# Magnetic Cellular Switches

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*Abstract*—**This paper focuses on the development of magnetic cellular switches to enable magnetic control of intracellular functions in living mammalian cells, including receptor signal transduction and gene transcription. Our approach takes advantage of the mechanosensitivity of adenosine 3′,5′-monophosphate (cAMP) induction and downstream transcription controlled by the cAMP regulatory element (CRE) to engineer gene constructs that optically report gene expression in living cells. We activate transcription of these gene reporters by applying magnetic (mechanical) stress to magnetic microbeads bound to cell surface integrin receptors. In these gene reporter constructs, CRE motifs drive the expression of fluorescent proteins or enzymes that produce fluorescent products, such as DsRed and β-lactamase (BLA), respectively. We demonstrate that a chemical inducer of cAMP (forskolin) increases expression of CRE-DsRed in living cells. More importantly, a threefold increase in CRE-BLA expression is induced by application of mechanical stress to magnetic microbeads (4.5 μm) bound to cell surface integrin receptors. Induction of cAMP could be detected within 5 min using a protein fragment complementation assay involving interactions between the KID and KIX domains of the CRE binding protein linked to complementary halves of the BLA enzyme. These studies confirm that application of magnetic stress to integrins induces gene transcription by activating the cAMP-dependent transcription factor CREB. Ongoing studies focus on optimizing sensitivity and reducing signal-to-noise by establishing stable cell lines that express these gene reporters. These studies collectively demonstrate the feasibility of using magnetic technologies to control function in living mammalian cells and, hence, support the possibility of developing magnetically-actuated cellular components for use in future micro- and nanotechnologies.**

*Index Terms*—**Biological cells, biological control systems, biological signal transduction, biomagnetics.**

## I. Introduction

LIVING CELLS exhibit a capacity for signal detection and information processing far beyond that of man-made technologies. Therefore, it may be of great value to integrate cells into future biosensors, diagnostic devices, and therapeutic approaches. For example, mammalian cells may have value as signal processing elements within microelectronic devices, as interfaces between man and machine, and as sensors for human biopathogens. One of the major limitations in this area has been the lack of a suitable control interface between cells and existing microtechnologies. This interface should be biocompatible, robust, reconfigurable, and exhibit low power requirements. Magnetic interfaces that link to physiological cellular signal transduction mechanisms would provide this type of functional control. Therefore, we set out to demonstrate the feasibility of developing magnetic cellular switches that permit the actuation of specific cellular behaviors with high fidelity.

Our approach is based on past work which demonstrated that mechanical forces are fundamental regulators of cell growth and function [1]–[3] and that mechanical stress can be applied to cells using magnetic microparticles [4], [5]. Cells sense mechanical stress through a family of transmembrane receptors known as *integrins* [4], [6] that mediate cell adhesion to extracellular matrix (ECM) proteins, which link cells together within all solid tissues [7]. Ligation and clustering of integrins by ECM induces the formation of local adhesion complexes on the cell surface, known as focal adhesions, that physically link the cytosolic portion of integrin receptors to the filamentous cytoskeletal network inside the cell [8], [9]. This mechanical coupling is responsible for changes in cell mechanics (e.g., strain-hardening behavior) that are observed when cells are mechanically stressed [4], [10]. Focal adhesions also contain multiple proteins involved in biochemical signaling [8], [11]–[13] that act to transduce mechanical stress applied to integrins into biochemical responses that affect signal transduction and gene expression [14]–[17].

Previous work from our laboratory has identified that intracellular signaling and gene transcription can be activated in a force-dependent manner by magnetically applying twisting forces to cell surface integrin receptors using ferromagnetic microbeads (1–5 μm diameter) coated with specific receptor ligands, such as synthetic RGD-containing peptides from the cell binding region of the ECM protein fibronectin [4], [11], [15]. Specifically, magnetic twisting of integrins that are clustered within focal adhesions results in a rapid increase in the production of the second messenger, adenosine 3′,5′-monophosphate (cAMP), inside the cell [15]. This increase in cAMP induces downstream signaling through protein kinase A and leads to the formation of a protein transcription complex that binds to an eight-nucleotide DNA sequence, known as the cAMP regulatory element (CRE), and promotes transcription of neighboring genes [18]. In this project, we leverage this previous work involving the mechanosensitivity of cAMP induction to develop magnetic cellular switches that enable the control of gene expression and cellular biochemistry in living mammalian cells using applied magnetic forces.

## II. Methods

### A. Optical Reporters of Gene Activation

To optically detect magnetically-induced changes in gene transcription, we engineered gene reporter constructs encoding either the red fluorescent protein DsRed or the enzyme β-lactamase (BLA) driven by three tandem CRE motifs, referred to as CRE-DsRed and CRE-BLA, respectively. BLA cleaves a fluorescent substrate (CCF-2) that, upon cleavage, fluoresces blue rather than green when excited at 409 nm. These reporter constructs were generated using CRE-d2EGFP (Clontech) that was modified by enzymatically removing the gene-encoding enhanced green fluorescent protein (EGFP) and inserting the gene encoding either BLA or DsRed.

For gene transfection studies, bovine capillary endothelial (BCE) cells were plated in glass coverslip-bottomed 35-mm dishes (MatTek) precoated with 500 ng/cm$^2$ fibronectin [19].

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Twelve to 24 h later, cells were incubated for 6 h with 0.5 $\mu$g plasmid DNA using Effectene reagent (Qiagen). The transfection was stopped by multiple washes with phosphate buffered saline (PBS) and the cells were then placed in defined medium (low-glucose Dulbecco's modified Eagle medium containing 1% bovine serum albumin, 20-mM HEPES buffer, 2-mM glutamine, 100-U/ml streptomycin, 100-U/ml penicillin, 10-$\mu$g/ml transferrin, and 10-$\mu$g/ml high density lipoprotein) for 6–12 h before carrying out experiments. To allow sufficient time for transcription and folding of BLA or DsRed after chemical or magnetic stimulation, cells were returned to growth medium containing 10% fetal calf serum for 6 h (or 20–24 h for DsRed) following stimulation. To account for low transfection efficiencies (typically 10% to 20%), cells transfected with BLA were cotransfected with a second plasmid encoding a constitutively-active DsRed (CMV-DsRed) as a marker to identify those cells that internalized plasmids.

To detect increases in intracellular cAMP more rapidly, we adapted a protein fragment complementation system in which two halves of a split enzyme (BLA) are fused to protein segments that are induced to bind one another in the presence of cAMP, and, thus, produce an immediate fluorescent readout [20]. The segments are the kinase inducible domain (KID) of CREB and the KIX domain of CREB-binding protein. In these studies, BCE cells were cotransfected with CMV-DsRed (marker), KID-BLAc (C-terminal portion of BLA), and KIX-BLAn (N-terminal). Following transfection, the cells were loaded with CCF-2 substrate (see below) and incubated with 100-$\mu$M forskolin to chemically activate cAMP signaling and assess BLA activity. pKIX-BLAn and pKID-BLAc were kindly provided by M. Greenberg, Children's Hospital, Harvard Medical School, Boston, MA.

### *B. Micromagnetic Actuation of Gene Transcription*

To apply mechanical stress to integrins, 4.5-$\mu$m ferromagnetic beads (Spherotech) were coated with RGD peptide (Peptite-2000, Integra Life Sciences) as described previously [4], [15]. Magnetic microbeads were allowed to bind to cells ($\sim$20 beads per cell) for 10–30 min and then twisted using magnetic twisting cytometry (MTC). In this technique, a brief (10 $\mu$s), but strong (1000 G), horizontal magnetic field is applied to align magnetic moments followed by a weaker (15 G) vertical magnetic field for 10 min that twists the magnetic particles [4]. BLA activity was assessed 6 h after twisting by incubating the cells with 2 $\mu$M CCF-2 for 45 min and quantitating cleavage of CCF-2, measured as a FRET-based color shift from green to blue fluorescence when excited at 409 nm. Images were obtained using a RT-Slider color digital CCD camera (Diagnostic Instruments), and the green and blue channels were digitally split using IPLab software (Scanalytics). The fluorescence intensity of each channel was quantitated by summing the pixel values within the projected area of each cell. The ratio of fluorescence intensity in the blue versus green channels was then used as a quantitative measure of cellular BLA activity.

## III. Results and Discussion

Our laboratory has previously demonstrated that twisting magnetic microbeads bound to ligated integrin receptors increases signaling through cAMP in a stress-dependent manner, whereas twisting other transmembrane receptors on the same cell (e.g., metabolic receptors and histocompatibility antigens) has no effect [14], [15]. In the current study, our goal was to leverage cAMP signaling through integrins to develop techniques for magnetic control of signal transduction and gene transcription in living mammalian cells that may be applied in the future toward the development of cell-based microtechnologies. To do this, we engineered gene constructs that take advantage of the mechanosensitivity of cAMP induction and transcription by CRE in order to actuate gene expression through magnetic (mechanical) stress applied to integrin-bound magnetic microbeads. In these constructs, three tandem CRE motifs drive the expression of optical reporter genes encoding either the red fluorescent protein DsRed or BLA which, when expressed and translated into protein, enzymatically cleaves the fluorescent substrate CCF-2 to induce a visible color change from green to blue. To first confirm that these reporter constructs respond to increases in cAMP, BCE cells were transfected with CRE-DsRed and treated with 100-$\mu$M forskolin to chemically activate adenylyl cyclase and increase intracellular cAMP. After 20 to 24 h, the cells treated with forskolin exhibited a significant increase in red fluorescence relative to untreated cells (Fig. 1).

To assess magnetic actuation of gene expression, RGD-coated magnetic microbeads were allowed to bind BCE cells that were cotransfected with CRE-BLA and CMV-DsRed; the constitutively active CMV-DsRed was used as a marker for plasmid uptake. Magnetic twisting was applied through integrin-bound RGD-beads for 10 min, and 6 h later cells were incubated with CCF-2 substrate and fluorescently imaged to assess BLA expression. Transfected (DsRed-positive) cells that were magnetically stressed exhibited a threefold increase in BLA activity compared to unstressed cells (Fig. 2), as measured by the mean ratio of blue-to-green fluorescence intensity. These results demonstrate that gene expression can be magnetically activated and detected optically in living cells.

A limitation of these and past studies is that it takes multiple hours for the reporter construct to be transcribed and translated into protein before enzyme activity can be measured. A true cellular switch would require a more rapid response to signal activation. To demonstrate the feasibility of a rapid magnetic readout, we utilized a fluorescent protein fragment complementation system in which two halves of the BLA enzyme are fused to two different intracellular proteins: the KID domain of CREB and the KIX domain of CREB-binding protein [20]. These domains normally mediate cAMP-dependent binding of CREB and CREB-binding protein during cellular cAMP signaling. Thus, with these engineered protein fragments, the presence of cAMP can be detected because cAMP-dependent binding of the KID-KIX domains brings the two halves of the BLA enzyme in close proximity so that they may refold into the active form of the enzyme. When cells expressing KID-BLAc (COOH-terminal of BLA) and KIX-BLAn ($NH_2$-terminal of BLA) were stimulated with 100-$\mu$M forskolin, cleavage of CCF-2 could be detected within 5 min after stimulation, with maximal levels achieved by 30 min (Fig. 3). These data demonstrate that this reporter construct provides a real-time fluorescent readout of cAMP induction in living endothelial cells, which is consistent with previous findings in neurons [20]. Current efforts in our laboratory are focused on applying this protein fragment complementation technology to develop living cellular switches that can be rapidly actuated magnetically. We are also optimizing sensitivity and reducing signal-to-noise by establishing stable cell lines that express high levels of these gene reporters.

Taken together, these studies collectively demonstrate the feasibility of using applied magnetic fields to control signal transduction and gene expression in living mammalian cells. This ca-

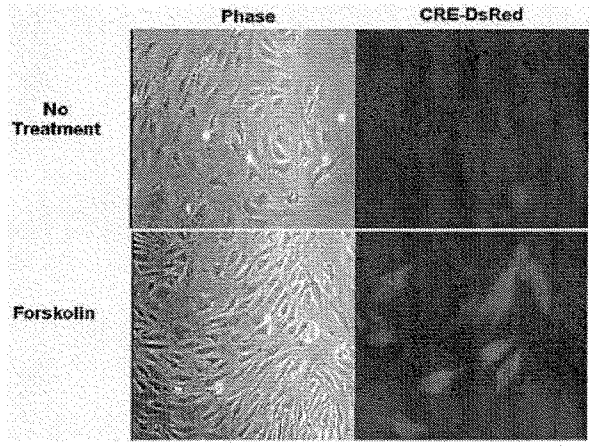


Fig. 1. Chemical activation of CRE-DsRed transcription. Phase contrast and fluorescence micrographs of BCE cells expressing CRE-DsRed treated in the presence or absence of forskolin (100 $\mu$M) for 24 h. Note the large increase in the number of DsRed-positive cells after forskolin stimulation relative to untreated controls, indicating successful induction of CRE-DsRed transcription by cAMP.

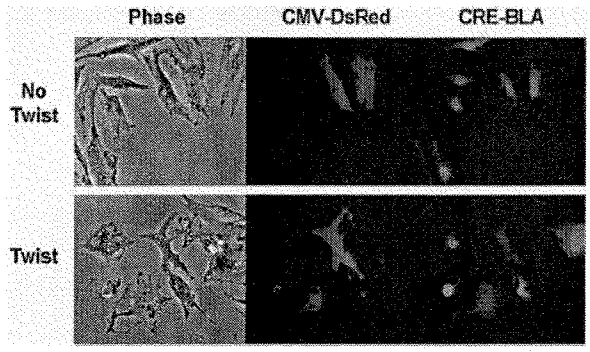


Fig. 2. Magnetic actuation of CRE-BLA transcription in living cells. Phase contrast and fluorescence micrographs of BCE cells that were cotransfected with CRE-BLA and CMV-DsRed (marker). Cells were bound to RGD-coated magnetic microbeads (4.5 $\mu$m) and magnetically twisted for 10 min. Six hours later, cells were loaded with CCF-2 and imaged using a color camera with 409-nm excitation. Expression of BLA and cleavage of CCF-2 (as indicated by a shift from green to blue fluorescence) increased threefold in plasmid-containing (DsRed-positive) cells that were magnetically twisted. These data were obtained from ten to 12 different cells for each condition; similar results were obtained in two independent experiments.

pability provides a potential mechanism to create novel magnetically-actuated cellular switching components for use in micro- and nanotechnologies. Because mechanical stress applied to integrins also activates other signaling pathways (e.g., tyrosine kinases and small GTPases), future magnetic cellular switches that work through integrins may also exploit different signal transduction mechanisms and readouts. It is also possible that magnetic stress application to mechanosensitive ion channels could directly induce transmembrane ion flux. However, regardless of their mechanism, the challenge for the future will be to create more robust magnetically-actuated cellular switches that can be removed from the laboratory environment and integrated within other microtechnologies so that they may be used in the field for applications in biotechnology, as well as biodefense.

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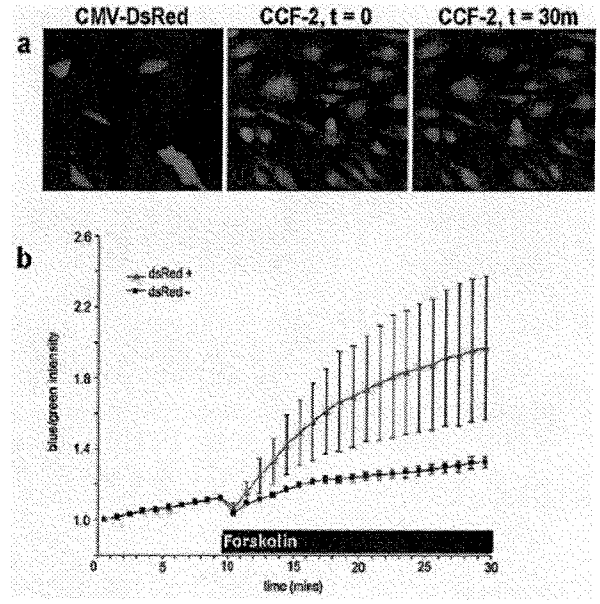


Fig. 3. Rapid readout of cAMP via induction of BLA activity using protein fragment complementation. BCE cells were transfected with CMV-DsRed (marker), KIX-BLAn, and KID-BLAc. Cells were loaded with CCF-2 and then treated with 100 $\mu$M forskolin. (a) Plasmid-containing (DsRed-positive) cells demonstrated increased CCF-2 cleavage 30 min after stimulation, seen as a change from green to blue cytoplasmic staining, indicating successful reconstitution of BLA enzyme activity via KID/KIX binding. (b) Quantitation of the relative change in blue-to-green fluorescence intensity in individual cells revealed that BLA activity can be detected within 5 min following treatment with forskolin to increase intracellular cAMP. Error bars represent SEM computed from either 5 DsRed-positive cells or 20 DsRed-negative cells from the experiment shown in (a).

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