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Synthetic mechanobiology: engineering cellular force generation and signaling

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Mechanobiology seeks to understand and control mechanical and related biophysical communication between cells and their surroundings. While experimental efforts in this field have traditionally emphasized manipulation of the extracellular force environment, a new suite of approaches has recently emerged in which cell phenotype and signaling are controlled by directly engineering the cell itself. One route is to control cell behavior by modulating gene expression using conditional promoters. Alternatively, protein activity can be actuated directly using synthetic protein ligands, chemically induced protein dimerization, optogenetic strategies, or functionalized magnetic nanoparticles. Proof-of-principle studies are already demonstrating the translational potential of these approaches, and future technological development will permit increasingly precise control over cell mechanobiology and improve our understanding of the underlying signaling events.

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Cells sense mechanical and other biophysical properties of their environment, altering their morphology, migration and differentiation in response. In turn, cells also influence microenvironmental structure and mechanics by secreting, digesting and remodeling matrix components. This dynamic mechanobiological relationship features centrally in development, tissue homeostasis, and disease progression. To explore and control these processes, a wide range of approaches for engineering the cellular microenvironment have been developed. It is only in more recent years that the field has begun to create complementary strategies to control mechanobiological signaling pathways from within the cell. These 'inside-out' approaches have been greatly accelerated by incorporating tools and concepts from synthetic biology, a field that seeks to build novel systems from living components.

Complex phenotypes often arise from the activation of signals at specific times and places within tissue (or nanoscale regions of cells), and so comprehensive control of mechanobiological behaviors requires precise modulation of these signals over a wide range of spatial and temporal scales. For instance, whereas stress-induced activation of Src occurs over a few hundred milliseconds [1], mechanically-driven stem cell differentiation responds to stimuli presented over the course of several hours or days. Furthermore, because signaling events are frequently associated with accumulation of a molecular effector to some critical local concentration [2–4], the duration of a signaling event is important in driving phenotype [5]. Similarly, gradients in biophysical cues and signaling molecules are important for facilitating cell polarity and directing migration [6,7]. Finally, mechanobiological signaling pathways and cell phenotype are typically strongly dependent on the dimensionality of a cell's growth environment. A complete synthetic mechanobiology toolbox therefore requires approaches that can permeate tissue scaffolds or even take advantage of three-dimensional biomaterials, in addition to controlled temporal and spatial cues.

In this review, we explore inside-out control of mechanobiological signaling and phenotype (summarized in Table 1), with emphasis on spatial specificity and temporal dynamics. First, we will discuss studies that direct cell behavior by changing the expression of a target protein. Second, we will explore strategies that control behavior by changing the activity of a target protein. In the former category, we focus on inducible/repressible gene expression systems in which mechanotransductive signals are placed under the control of soluble inputs. In the latter category, we emphasize small-molecule induction of protein complexation. We then consider technologies in which nominally mechanotransductive signaling systems are re-engineered to be induced by non-mechanical inputs such as light and magnetic fields.

Controlled induction of gene expression

Gene transcription represents an early point of control in regulating protein abundance and therefore activity. A range of conditional promoter systems have been deployed in mammalian cells, most of which place the

Table 1

Major mechanobiological pathways manipulated by synthetic biology tools

Mechanobiological process	Key signaling molecules	Engineering approach
Actomyosin contractility	RhoA, ROCK, non-muscle myosin activation	Genetic [16,17••,18], Chemical [23], Optical [38,47]
	Non-muscle cells: MLCK, pMLC	Genetic [16,18]
	Muscle cells: Ca^{2+}-CaM, MLCK, caldesmon, pMLC	Optical [42,44,45•,46]
Actin polymerization	Tiam1, Rac1, WAVE, PAK1, Arp2/3	Genetic [17••], Chemical [23,29,30,31,33,34,36,37], Optical [38,39,41,43,48], Magnetic [53••]
	Cdc42, N-WASP, Arp2/3, Intersectin	Chemical [23,26], Optical [48], Magnetic [53••]
Focal adhesion assembly	Src, FAK, p130Cas, Paxillin	Chemical [24,32]
Microtubule assembly	RCC1, RanGTP, Microtubule associated proteins (MAPs)	Magnetic [54,56•]

transcription of specific genes under the control of light [8,9] or small molecules that can be added to the culture medium, such as antibiotics [10,11], steroid hormones [12,13], or metabolites [14,15]. These systems are typically reversible, such that removal of the stimulus restores expression to basal levels. While these systems allow control of expression rates, they neither directly control protein activity levels nor evade native cell regulatory mechanisms.

To apply these strategies to mechanobiological signaling while circumventing endogenous feedback regulation, our laboratory has placed constitutively active (CA) mutants of key mechanotransductive genes under the control of conditional promoters. In an early effort, we used lentiviral delivery to create stable human glioma cell lines that express CA RhoA or CA myosin light chain kinase (MLCK) under the control of a tetracycline-repressible promoter. By varying the concentration of tetracycline in the medium, we achieved stably graded expression levels of these proteins. Moreover, because both RhoA and MLCK promote activation of the actin cytoskeletal motor non-muscle myosin II, we were able to apply this strategy to control a variety of mechanobiological phenotypes in a graded and stable way (Figure 1), including random migration speed, cortical stiffness and traction force generation [16].

Provided mutually orthogonal promoters are selected, this strategy can be multiplexed to independently and simultaneously control several target proteins. This approach could allow for engineering more complex mechanobiological behavior, or permit one to map the 'phase space' that describes how multiple proteins interact to control cell phenotype. For example, we used dual lentiviral transduction to simultaneously express CA RhoA and CA Rac1 under the control of a doxycycline-inducible promoter and a cumate-inducible promoter, respectively [17••]. These GTPases are canonically regarded to regulate opposing aspects of cell motility and mutually antagonize one another at several levels, making it challenging to independently manipulate them. By using this orthogonal promoter strategy, we circumvented this crosstalk and mapped the range of phenotypes observed in the otherwise inaccessible state of high-RhoA activation and high-Rac1 activation.

Inducible/repressible promoter strategies offer a number of important advantages, including highly stable expression and the ability to uniformly control gene expression in an entire population of cells, and in an easily scalable way. These features can be leveraged to study and control the biomechanical role of target proteins in mice grafted with genetically engineered cells [18]. However, there are also a number of limitations, perhaps the most important of which is the slow dynamics of the expression system and the protein of interest. While in some scenarios, cells respond phenotypically within six hours [16,19••], some systems may take as long as ten days to reach a steady-state response [19••,20]. This is compounded by system-to-system variations in the kinetics of transcriptional activation, protein folding and post-translational modifications, protein transport, and protein degradation, all of which may be key to the final phenotype.

Additionally, this strategy has inherently limited spatial resolution. Once the gene has been transcribed, there is no control over subcellular protein localization. However, several approaches for spatial control of gene expression at the cell population level have been proposed. For instance, inducers and repressors can be restricted to certain areas of a cell population through microfluidic control [20], by occlusion of membrane pores [19••], or by sequestration of the agent within the material scaffold [21,22]. Several factors influence the extent of control over spatial activation of gene expression and thus pattern fidelity. Cell migration and slow delivery, induction, and expression kinetics may disrupt intended patterns. Shorter lag times between introduction of the inducer/repressor and protein expression allows for more faithful pattern formation [19••].

Controlled activation of protein activity

While modulating gene expression can produce graded and reversible changes in cell mechanobiology, the response time of this system is limited by transcription and translation rates as well as by protein and mRNA degradation rates. As a result, these approaches are most relevant for