constantly exposed to a spectrum of mechanical forces, such as blood flow-induced fluid shear stress, compression, stretches, differential tissue rigidity, and strain. Those forces modulate gene expressions and cellular functions to influence and govern physiology and pathophysiology in health and disease. Epigenetic modifications play crucial roles in these gene expressions to not only respond to the external mechanical cues but also to allow cells to adapt to differential mechanical environments (Chen *et al*. 2013; Miroshnikova *et al*. 2017). Here, we reviewed current understanding of how mechanical and epigenetic modifications couple together to regulate molecular and cellular functions.

One current understanding is that mechanical stress directly induces chromatin remodeling through epigenetic modifications to alter gene expressions and cellular functions. For example, shear stress (SS) is defined as the frictional force generated by blood flow in the endothelium. Endothelial cells (ECs) sense the changes of SS and activate intracellular signaling pathways, leading to the transcription of specific genes. Those genes include mechanosensory complexes of platelet-endothelial cell adhesion molecule 1 (PECAM-1) which directly transmits mechanical force, vascular endothelial cadherin (VE-cadherin) which functions as an adaptor, and vascular endothelial growth factor receptor 2 (VEGFR2) which activates phosphatidylinositol-3-OH kinase (Tzima *et al*. 2005). The expression of those important genes is regulated by the modification of chromatin structure, which depends on the activity of histone modification enzymes, including histone acetyltransferase (HAT) and histone deacetylase (HDAC) (Jenuwein 2001). HDACs are sensitive to hemodynamic forces and are mainly divided into three groups: Class I (HDAC-1/2/3 and HDAC-8), Class II (HDAC-4/5/6/7 and HDAC-9/10), and Class III sirtuins (SIRT) (Chen *et al*. 2013). Among the HDACs, it is worth noting that Class III HDACs play a protective role in atherosclerosis (Stein and Matter 2011). Besides, eNOS plays a key role in vascular wall homeostasis and regulation of vasomotor tone. SS can also remodel chromatin structure particularly on histone H3 and H4 by altering the state of enzymes like phosphatidylinositol 3-kinase and HDAC (Class II and III), resulting in modulating the endothelial nitric oxide synthase (eNOS) gene expression at the transcriptional level (Chen *et al*. 2010; Fish *et al*. 2005; Illi *et al*. 2003, 2008). Further research indicates that SS can increase H3K27ac level to open up chromatin and subsequently activate the inositol 1,4,5-trisphosphate receptor 3 (ITPR3)–eNOS axis in Ecs via krüppel-like factor 4(KLF4)-regulated ITPR3 transcription, which is a principal mechanosensitive cue of atheroma protective flow (He *et al*. 2019). Coupling shear stress and epigenetic modifications together to regulate vascular marker gene expression eventually can affect phenotypes (Gomez *et al*. 2015). For example, mechano-induced DNA demethylase ten-eleven translocation-2 (TET2) expression can modulate vascular tone by guiding the phenotype of vascular smooth muscle cells (VSMCs) between contractile and expansible (Liu *et al*. 2013). The blood pressure (BP) can, subsequently, be changed by the phenotype of vascular VSMCs (Brozovich *et al*. 2016). A trans-ancestry genome-wide study shows 12 genetic loci influencing blood pressure and implicates a role for DNA methylation, with four of them (IGFBP3, KCNK3, PDE3A and PRDM6) related to the vascular smooth muscle function (Kato *et al*. 2015; Liu *et al*. 2015). An array of cardiovascular diseases associated with epigenetic modifications caused by mechanotransduction are listed in Table 2 (Bauer and Martin 2017; Haberland *et al*. 2009; Koentges *et al*. 2016; Kumar *et al*. 2013; Lee *et al*. 2012; Liu *et al*. 2013; Nakamura and Sadoshima 2018; Ohtani *et al*. 2011; Oka *et al*. 2011; Shao *et al*. 2017; Shpargel *et al*. 2014; Zhang *et al*. 2011; Zheng *et al*. 2011; Zhu *et al*. 2016; Zhuang *et al*. 2017).

Another current understanding is that mechanical force mediated by nuclear lamina can indirectly organize chromosome through epigenetic modifications to regulate gene expression. Lamin A/C is a protein network that connects the linker of nucleoskeleton and cytoskeleton (LINC) complex to chromosomes. As a mechanosensory structure, it can respond to mechanical forces and regulate gene expressions through epigenetic modifications (Chowdhury *et al*. 2010; DuFort *et al*. 2011). Previous reports show that stem cell differentiation into bone on stiff matrix and into fat on soft matrix can be enhanced by the high and low lamin-A levels corresponding to high and low H3K9m3 levels on chromosomes, respectively (Swift *et al*. 2013). This result is also confirmed in melanoma cells. People found that soft fibrin matrices indeed promote H3K9 demethylation and increase Sox2 expression and self-renewal, while stiff fibrin has an opposite effect (Tan *et al*. 2014). A recent study demonstrated how mechanical force stretches chromatin to affect transcription. A green fluorescent protein (GFP)-tagged bacterial chromosome dihydrofolate reductase (DHFR) and an Arg-Gly-Asp-coated magnetic bead were

Table 2 Cardiovascular disease associated with epigenetic modifications caused by mechanotransduction

Epigenetics regulation	Modifiers	Residues	Targets	Diseases
Histone acetylation	(↑) P300, CBP (Bauer and Martin 2017)	Histones and non-histone proteins	Cardiomyocytes, VSMCs	Cardiomyocyte proliferation and hypertrophy
Histone deacetylation	(↑) HDAC-1-9 (Haberland *et al*. 2009; Zheng *et al*. 2011; Nakamura and Sadoshima 2018; Zheng *et al*. 2011)	Histones and non-histone proteins	Cardiomyocytes, VSMCs	Cardiac hypertrophy, failure fibrosis, ischemia
	(↑) SIRT (Oka *et al*. 2011; Koentges *et al*. 2016, p. 3; Oka *et al*. 2011)			
Histone demethylation	(↑) UTX(KDM6A) (Lee *et al*. 2012; Shpargel *et al*. 2014, p. 6)	H3K27	Embryonic stem cells (ESCs)	Cardiovascular malformation
	(↑) JMJD2a (KDM4A) (Zhang *et al*. 2011; Zheng *et al*. 2011, p. 4)	H3K9, H3K36	Cardiomyocytes	Cardiac hypertrophy, ischemic cardiomyopathy (ICM)
	(↑) JMJD1a (KDM3A) (Ohtani *et al*. 2011)	H3K9	Cardiomyocytes	ICM, idiopathic dilated cardiomyopathy (IDCM)
Histone methylation	(↓) EZH2 (Kumar *et al*. 2013; Shao *et al*. 2017; Zhu *et al*. 2016, p. 1; Shao *et al*. 2017)	H3K27	VSMCs, cardiomyocytes, cardiac myofibroblast	Atherosclerosis, cardiac hypertrophy, fibrosis
DNA methylation	(↓) DNMT (Zhuang *et al*. 2017)	DNA cytosines	VSMCs	Atherosclerosis
DNA hydroxymethylation	(↑) Ten-eleven translocation (Liu *et al*. 2013)	DNA methylcytosine	VSMCs	Hypertension

"↑" denotes activating and "↓" denotes suppressing

used to demonstrate that local stresses can be propagated from the tensed actin cytoskeleton to the LINC complex via integrins and then stretch chromatin and upregulate transcription via lamina-chromatin interaction (Tajik *et al*. 2016). So, mutations in lamins can result in a wide array of pathologies, collectively referred to as laminopathies. Such diseases include Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, dilated cardiomyopathy, familial partial lipodystrophy, Charcot-Marie-Tooth, and the accelerated aging disorder Hutchinson-Gilford progeria syndrome (HGPS) (Schreiber and Kennedy 2013). In fact, HGPS is caused by progerin which is a truncated version of the lamin-A protein. However, progerin remains permanently farnesylated and carboxymethylated since it lacks the second site for endoproteolytic cleavage. The accumulation of progerin leads to altered H3K27me3 through the downregulation of enhancer of zeste homolog 2 gene (EZH2) and disrupts heterochromatin–lamina interactions, which eventually lead to cellular and organismal decline (McCord *et al*. 2013). As a result, the senescence in HGPS may be delayed if the post-translational modifications of progerin are changed. Further research also showed that, blocking farnesylation of progerin (Gonzalo *et al*. 2017), via prenylation inhibitors (Moiseeva *et al*. 2016; Varela *et al*. 2008) and isoprenyl cysteine carboxyl methyltransferase inhibitors (Ibrahim *et al*. 2013), can lead to effective therapeutic treatment of HGPS. Another reported disease connected to lamin-A is Dunnigan-type lipodystrophy, namely, familial partial lipodystrophy type 2 (FPLD2), which can be caused by the R482W mutation on lamin-A, preventing lamin-A's binding at MIR335 locus. This locus determines the aberrant transcription of the anti-adipogenic miR-335 with subsequent inhibition of adipogenic differentiation (Oldenburg *et al*. 2017). Lamina-associated domains (LADs) are redistributed for LMNA mutations in association with markedly altered CpG methylation and gene expression. As such, LADs can contribute to the pathogenesis of dilated cardiomyopathy (DCM) through genomic alterations and epigenetic regulation (Cheedipudi *et al*. 2019).

FRET IMAGING OF EPIGENETIC REGULATION IN SINGLE LIVING CELLS

Mechanical cues can serve as potent regulators of epigenetics, gene expression, and cell fate. In order to bridge mechanical regulation with epigenetic regulation at molecular levels, people have developed technologies

to study the dynamic manipulation and regulation. For example, Guohong Li's group has reported that the tetranucleosomes-on-a-string appears as a stable secondary structure during hierarchical organization of chromatin fibers using single-molecule magnetic tweezers and force spectroscopy. This stability is enhanced by histone chaperone facilitates chromatin transcription (FACT) *in vitro* (Li *et al*. 2016). They further reported that FACT displays dual functions in destabilizing the nucleosome and maintaining the original histones and nucleosome integrity at the single-nucleosome level (Chen *et al*. 2018). As we mentioned in "Epigenetic regulation in mechanobiology" section, Ning Wang's group used 3D magnetic twisting cytometry to apply local stresses on the cell surface via an Arg-Gly-Asp-coated magnetic bead to deform chromatin as well as force-induced gene expression in a living cell (Tajik *et al*. 2016). Cheng Zhu's group developed a DNA-origami tension probe (DOTP) that maps the piconewton forces generated by living cells at the nanometer-length scale (Dutta *et al*. 2018). However, the molecular pathways and mechanisms for mechanical and epigenetic coupled regulations remain unclear, requiring further studies using more advanced technologies like FRET epigenetic biosensors.

FRET imaging

Understanding the molecular basis of physiological regulations is crucial to understanding the pathways of pathological events to find treatments. However, many detection methods like genomic sequencing lack spatial and temporal resolutions (Wang and Wang 2009). Furthermore, those methods typically provide little information on epigenetic modifications. As a result, an imaging method that can simultaneously provide both spatial and temporal information is much needed. Fluorescence resonance energy transfer (FRET) emerged as a recent advance in molecular detection, offering high spatiotemporal resolutions (Wang *et al*. 2008).

FRET technique uses a pair of fluorescence proteins (FP) to detect molecular signals in organisms. The first discovered and isolated FP is the GFP from aequorin (Shimomura *et al*. 1962), after which numerous other natural and engineered FPs were discovered (Tsien 2005). Among those FPs, cyan-colored ECFP and yellow-colored EYFP and its various derivatives are believed to be the best FRET pairs (Miyawaki *et al*. 1997). There are three main types of FRET biosensors. In the first type, a donor FP and an acceptor FP are pulled by two interacting sensing molecules (Fig. 4A). When activated, the two sensing molecules bind together to change the distance and orientation of FPs, resulting in a FRET signal change. In the second type, only one sensing molecule is present (Fig. 4B). This sensing molecule can change conformation when activated, causing FRET change. FRET biosensors with two interacting molecules fused separately with the donor and acceptor FPs are also possible (Fig. 4C). The interaction of the two sensing domains can bring two FPs closer in the distance, resulting in a signal which measures this interaction (Wang *et al*. 2008). Three main factors influencing the efficiency of FRET imaging are the distance between FPs, the relative orientation between the donor and acceptor FPs, and the excitation/emission spectrum of FPs. High FRET efficiency requires close distance, correct orientation, and overlapping between emission wavelengths of donor protein and excitation wavelengths of acceptor protein (Clegg 1995; Wang and Wang 2009).

One application of a FRET biosensor is to monitor kinase activity in living cells. For example, biosensors using FRET can monitor the mechanical activation of Src (Wang *et al*. 2005). This biosensor consists of an N-terminal ECFP, an SH2 domain from Src kinase, a flexible linker, a substrate-binding domain derived from p130cas which is sensitive to Src phosphorylation, and a C-terminal citrine (a variant of YFP) (Wang *et al*. 2005, 2008; Wang and Wang 2009). Recently, improved FRET biosensors with enhanced specificity are being developed. For example, a biosensor that monitors the activation of Fyn, a member of the Src family, is reported. This biosensor used a similar design with previously reported Src biosensor and was altered only in the substrate peptide derived from p34cdc2. *In vitro* kinase assays suggest that this biosensor has a clear preference of the activation of Fyn over other Src family kinases like Src, Yes, and Abl (Ouyang *et al*. 2019). Other enzymatic activities besides Src family kinases can also be detected using FRET biosensors. For example, using a FRET biosensor, Pan *et al*. reported that stronger EphA4 activation might occur in non-raft regions than raft regions on the plasma membrane (Pan *et al*. 2019).

FRET-based epigenetic biosensors

Recently, FRET-based biosensors capable of monitoring histone epigenetic modifications have been developed to study the effect of epigenetic events on cell fate. Lin and Ting first developed a histone H3S28 phosphorylation biosensor (Lin and Ting 2004), and then, Lin *et al*. reported a histone H3K9me3 and H3K27me3 biosensor which can visualize the histone methylation both *in vitro* and in single living cells (Lin *et al*. 2004). Later, Chu *et al*. developed another FRET-based and centromere-targeted

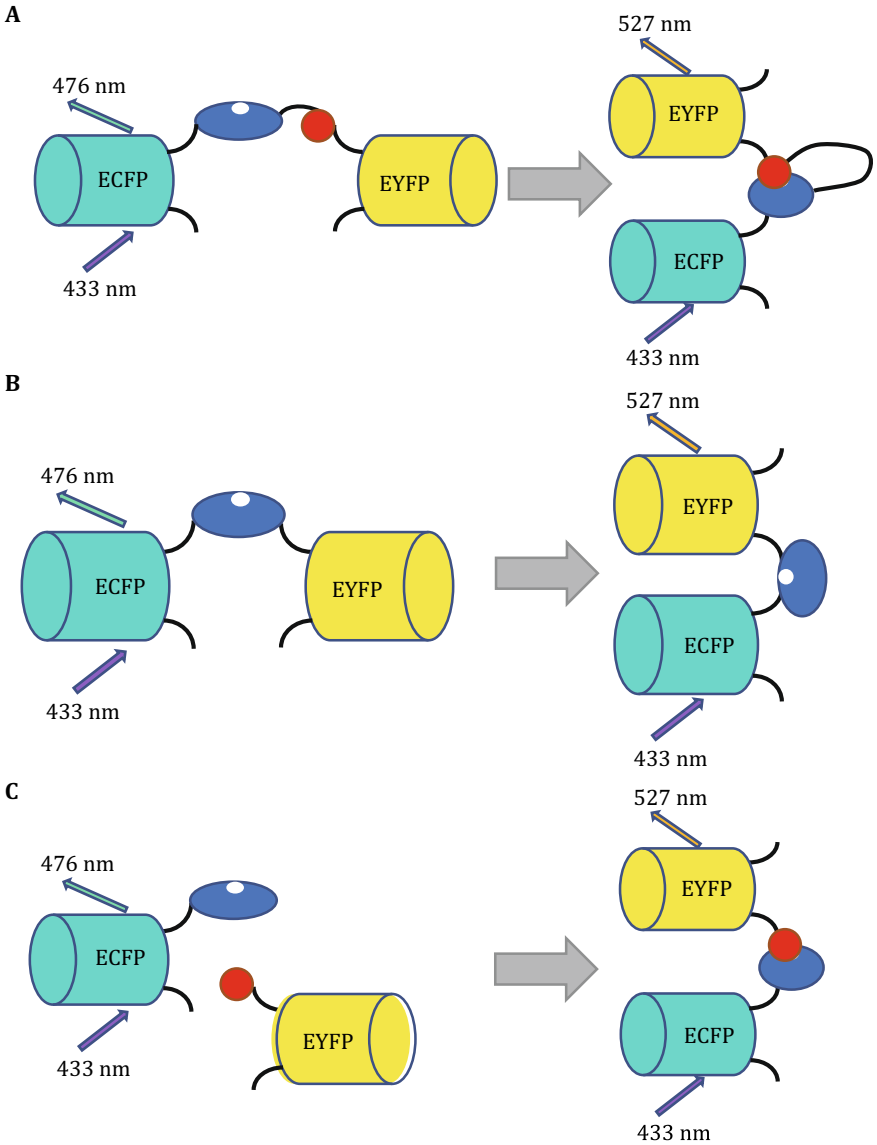

Fig. 4 Three designs of FRET biosensors. **A** Intramolecular FRET biosensor with two interacting sensing domains. The two sensing domains interact with each other upon activation, resulting in FRET. **B** Intramolecular FRET biosensor with one target sensing domain. The sensing domain changes conformation upon activation, bringing the two FPs into close distance and correct orientation. **C** Intermolecular FRET biosensor. One molecule is fused to donor FP, and the other to the acceptor FP. Upon activation, two molecules interact with each other to induce FRET changes

H3K9me3 biosensor to visualize the methylation dynamics during chromosome segregation (Chu *et al.* 2012). As for acetylation, Sasaki *et al.* developed an H4K5 and H4K8 biosensor, followed by an H4K12 biosensor developed by Ito *et al.* (Ito *et al.* 2011; Sasaki *et al.* 2009). Recent studies further reported the monitoring of H3K9ac and H3K14ac by FRET biosensors in 2016 (Nakaoka *et al.* 2016; Sasaki and Yoshida 2016).

FRET epigenetic biosensors have many applications. For example, by using an H3K9me3 FRET biosensor, it was reported that a low level of H3K9me3 is present in tumor-repopulating cells that are not sensitive to matrix stiffness and applied forces (Tan *et al.* 2014). Using an H3K9 tri-methylation biosensor together with an H3S10 phosphorylation biosensor in the same cell, Peng *et al.* found an anticorrelation between H3K9me3 and H3S10p during cell cycles. Further studies revealed that this coordinated regulation might allow increased access of remodeling complexes to the chromatin in preparation of the global reorganization of chromatin during mitosis (Fig. 5) (Peng *et al.* 2018). To study the dynamic epigenetics in mechano-regulation, epigenetic

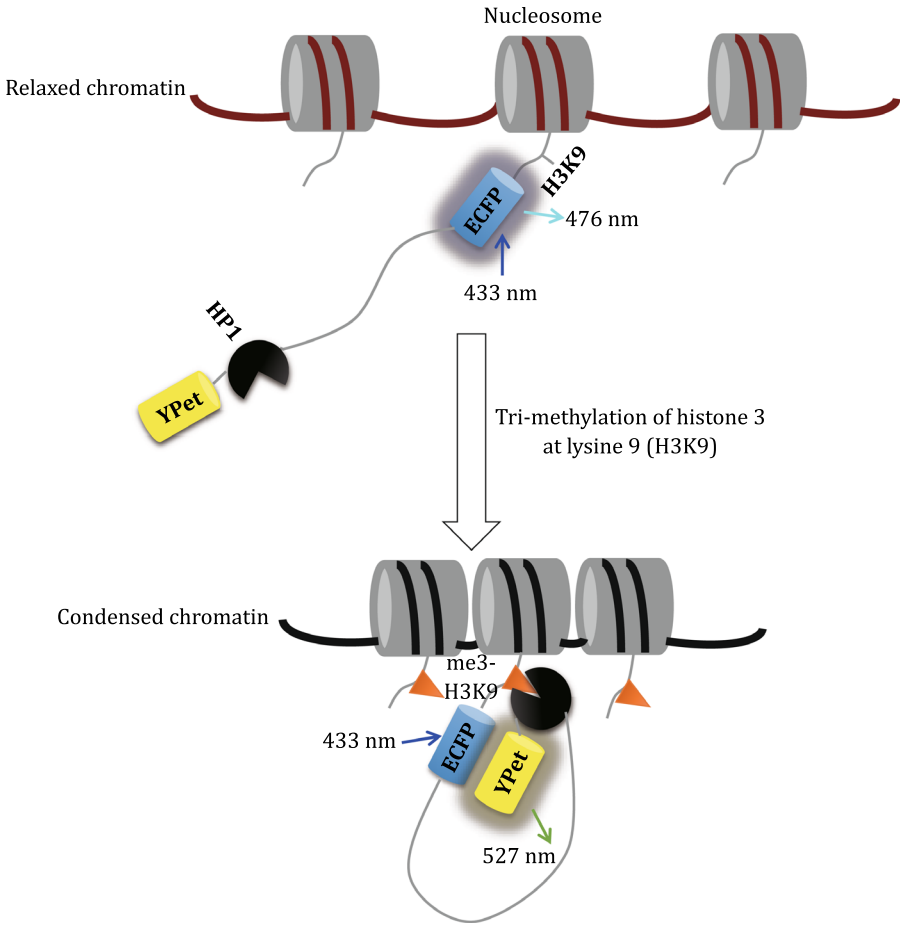

Fig. 5 The designed activation mechanism of H3K9 tri-methylation FRET biosensor. The H3K9me3 tri-methylation biosensor contains a full-length histone H3, an ECFP (donor), a flexible EV linker (120aa), a heterochromatin protein 1 (HP1), and a YPet (acceptor). At rest state, the H3K9me3 biosensor has an open conformation with low FRET. When the histone H3 lysine 9 is methylated by an upstream methyltransferase, such as SUV39H1, HP1 binds to the tri-methylated H3K9, causing a strong FRET. The excitation of ECFP at 433 nm then results in the emission from YPet at 527 nm

FRET biosensors can be introduced into endogenous genome to create stable cell lines by using CRISPR/Cas9, such as EC stable cell line. Combining with liveFISH labeling technology (Wang *et al*. 2019), the transcription and epigenetic dynamics at specific genomic loci in EC stable cell lines can be visualized in real time under shear stress stimulation. As such, epigenetic visualization will provide more mechanistic understanding on mechano-epigenetic regulation in gene expression at single living cell levels.

Even though several FRET biosensors have been developed to study epigenetic events, the actual usage of the FRET epigenetic biosensor is still limited. The sensitivity of most epigenetic biosensors is typically low comparing to other types of chemical or biological imaging probes (Sasaki *et al*. 2009; Tan *et al*. 2014). Some strategies to optimize the FRET biosensor are proposed. For example, peptide scaffold (*e.g*., monobody)-based directed evolution will be a promising technology to increase the specificity and sensitivity of the FRET biosensors (Limsakul *et al*. 2018). Tools other than the FRET biosensor are also helpful in understanding the relationship between epigenetics and mechanobiology. For example, methods such as fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP), and split fluorescence-activating and absorption shifting tag (splitFAST) are used to monitor protein dynamics (Liu and Irudayaraj 2019; Tebo and Gautier 2019). Should more FRET biosensors with increased specificity and sensitivity become available in the future, mechanobiological studies using FRET biosensors will thrive.

SUMMARY AND PERSPECTIVES

In general, there are multiple concurrent epigenetic modifications involved in cellular processes. These modifications synergistically cooperate to govern cellular functions at different subcellular compartments, *e.g*., in the nucleus. As such, developing more FRET epigenetic biosensors for the visualization and quantification of histone and other epigenetic modifications is in great need. Simultaneous imaging of two or more histone modifications in the same live cell, together with the associated traction force map, could allow us to

generate a spatiotemporal landscape of multiplex histone orchestration in association with the corresponding mechanical stress distribution. Moreover, by combining the CRISPR/dCas9 labeling system, these biosensors should allow the more precise monitoring of spatiotemporal epigenetics at specific genomic loci in response to mechanical force. In summary, the single-cell study of epigenetic regulation should not only advance our precise understanding of the dynamic coordination of epigenetics and genomic regulations, but also identify new therapeutic targets in response to mechanical cues in diseases.

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Compliance with Ethical Standards

Conflict of interest Yingxiao Wang is a scientific co-founder of Cell E&G Inc. and Acoustic Cell Therapy LLC. However, these financial interests do not affect the content of this review article. Shitian Li, Dingyi Yang, Li Gao, and Qin Peng declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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