Programming Houses and Mechanobiological Circuit Design

Cells, the complex thermocontrol systems built of biopolymers that give rise to the hierarchical phenomena of life, are composed of myriad interconnected molecular feedback systems that in greater numbers have started to be approximated into circuits adhering to digital logic. Of natural cellular processes/systems, mechanical forces produce key inputs that modulate cellular behavior, be it at the level of rapid changes in mechanosensitive proteins or longer term architectural memory in the cytoskeleton. Many of the cellular structures that directly interact with and respond to these forces are modulated biochemically by various proteins, which subjects these processes to the methods of gene circuit design. Likewise, the rapidly advancing knowledge of cellular and subcellular-level biomechanical properties bridges the gaps needed for implementing gene circuits with mechanical forces as inputs/outputs. This mini-review briefly discusses recent advances in synthetic and mechanobiology with a particular focus on cytoskeletal mechanochemical signaling and circuit design. Although the inclusion of mechanical forces into gene circuits has only started to be explored, this coupling is likely to synergize into a novel engineering paradigm that takes advantage of the additional level of feedback and control using integrated circuits at multiple scales to produce intricate and dynamic biomaterials and cellular behaviors.

“After all, tiny life forms, driven solely by their own natural DNA, have, just by themselves, produced large, complex objects: elephants, whales, dinosaurs… A minuscule fertilized whale egg produces an object as big as a house. So maybe one day we can program an organism, or a batch of them, to produce not the whale but the actual house.” George Church in *Regenesis* [1].

Synthetic biology utilizes an interdisciplinary engineering framework to computationally model and then experimentally construct genetic circuits based off of digital logic (**Figure 1**) [2] that create complex, dynamic behaviors inside of living cells. Since the original toggle switch [3] and oscillator [4] designs, genetic circuits have been constructed that generate patterns [5], count discrete cellular events [6], and oscillate synchronously [7]. This forward engineering approach is extremely useful in both improving mechanistic understanding of biological systems as well as in the generation of design specifications for a variety of applications. Even the creation of gene circuits has recently started on what may become an exponential acceleration process of complexity, due to software systems such as Cello that have demonstrated the capability of designing dozens of circuits with >90% output accuracy (**Figure 2**) [8].

Despite these rapid advances, synthetic biology has yet to take notice of and incorporate many key physical properties that influence gene expression, protein/polymer assembly, various cellular behaviors and so on. Although gene circuits have been designed to include external control and tuning mechanisms such as temperature [9], the mechanical properties of cells have only recently started to be explored under the umbrella of circuitry. Yet these properties, such as shear stress, tensional and compressive forces, and cytoskeletal tensegrity-generated isometric tension, are intricately linked to myriad cellular functions, often through transcriptional regulation

[10]. The diversity of mechanically-linked genes/functions is vast, and mechanosensitivity becomes particularly important during the development of an organism due to dramatic spatiotemporal changes. Overall, the influences of mechanical forces billow out from embryogenesis to organogenesis; from pluripotency maintenance to lifetime cell and tissue maintenance (and failures in maintenance – disease) [10]. Of these influences, some rely less on hierarchical structural assembly and instead correspond more directly to actions at the level of individual proteins. Examples are as varied as the mechanosensitive protein channels in *Drosophila* photoreceptors, that rapidly act on cell membrane lipids to create large cellular contractions in response to light [11], or single talin protein rods in the cytoplasm, which expose additional binding sites when stretched that lead downstream to cytoskeletal rearrangement [12]. Manipulation of such mechanosensitive proteins has even resulted in neurons sensitized to ultrasound, helping to spearhead “sonogenetics” [13]. Many other influences from mechanical forces involve direct or indirect transcriptional coupling to cytoskeletal architecture.

One key element of eurkaryotic cellular architecture, the actin protein, to which over 150 other proteins can bind, often takes the form of a tree’s branches, and can push outwards on the plasma membrane while steadily elongating to provide sustained forces for a cell’s leading edge (as in the case of crawling leukocytes) [14]. In the case of leukocytes, actin assembly is mediated through biochemical signaling intermediates that lie between the processes of receptors on the cell surface responding to chemotaxis, and actin polymerization [15]. Likewise, organization of the stiffer, rapidly assembling and disassembling microtubules is often mediated by motor proteins (which influence actin as well) that transverse these transient, *Inception*-esque highways [14]. Intermediate filaments, the least stiff of the cytoskeletal trio, are dramatically favored for tensile force resistance, and through plectin proteins can be crosslinked to other intermediate filaments, as well as actin and microtubules, often in response to mechanical stress [14, 16]. Still deeper into the [typical eurkaryotic] cell, polymerized lamin proteins (a type of intermediate filament) help stabilize the structure of the nucleus and provide an interface between the cytoskeleton and DNA, and can contribute to diseases such as progeria and various cancers when genetic mutations arise [14, 17, 18]. These and other nuclear envelope proteins, linked to the cytoskeleton (and thus indirectly to the extracellular environment) via nesprin, can bind directly to transcription factors and thus may be able to act as a “mechanostat” and modulate gene expression upon contact with various forces (**Figure 3**) [10, 19, 20].

Yet it is not only eukaryotes that carry the privilege of dynamic cytoskeletons. Bacteria such as *E. Coli*, the current workhorses of synthetic biology, not only contain their own prokaryotic versions of intermediate filaments, actin and tubulin, but additionally have unique cytoskeletal elements separate from eukaryotes with considerable cross-species diversity [21, 22]. MreB, for example, forms cytoskeletal filament bundles in bacteria and helps determine whether overall cell morphology is spherical or rod-like [23]. Furthermore, as with eukaryotes, bacteria contain a variety of mechanosensitive proteins that can be activated or deactivated by forces such as tension as well as various amphipaths [24], offering the possibility of more precise and direct control over hypothetical gene circuits that blend mechanical and chemical processes. Indeed, all of these chemical intermediates (as well as the cytoskeletal proteins themselves) which can be manipulated by altering the flux of RNA polymerase onto and off of DNA (**Figure 1**) become potential targets for interventions via genetic circuitry – for temporarily or permanently changing the ways in which cells sense and respond to mechanical forces in

their environment. A bacterial strain, for example, engineered with biochemical logic gates to produce a therapeutic once inside (and only once inside) a tumor's necrotic core could be programmed to release additional signaling molecules upon encountering further pressure – such as triggering synchronized lysis (Din & Danino *et al.*, in press).

Although (to the best of my knowledge) a gene circuit and corresponding logic gate(s) incorporating a mechanical force as an input and/or output has yet to be designed and physically implemented into a prokaryotic or eukaryotic cell line via plasmids, CRISPR, etc., **Figures 4 and 5** offer early examples of what these hypothetical circuits may begin to resemble. In **Figure 4**, the process of bone formation is linked to matrix stiffness through various proteins / signaling intermediates, beginning with forces acting upon lamin A [25] – similar to the tension-induced dephosphorylation of lamin A and subsequent signaling shown in **Figure 3** [20]. In **Figure 5**, protein synthesis and degradation are incorporated into increasingly complex circuits [26]. For implementation into logic gates, an additional molecule (such as a *LMNA* transcription factor) could be incorporated to compliment tension as a possible AND gate.

In order to adequately face off against the sheer complexity of (controlled or automated) DNA-encoded macro-manipulation of cellular movement, or of programmed cellular responses to mechanical stimuli, rigorous mathematical modeling will be required, as in **Figure 5B & C**. Such will not only allow for computational hypothesis validation of individual circuit behaviors, but will also permit biomechanical circuits to be designed, tested and integrated with numerous other circuits in order to generate complex behaviors. Likewise, is a gene circuit that has tension as an input still a gene circuit? Incorporating such forces quickly leaves the realm of commonplace genetic circuit schema (**Figure 1**), and may require a new, systems based design approach. A primitive example of what systems-based gene circuits could begin to resemble is shown in **Figure 6**, [17], allowing factors such as tension to be incorporated under "sensing", "process", etc., to more accurately represent biological circuits as they are – processes. Although there is great difficulty in discerning just how physical forces will be integrated into gene circuits, and what these circuits will actually look like, the outcome will surely hold many crucial implications in the design of dynamic, large-scale biomaterials (buildings?), biosensors, health and disease, and biological control.

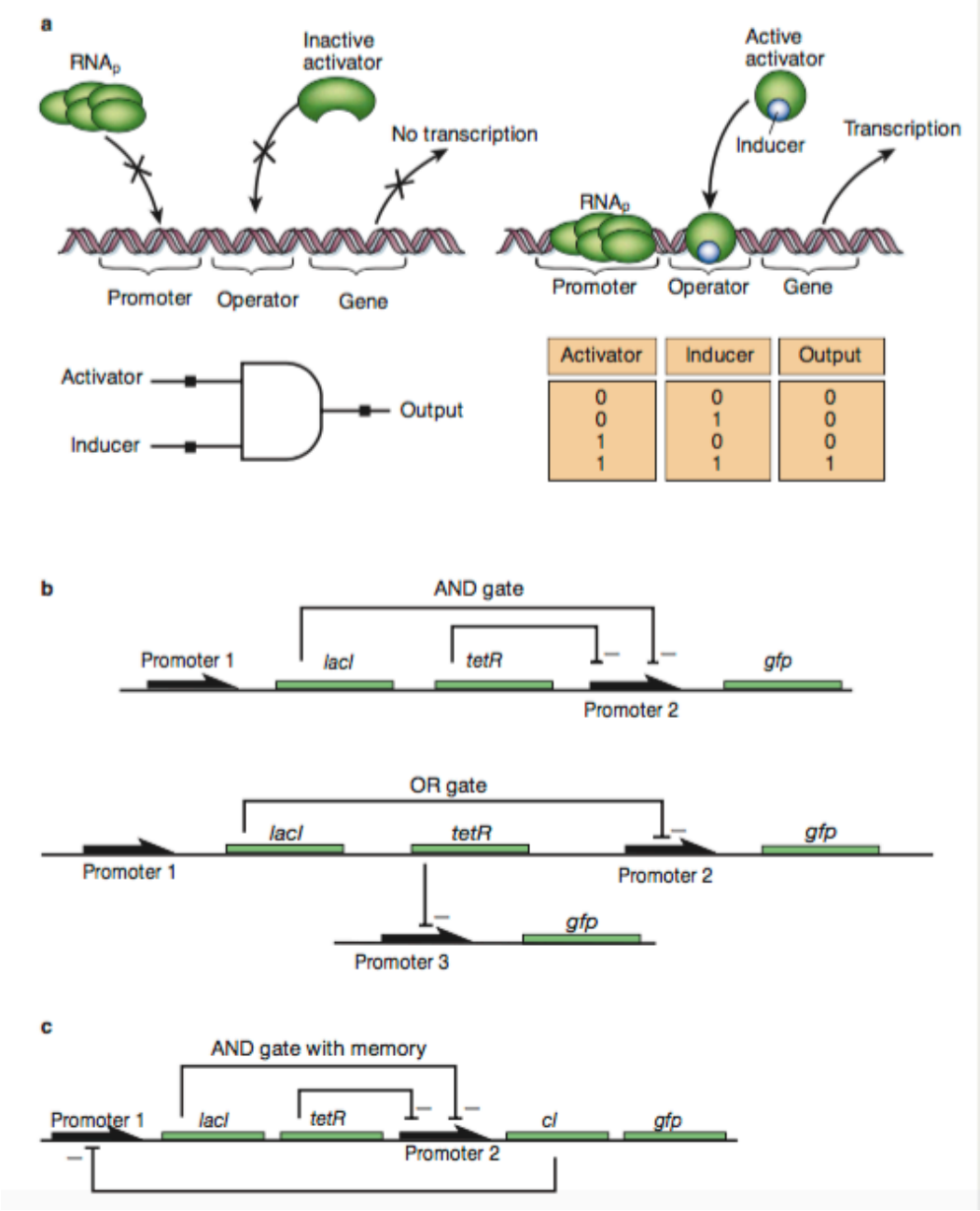


Figure 1. *Basic logic gate construction with gene circuits*. Adapted from Hasty et al, 2002, Engineered gene circuits. **a**, Genetic/electronic circuit diagrams of an AND gate, where both the binding of an inducer to an activator protein, and subsequent binding of an activator protein to the operator region to recruit RNA polymerase is required for activation / "ON". **b**, Alternative AND / OR gates requiring inducers to remove downstream, constitutively-expressed repression on a GFP promoter (inducers marked as dashes, ---). **c**, AND gate with memory, where the inclusion of the additional cI gene under the control of the second promoter can repress the first promoter, and keep the system in a state of LacI and TetR repression.

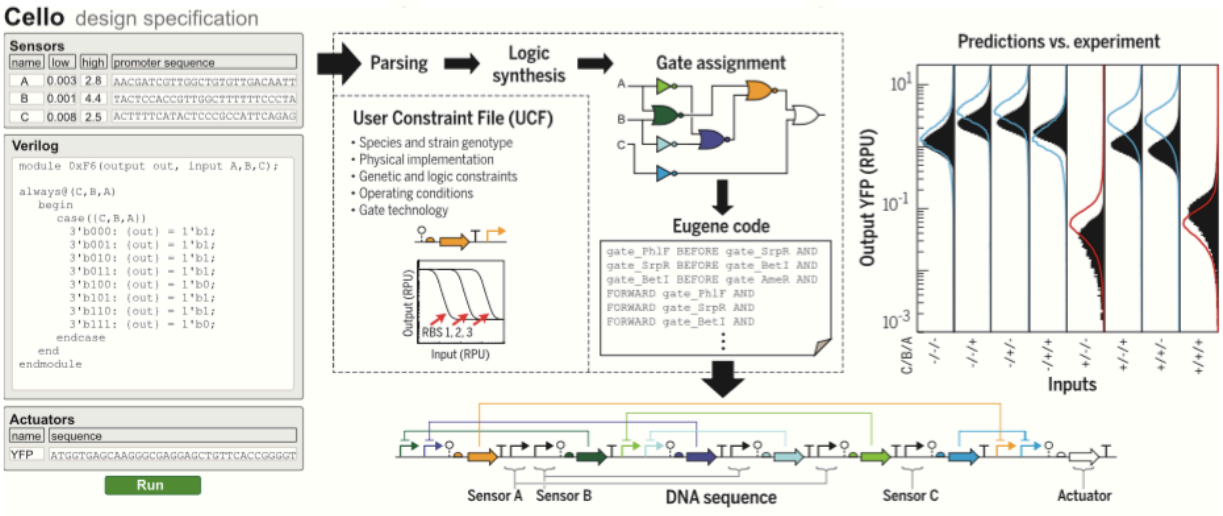


Figure 2. *Open-source software Cello as a tool for rapidly constructing complex genetic circuits*. Adapted from Voigt et al, 2016, Gene circuit design automation. Genetic programming using the Cello software. The desired function of the gene circuit in entered in Verilog code, which is then turned into a sequence of DNA. An example circuit is shown; Red and blue curves = predicted output states for cell populations, solid black = experimental flow cytometry data. The outputs are shown for all combinations of sensor states; plus and minus signs indicate the presence or absence of input signal. RBS, ribosome binding site; RPU, relative promoter unit; YFP, yellow fluorescent protein.

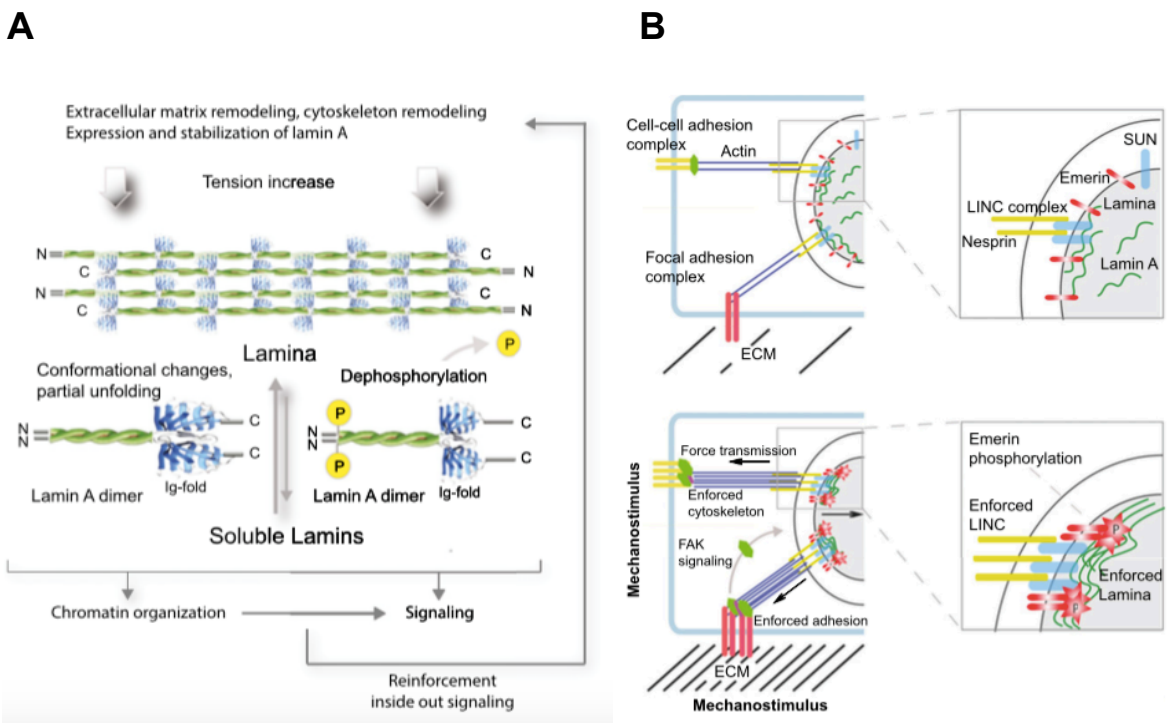


Figure 3. *Lamins couple mechanical and chemical signaling*. Adapted from Osmanagic-Myers et al, 2016, Lamins at the crossroads of mechanosignaling. **A**, Increasing tension partially unfolds lamin A, resulting in phosphorylation and additional downstream signaling, including the assembly of soluble lamin A into the lamina. **B**, Tension-sensing focal adhesion and cell-cell adhesion complexes are physically linked to the cell nucleus through the cytoskeleton, LINC complexes, and the nuclear lamina, resulting in mechanoresponsive gene expression. Mechanostimuli (i.e. increased tension) result in the reinforcement of the lamina, adhesion and LINC complexes, and the cytoskeleton through actin filament assembly, increased adhesion/ LINC complex protein recruitment, and A-type lamin assembly. Additionally, tension can activate signaling cascades (such as FAK signaling) on cell membrane adhesion complexes, allowing the forces involved in mechanosensitive gene expression to sometimes bypass the nucleus.

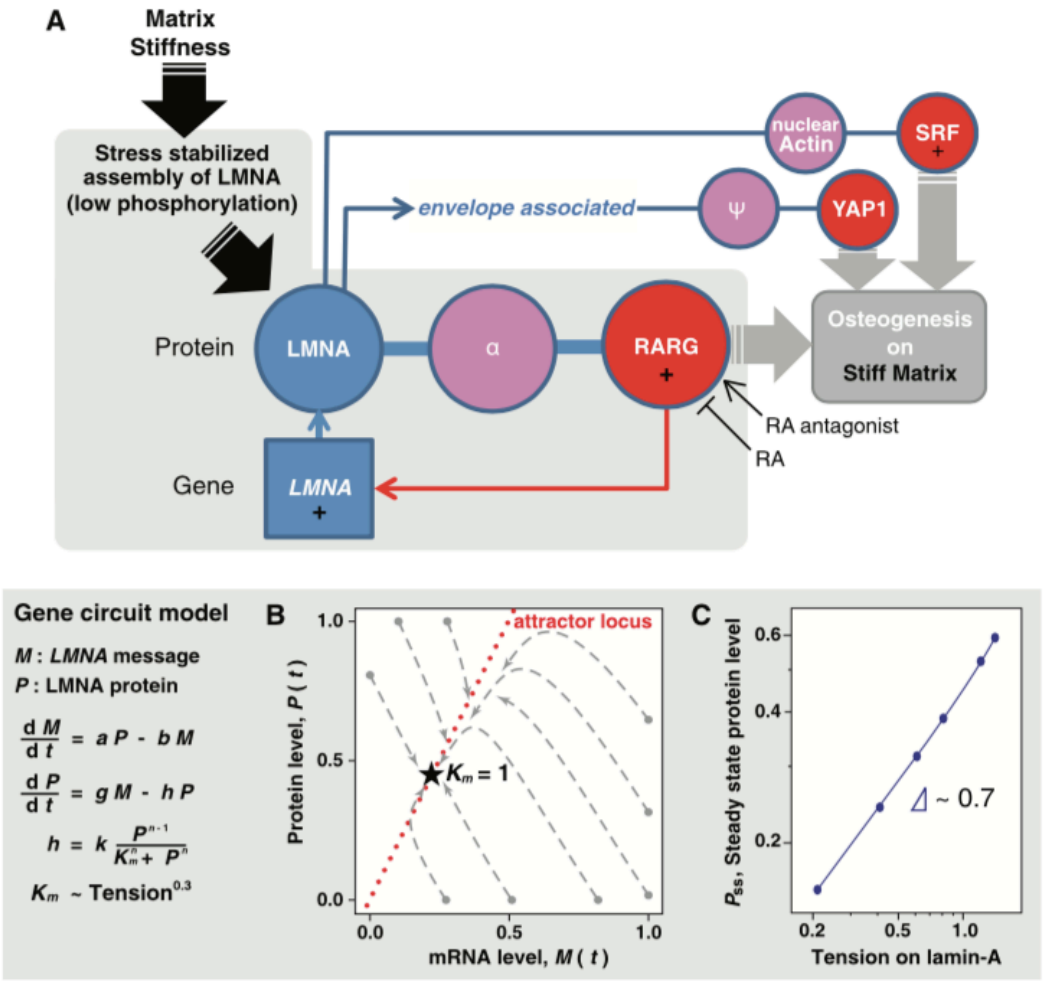
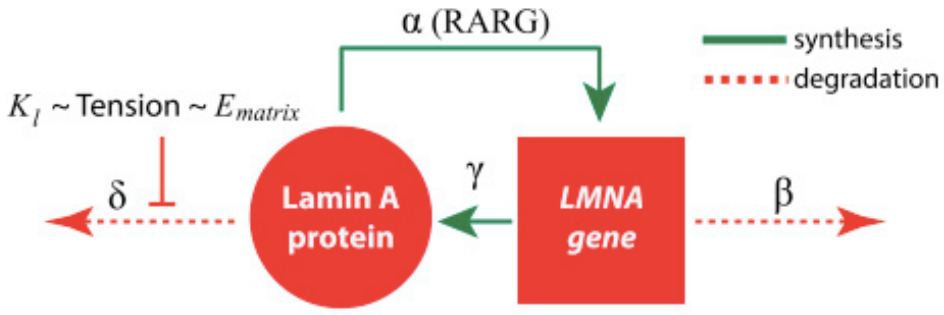


Figure 4. *Gene circuits as a linker between mechanical properties and cellular differentiation*. Adapted from Discher et al, 2013, Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-Directed Differentiation. **A**, Osteogenesis connected to matrix stiffness via gene circuit (an overlapping circuit for adipogenesis on soft matrix is not shown). Lamin A protein level (encoded for by *LMNA*) is regulated by a phosphorylation mechanism sensitive to stress. The interaction of RARG with the *LMNA* transcript allows for an additional degree of control through agonist/antagonist regulation. Lamin A also influences location of YAP1 and regulates SRF through interaction with nuclear actin. **B**, Lamin-A mRNA and protein converge from initial conditions to a single steady-state solution appropriate to the tension. **C**, Setting the kinase/ protease binding coefficient, Km, to be proportional to tension^0.3 results in steady-state lamin scaling.

A



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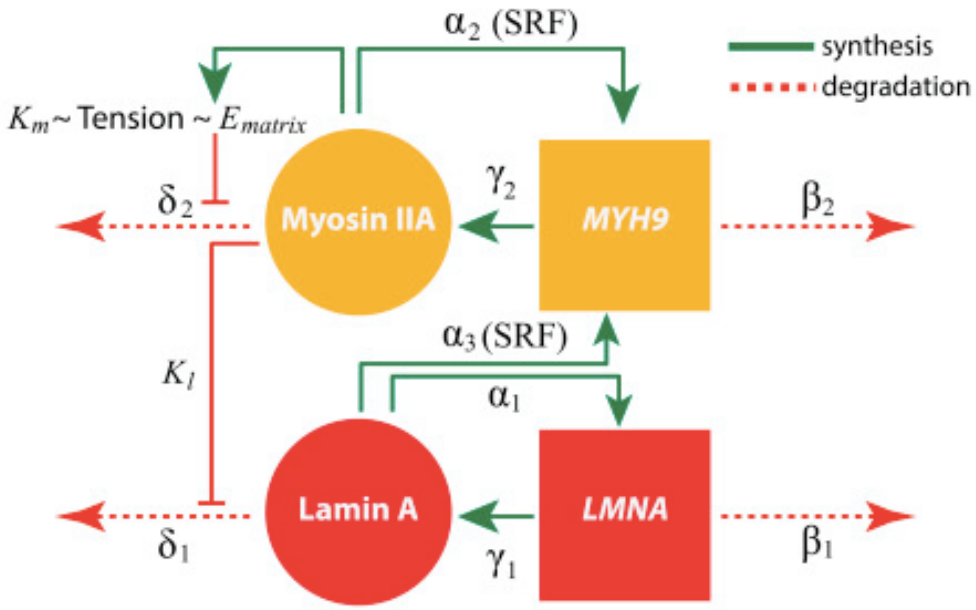
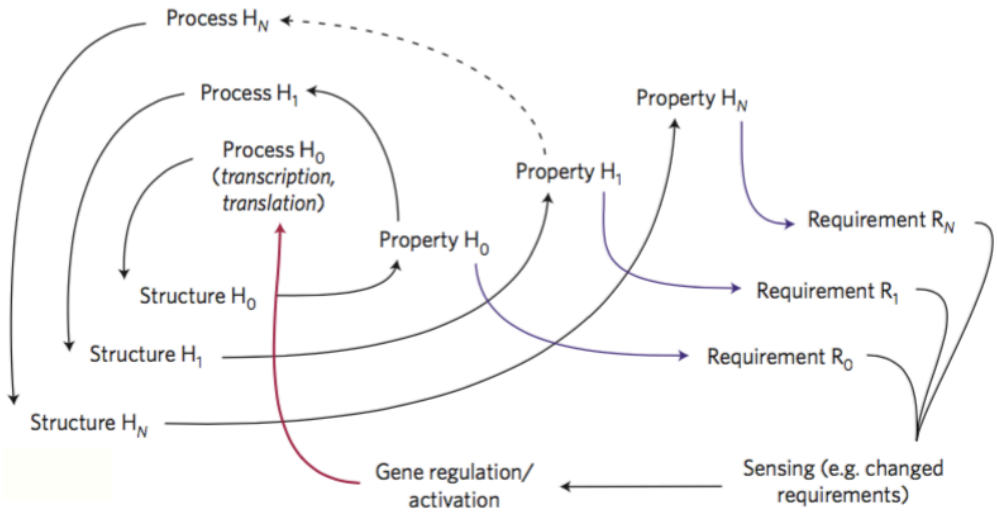


Figure 5. *Increasing complexity of mechanobiological circuits*. Adapted from Dingal and Discher, 2014, Systems Mechanobiology: Tension-Inhibited Protein Turnover Is Sufficient to Physically Control Gene Circuits. Beta = mRNA degradation, delta = protein degredation. **A**, Lamin-A protein, in addition to exhibiting positive feedback via the regulation of its transcription factor retinoic acid receptor-gamma (RARG), assembles through the application of tension (which inhibits Lamin-A protein degredation). **B**, A similar tension-inhibited mechanism of degradation influencing the coupling of cytoskeletal myosin and nucleoskeletal lamin A where each regulates their own message. Lamin A has additionally been found to regulate SRF-target genes (α_3), such as *MYH9*.

A



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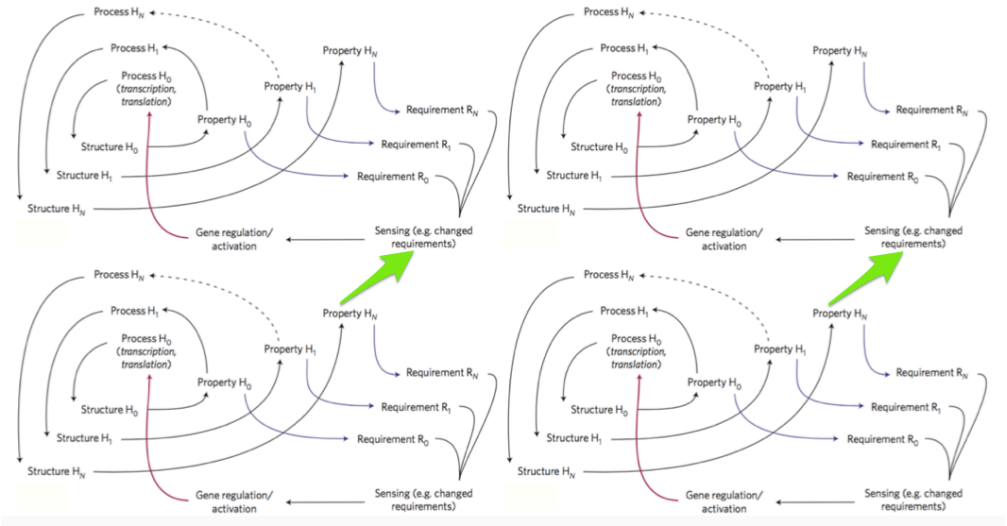

Figure 6. *Scaling systems-based gene circuits*. Adapted from Buehler and Yung, 2009, Deformation and failure of protein materials in physiologically extreme conditions and disease. **A**, A schematic exhibiting the practical crossover of materials science into biology, incorporating multiple hierarchical structures (H = hierarchy levels, R = material property requirements at each level). **B**, Multiple interconnected, materials-science-based feedback systems where particular hierarchical properties influence outside gene expression. Such is intended as an early representation of a novel form of genetic circuit design, where multiple hierarchical levels are taken into account to build larger, complex, systems-based “circuit boards”.

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