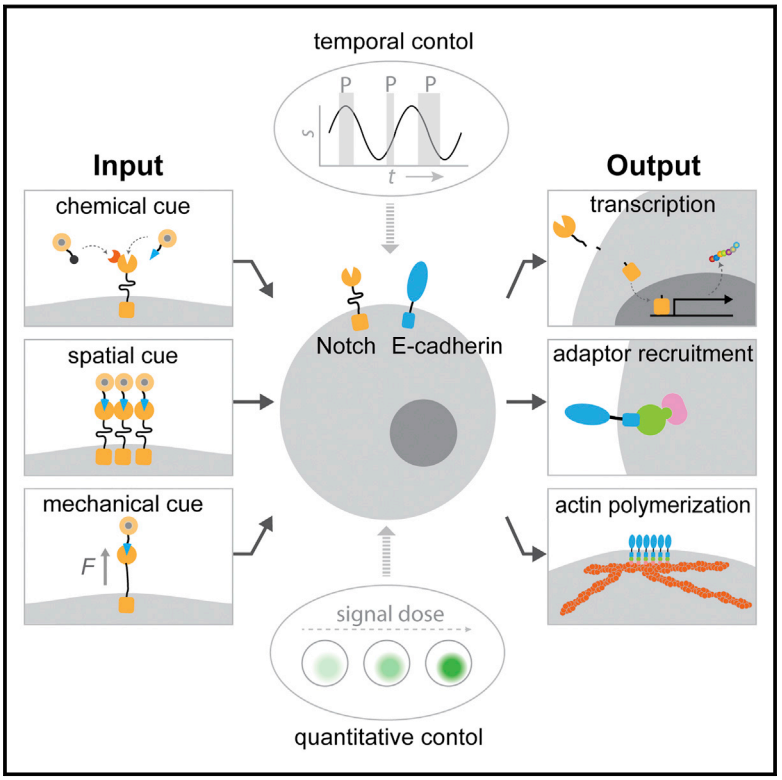
Resource

Cell

A Mechanogenetic Toolkit for Interrogating Cell Signaling in Space and Time

Graphical Abstract



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In Brief

Mechanogenetics, a new approach that uses nanoparticles with imaging, localizing, and mechanically loading capabilities to activate targeted proteins with high spatiotemporal resolution, reveals how chemical, spatial, and mechanical cues cooperate to direct activation dynamics of Notch and E-cadherin receptors.

Highlights

- Development of a mechanogenetic single-cell perturbation approach
- Interrogation of the spatial, chemical, and mechanical responses of Notch receptors
- Identification of the roles of spatial and mechanical cues on E-cadherin signaling
- Spatiotemporal and quantitative control of single-cell transcription by nanoprobes

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A Mechanogenetic Toolkit for Interrogating Cell Signaling in Space and Time

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SUMMARY

Tools capable of imaging and perturbing mechanical signaling pathways with fine spatiotemporal resolution have been elusive, despite their importance in diverse cellular processes. The challenge in developing a mechanogenetic toolkit (i.e., selective and quantitative activation of genetically encoded mechanoreceptors) stems from the fact that many mechanically activated processes are localized in space and time yet additionally require mechanical loading to become activated. To address this challenge, we synthesized magnetoplasmonic nanoparticles that can image, localize, and mechanically load targeted proteins with high spatiotemporal resolution. We demonstrate their utility by investigating the cell-surface activation of two mechanoreceptors: Notch and E-cadherin. By measuring cellular responses to a spectrum of spatial, chemical, temporal, and mechanical inputs at the single-molecule and single-cell levels, we reveal how spatial segregation and mechanical force cooperate to direct receptor activation dynamics. This generalizable technique can be used to control and understand diverse mechanosensitive processes in cell signaling.

INTRODUCTION

Mechanosensitive cell-surface receptors allow cells to sense the physical properties of the extracellular environment, including mechanical force resulting from cell-matrix and cell-cell interactions (Vogel and Sheetz, 2006). These receptors integrate mechanical, chemical (i.e., ligand-receptor interaction), spatial, and temporal cues (Iskratsch et al., 2014) to activate downstream signaling pathways implicated in development, homeostasis, and disease. Recent advances in imaging and force-sensing tools have extended our understanding of how mechanosensitive receptors transduce force from the cell exterior to the cytosol. However, little is known about how mechanosensitive receptors integrate mechanical signals with chemical, spatial, and temporal cues to differentially regulate downstream signaling pathways.

Single-cell perturbation techniques are required to understand how spatial and dynamic cues regulate downstream signaling pathways (Banghart et al., 2004; Deisseroth, 2011; Miesenböck, 2009; Toettcher et al., 2011). The key to this approach is the ability to quantitatively deliver a specific biochemical cue to any desired location and at any given time (Toettcher et al., 2011). Exemplary of this approach are optogenetic methods, where spatial and conformational control of light-sensitive proteins has provided systems-level mechanistic insight into a variety of neuroelectrical and biochemical signaling processes (Banghart et al., 2004; Deisseroth, 2011; Miesenböck, 2009; Toettcher et al., 2011). However, analogous methods do not exist for mechanosensitive signaling proteins. Microprobe-based force microscopy tools can deliver a specific force to purified biomolecules (Dufrêne et al., 2011; Neuman and Nagy, 2008), but, due to the large size and multivalent character of microprobes, these tools are not ideal for spatial control of individual membrane proteins in live cells because of their propensity to cluster upon binding (details are given in Results). Magnetic nanoparticles were previously used for controlling the subcellular distribution of proteins (Bharde et al., 2013; Cho et al., 2012; Etoc et al., 2013; Hoffmann et al., 2013; Mannix et al., 2008; Tseng et al., 2012) but have not been used for the mechanical loading of single biomolecules with controlled force. Therefore, new experimental tools are needed to probe the spatial, temporal, chemical, and mechanical regulation of mechanosensitive proteins in a single and integrated platform.