Mechanical force induced gene expression relies on epigenetic modification

Preview on "Sun, J., Chen, J., Mohagheghian, E., and Wang, N. (2020). Force-induced gene up-regulation does not follow the weak power law but depends on H3K9 demethylation. Science Advances *6*."

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Introduction:

Almost all living cells and tissues sense physical forces from their environment and respond appropriately to maintain proper biological function. In the human body, those forces vary among different cells and tissue types, and have different magnitudes, frequencies and durations (e.g., hearing, breathing, heartbeats, walking, running and jumping) [1]. The extracellular matrix (ECM) is fundamental to surround and support cells, and its mechanical properties are essential for cells to maintain integrity and functionality.

In the early 1980s, Ingber *et al.* [2] firstly demonstrated that the ECM components could regulate cell growth and differentiation. The molecular connectivity from ECM to the cell surface and the nucleus have been discovered [3]. At the exterior, ECM scaffolds are coupled to transmembrane integrins. Integrins link to the cytoskeleton by binding to actin-associated protein, which leads to the focal adhesion formation. These cytoskeletal molecular couplings can function together as cytoskeleton filaments and then be connected to the linker of nucleoskeleton and cytoskeleton (LINC complex), which contains nesprins, sun and lamin proteins. Through LINC complex, the mechanical forces can be propagated from ECM to nucleus lamins which are attached with chromatin and DNA. Chen *et al.* [4] proposed that the linkage of integrin and cytoskeleton can modulate gene expression inside the cells. The forces could act at a distance to induce gene alterations inside the nucleus by mechanochemical conversion. However, little is known about how gene expression changes upon force dynamics.

In a recent research [5], the scientists utilized cells from Chinese hamster ovary (CHO) DG44 DHFR 10 subclone to measure the transcription regulation by force. Those cells contain inserted bacterial artificial chromosomes (BAC) together with gene dihydrofolate reductase (DHFR) and lac operator repeats into the same chromatin domain, and stably express EGFP-dimer lac repressor which enables visualization of DHFR. To applying sinusoidal stress to cells, they employed magnetic twisting cytometry (MTC) which used ferromagnetic bead coated with RGD (Arg-Gly-Asp) peptides to attach to the apical surface of the cells via integrins. Their work demonstrates that the externally applied force can be transmitted from the cell surface into the nucleus to stretch chromatin directly and then induce the activation of transgene DHFR rapidly. However, the underlying mechanism of this rapid transcription activation by force is still elusive and whether endogenous mechanoresponsive genes (e.g., *egr-1* (early growth response-1) and *Cav1* (caveolin-1)) can also be directly activated by force is still unknown.

Histone H3 lysine-9 (H3K9) trimethylation (H3K9me3) is associated with gene expression regions in heterochromatin. Upon mechanical stimulation, the condensed heterochromatin with H3K9me3 modifications will de-condense to a more loosely packed form with H3K27me3 modifications [6]. Nevertheless, whether force-induced gene expression change is correlated with H3K9me3 is not clear. In this paper, Sun *et al.* [7] explained how H3K9me3 played a role in regulating gene expression in response to the external force.

Results:

The authors first set to examine whether the force frequency can affect the transcription activation. With the magnetic twisting cytometry, the authors tested stress frequency at a range from 0.3 to 100 Hz. As the force frequency increased, the magnetic bead displacements became smaller, so as the extent of the chromatin stretching. This indicates that the effect of chromosomal stretching comes directly from the force stress, while higher frequency may lead to cell stiffness so that hinders the chromatin deformation effect. To further test whether the force of different frequencies can elicit gene expression change, researchers performed fluorescence in situ hybridization (FISH) on DHFR gene. Indeed, the transcription of DHFR increased proportionally with the chromatin stretching and bead displacement distance, with a peak at low frequencies. This activation was also dependent on the angle of the stress, as the angle between the magnetic bead movement and the cell long axis was almost proportional to the level of transcription up-regulation at all frequencies. Strikingly, the stress angle-dependent activation of gene transcription was also observed in two previously implicated mechanoresponsive endogenous genes: egr-1 and Cav1. Similarly, the most profound activation effect was observed at lower frequencies. Meanwhile, ChIP results indicated that the RNA polymerase occupancy was also significantly higher in the genes that showed force-induced transcriptional activation, suggesting that the activation was mediated by the enhanced recruitment of RNA polymerase. Conversely, the genes not responding to the mechanical stress were null of all the changes as well. Then what gives rise to such a difference? The authors found that the three genes they identified that responded to force were at nuclear center, while the control non-responding genes were closer to the nuclear periphery. Consistent with the observation, the transcription activation of the force responding genes happened only when their loci were at the center region. This distribution manner corresponded well with an inhibitory histone modification H3K9me3, which was highest near the nuclear periphery. To prove that H3K9me3 modification can mediate the force-induced gene activation, the authors performed a series of pharmaceutical and siRNA interventions on this modification. Indeed, inhibiting H3K9me3 lead to gene up-regulation regardless of the stress, while inhibiting the demethylation abolished all the gene activation in response to force. Taken together, these results demonstrate that demethylation of H3K9me3 is necessary for force-induced transcription up-regulation. Finally, the authors demonstrated that all the RNA polymerase occupancy results were in accordance with the perturbation of H3K9 methylation and gene activation.

Conclusion and Significance:

Sun *et al.* [7] set to elucidate the mechanisms of rapid transcription up-regulation upon mechanical strain stimulation. They revealed that the high force frequency (>50Hz) didn't induce rapid transcription activation. They found that endogenous mechanoresponsive genes *egr-1* and *Cav1* can be rapidly activated by force. And this up-regulation didn't follow the weak power-law with force

frequency but depended on H3K9 demethylation. It is notable that, the force-induced transcription activation of *egr-1* and *Cav1* at the nuclear interior was correlated with demethylation of H3K9me3, whereas near nuclear periphery, on the contrary, H3K9me3 hypermethylation inhibited RNA polymerase II so that there was no force-induced transcription. The findings in this paper are remarkable because they firstly provided evidence that endogenous genes can be directly activated by external force. Furthermore, they suggested that the force-induced gene activation relied on the demethylation of H3K9me3 instead of correlating with the rheology in living cells, which helps to explain how cells can respond quickly to the changes in their environments.

The paper leaves several interesting questions for future research: 1. What is the general transcriptional change under the beed-conferred force? Are all of such changes mediated by the H3K9 methylation or in accordance to the spatial distribution? 2. Is the presence of H3K9me3 the only limitation for the forced-induced gene activation? Will most genes become more actively transcribed in response to force at such chaetocin condition? 3. Can this mechanical regulation of gene expression mechanism be applied to the cells constantly exposed to mechanical forces such as lung epithelial cells and heart muscle cells?

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