A Yeast-Based Rapid Prototype Platform for Gene-Control Elements in Mammalian Cells

Kathy Y. Wei,1 Yvonne Y. Chen,2,3 Christina D. Smolke1

1Department of Bioengineering, Stanford University, 473 Via Ortega, MC 4201, Stanford, CA 94305; telephone: 650-721-6371, fax: 650-721-6602; e-mail: csmolke@stanford.edu
2Harvard Society of Fellows, Harvard University, Cambridge, MA
3Department of Systems Biology, Harvard Medical School, Boston, MA 02115

ABSTRACT: Programming genetic circuits in mammalian cells requires flexible, tunable, and user-tailored gene-control systems. However, most existing control systems are either mechanistically specific for microbial organisms or must be laboriously re-engineered to function in mammalian cells. Here, we demonstrate a ribozyme-based device platform that can be directly transported from yeast to mammalian cells in a “plug-and-play” manner. Ribozyme switches previously prototyped in yeast are shown to regulate gene expression in a predictable, ligand-responsive manner in human HEK 293, HeLa, and U2OS cell lines without any change to device sequence nor further optimization. The ribozyme-based devices, which exhibit activation ratios comparable to the best RNA-based regulatory devices demonstrated in mammalian cells to-date, retain their prescribed functions (ON or OFF switch), tunability of regulatory stringency, and responsiveness to different small-molecule inputs in mammalian hosts. Furthermore, we observe strong correlations of device performance between yeast and all mammalian cell lines tested ($R^2 = 0.63–0.97$). Our unique device architecture can therefore act as a rapid prototyping platform (RPP) based on a yeast chassis, providing a well-developed and genetically tractable system that supports rapid and high-throughput screens for generating gene-controllers with a broad range of functions in mammalian cells. This platform will accelerate development of mammalian gene-controllers for diverse applications, including cell-based therapeutics and cell-fate reprogramming.

Introduction
Advances in synthetic biology have highlighted the importance of precise gene expression control in the design of genetic circuits encoding complex functions (Anderson et al., 2007; Basu et al., 2005; Bonnet et al., 2012; Danino et al., 2010; Gardner et al., 2000; Stricker et al., 2008). Accordingly, significant effort has been directed toward the development of molecular tools that support the tight control of protein levels and activities within living cells (Alper et al., 2005; Ham et al., 2006; Isaacs et al., 2004; Salis et al., 2009; Win and Smolke, 2007). However, the majority of these undertakings have been directed toward microbial systems, while fewer tools exist for mammalian cells. The efficient engineering of mammalian cells that exhibit user-defined functions, including cell-fate reprogramming and cellular therapeutics, will require flexible, adaptable, and tunable gene expression control systems.

Synthetic RNA-based control devices, or RNA devices, are a versatile class of gene-regulatory elements that link environmental or endogenous inputs to gene expression outputs in living cells (Chang et al., 2012; Isaacs et al., 2006; Wittmann and Suess, 2012). RNA devices generally couple a sensing function, encoded by an RNA aptamer, to a gene-regulatory function (Liang et al., 2011). As RNA aptamers can be generated de novo for diverse small molecule and protein ligands (Ellington and Szostak, 1990; Tuerk and Gold, 1990), the input to these RNA-based controllers can be tailored to the particular application and host organism of interest. The majority of RNA devices demonstrated to-date have been designed to function in microbial cells (Babiskin and Smolke, 2011; Isaacs et al., 2004; Sharma et al., 2011; Sinha et al., 2010; Wieland and Hartig, 2008) such as...
Escherichia coli and Saccharomyces cerevisiae. More recently, researchers have begun to focus on the development of RNA devices that function in mammalian cells through diverse regulatory mechanisms (Beisel et al., 2011; Chen et al., 2010; Culler et al., 2010; Saito et al., 2011; Xie et al., 2011). RNA-based control systems exhibit several unique properties advantageous to clinical applications in human cells. In particular, RNA-based devices are encapsulated within a compact RNA-only platform (avoiding the use of immunogenic protein components) and exhibit adaptability to a wide range of molecular inputs and regulatory targets, tunable regulatory stringency, and rapid response to input availability (Chen et al., 2012).

The ability to tune the quantitative properties of gene expression control systems is of particular importance for the construction of complex genetic networks (Anderson et al., 2007; Basu et al., 2005; Bonnet et al., 2012; Danino et al., 2010; Gardner et al., 2000). Mammalian systems present challenges for rapid optimization given their slower growth rate relative to microbial systems, limited propagation ability in many cases, and inability to stably maintain extrachromosomal DNA. One approach to reduce the development time associated with mammalian gene controllers would be to develop a rapid and iterative prototyping strategy in a microbial host, such as the budding yeast, and subsequently validate optimized devices in a mammalian host (Fig. 1). A rapid prototyping platform (RPP) based on a yeast chassis would provide a well-developed and genetically tractable system, supporting high-throughput screens to generate broad ranges of quantitative genetic functions (Liang et al., 2012). The use of prototypes to rapidly optimize devices or systems is well established in many engineering fields, such as electrical and civil engineering, but has not yet been systemically adopted for biological devices. One reason is that most genetic devices do not maintain function when directly transported between mammalian and microbial systems, thus limiting the ability to develop such RPPs.

Most RNA devices that have been developed for mammalian systems utilize gene-regulatory mechanisms specific to higher eukaryotic organisms, such as RNA interference (An et al., 2006; Beisel et al., 2008, 2011; Kumar et al., 2009). However, a class of RNA devices has been described that utilizes a ribozyme-based gene-regulatory element, which functions independently of cell-specific machinery and thus offers a potentially directly portable control system. Ribozyme-based devices have been described in bacteria, yeast, and mammalian systems (Auslander et al., 2010; Chen et al., 2010; Ogawa and Maeda, 2008; Wieland and Hartig, 2008; Wieland et al., 2009; Win and Smolke, 2007, 2008). The majority of these devices are integrated into transcripts such that the regulatory effects of the resulting control systems are specific to the host organism in which they are developed. However, a recent example demonstrated a ribozyme-based device architecture that exhibits functional activity in yeast as well as murine and human T cells without changes in device sequence or architecture layout (Chen et al., 2010). While these results highlight the potential of developing an RNA device platform that can directly transfer between mammalian and microbial hosts, a systematic and quantitative examination of device activities in different cellular environments is necessary to support the use of yeast as the chassis of an RPP for mammalian ribozyme-based controllers.

Here, we demonstrate the ability of ribozyme-based devices prototyped in yeast to regulate gene expression in a ligand-responsive manner in model mammalian cell lines. Specifically, functional activities of six distinct devices capable of either inducing or repressing GFP levels in response to theophylline or tetracycline are verified in HEK 293, HeLa, and U2OS cells. We show that the ribozyme-based devices can be implemented combinatorially to increase regulatory stringency. Importantly, we observe that device performance is strongly correlated between yeast and all three mammalian cell lines tested, thus enabling the use of yeast data as a predictive basis for device performance in mammalian systems. The described RPP will make the process of generating mammalian gene-regulatory devices faster, easier, and more reliable. This process will reduce the cost and time currently associated with synthetic biology research in mammalian cells, and facilitate the development
of complex systems required for regenerative medicine, production of highly glycosylated compounds, and cell-based therapeutics.

Materials and Methods

Plasmid Construction

Plasmids were constructed using standard molecular biology techniques (Sambrook and Russell, 2001). Oligonucleotides were synthesized by Integrated DNA Technologies and constructs were sequence verified (Elim Biopharmaceuticals, Inc., Hayward, CA). Details of plasmid construction are available in Supplementary Materials, including plasmid maps (Fig. S1) and ribozyme switch sequences (Table S1).

Cell Culture Maintenance and Stable Cell Line Generation

Details of cell line generation and culture maintenance are available in Supplementary Methods Section.

Transient Transfection and Fluorescence Quantification

HEK Flp-In 293 transient transfections were performed using FuGENE 6 (Roche, Mannheim, Germany) following the manufacturer’s protocols. Plasmids that simultaneously express EGFP (regulated by ribozyme switches located in its 3′ UTR) and DsRed-Express (expressed from a separate, constitutive promoter to serve as an independent indicator for transfection efficiency) were used in transient transfection studies (see Fig. S1 for plasmid maps). Cells were seeded at 0.05 × 10^6 and 500 μL/well, in 24-well plates 24 h prior to transfection. Each transfection sample received 250 ng of plasmid DNA at a FuGENE:DNA ratio of 4:1. HeLa transient transfections were performed using FuGENE HD (Promega, Madison, WI) following the same protocol as for HEK cells except that each sample received 500 ng of plasmid at a FuGENE HD:DNA ratio of 5:2. U2OS transient transfections were performed using FuGENE HD following the same protocol as for HeLa cells except that cells were seeded at 0.08 × 10^6 cells/mL and a FuGENE HD:DNA ratio of 7:2 was used. Theophylline and tetracycline stock solutions were added to wells at the time of transfection to the appropriate final concentrations. Fluorescence data were obtained 48 h after transfection using a Quanta Cell Lab Flow Cytometer (Beckman Coulter, Brea, CA) equipped with a 488-nm laser. EGFP and DsRed-Express were measured through 525/30-nm band-pass and 610 long-pass filters, respectively. All fluorescence data were collected in the linear range of the instrument, with detector PMT set at 2.80 and 3.02 for EGFP and DsRed-Express, respectively. Flow cytometry data were analyzed using the FlowJo software (Tree Star, Ashland, OR). Compensation between EGFP and DsRed-Express was set according to single-color controls (Fig. S2A). Viability was gated by side scatter and electronic volume, and viable cells were further gated for DsRed-Express fluorescence, which served as an internal control for transfection efficiency and gene expression level (Fig. S2B and C). Fluorescence values from DsRed-Express-positive populations were used to calculate % GFP expression as follows:

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\text{% GFP Expression} = \frac{(\text{Sample GFP}/\text{Sample DsRed})}{(\text{Control GFP}/\text{Control DsRed})}
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where GFP and DsRed are the geometric mean of EGFP and DsRed-Express fluorescence intensity, respectively; “Sample” is the ribozyme switch being tested; and “Control” is the inactive ribozyme sTRSV Ctrl cultured with the same concentration of the appropriate ligand molecule as the test sample (Fig. S2D). Mean values from triplicate samples were reported with an error range of ±1 standard deviation.

Theophylline Response Assay for Stable HEK Flp-In Cell Lines

Stably integrated HEK cell lines as well as the parental HEK Flp-In 293 cell line were each used to seed three 24-well plates with 500 μL of culture per well. Each plate was seeded at different densities (0.1 × 10^6, 0.05 × 10^6, and 0.02 × 10^6 cells/mL) to allow assaying at different time points through the 5-day time-course study. At each seeding density, one-third of the wells were fed with 1 mM and one third with 5 mM final concentration of theophylline. At 24, 72, and 120 h after seeding, one plate was analyzed by flow cytometry, starting with the plate seeded at the highest cell density. Data analyses were performed as described for transient transfections, except without normalizing to DsRed levels as there was no need to account for transfection efficiency in the stable cell lines.

Cell Toxicity Quantification

Details of cell toxicity quantification are available in Supplementary Methods Section.

Results

Ribozyme Control Devices Prototyped in Yeast Are Directly Transferable to Mammalian Cells

The ribozyme-based device platform previously developed in our laboratory provides a modular assembly strategy for building user-programmed gene-control devices. Ribozyme devices are composed of three functional domains: an actuator (sTRSV hammerhead ribozyme), a sensor (RNA aptamer), and a transmitter (rationally designed RNA sequence linking the sensor and actuator) (Win and Smolke, 2007). The transmitter is designed such that the RNA device
can adopt at least two different conformations, one of which results in ribozyme cleavage (Fig. 2A and B). The transmitter component relays ligand-binding information from the sensor to the actuator through a programmed competitive hybridization event that specifies the signal-processing function encoded in the device (i.e., ON or OFF switch) and contributes to tuning of the quantitative performance of the device (Win and Smolke, 2007). In an ON switch (Fig. 2A), the transmitter is designed such that ligand binding to the sensor shifts the thermodynamic distribution to favor the ribozyme-inactive conformation, whereas in an OFF switch (Fig. 2B), ligand binding favors the ribozyme-active conformation. The ribozyme-based device is integrated into the 3' untranslated region (UTR) of the target gene, where ribozyme cleavage results in degradation of the target transcript and down-regulation of gene expression. By inserting the RNA devices into the 3' UTR rather than the 5' UTR of the target gene, we avoid nonspecific translational inhibition caused by secondary structures located upstream of the translation start site (Pelletier and Sonenberg, 1985) and ensure that the gene-regulatory activity of the RNA device will be conserved across organisms with diverse translation mechanisms.

Our unique device architecture allows for the direct translation of ribozyme-based devices initially developed in microbial cells (yeast) to mammalian cells and thus provides the foundation for an RPP. In particular, the devices function without any change in the sequence and without the need to optimize its placement relative to the gene of interest. We characterized the gene-regulatory activities of a theophylline-responsive OFF switch and several theophylline- and tetracycline-responsive ON switches with distinct transmitter sequences that dictate different quantitative response properties (i.e., basal expression levels, dynamic ranges) through transient transfection assays in a human embryonic kidney cell line (HEK 293; Fig. 2C–E).

Specifically, device gene-regulatory activity was measured as normalized % GFP Expression in the absence and presence of the appropriate small-molecule input, where the inactive (i.e., non-cleavable, non-switch) ribozyme device sTRSV Ctrl cultured at matching ligand-input levels served as the normalizing control (Fig. S2D). Due to differences in ligand toxicities toward different cell types, ligand concentrations used in mammalian cell assays were lower than those used when characterizing the devices in yeast (Liang et al., 2012).

Our results demonstrate that the relative activities of ribozyme-based devices initially developed in yeast are maintained when transported into mammalian cells (Table S2). For example, among the theophylline-responsive ON switches harboring different transmitter sequences, L2b8(1x) exhibits the lowest basal expression level and

Figure 2. Ribozyme switches prototyped in yeast with both ON and OFF capabilities demonstrate the appropriate ligand-responsive gene expression knockdown in mammalian cells. A and B: Ribozyme switches are designed to sample alternative structural conformations. The most thermodynamically stable conformation changes with the availability of the cognate ligand molecule. Schematics for ligand-responsive ribozyme (A) ON switch and (B) OFF switch are shown. Transient GFP expression levels are reported for (C) theophylline-responsive ON switches L2b1, L2b8, and L2b9; (D) tetracycline-responsive ON switches L2b1tc and L2b18tc; and (E) theophylline-responsive OFF switch L2bOFF1. GFP fluorescence values were normalized to DsRed fluorescence values (marker for transfection efficiency) and to those of the inactive ribozyme control cultured at corresponding ligand concentrations. Reported values are mean ± SD from triplicates.
largest fold-change, whereas L2b1(1x) exhibits the highest basal expression level and smallest fold-change (Fig. 2C). In addition, of the two tetracycline-responsive ON switches harboring different transmitter sequences, L2b18tc(1x) exhibits the lower basal expression level and higher fold-change (Fig. 2D). The maintenance of relative gene-regulatory activities suggests that the tuning capability of the transmitter sequences is retained in mammalian cells. However, we also observed that basal levels of the ON switches and induced level of the OFF switch are generally higher in HEK 293 cells compared to the levels of the same switches in yeast. The observed reduction in knockdown efficiency is consistent with differences in gene-knockdown activities associated with the unmodified sTRSV hammerhead ribozyme in yeast (≈1%) (Win and Smolke, 2007) versus HEK 293 cells (≈5%; Table S2). This variation in gene-regulatory activity is likely associated with differences in the rates of cellular mechanisms associated with this gene-control platform between the two cell types, including ribozyme cleavage activity as well as transcript and protein synthesis and degradation. The dynamic ranges exhibited by ribozyme switches in HEK 293 cells are generally lower compared to yeast. The reductions can be attributed to the lower ligand concentrations used in mammalian cell characterizations (i.e., 5 mM vs. 10 mM theophylline), as previous studies have shown that these ligand concentrations do not result in saturation of the ligand-induced state of the ribozyme-based devices (Liang et al., 2012).

Taken together, the data indicate that ribozyme-based devices developed in yeast preserve all relative trends when directly transferred into a model mammalian cell line. The ribozyme-based switches are functional in HEK 293 cells with no modification to the device sequence and only facile changes to the genetic construct (i.e., modifying the promoter and terminator elements, using a mammalian codon-optimized GFP) to support expression in mammalian systems. The data support that ribozyme-based devices can be rapidly prototyped and optimized in a model microbial host and maintain function when transferred into a mammalian cell line, thus enabling an RPP.

Multi-Copy Ribozyme Switch Architectures Provide Predictably Tunable Gene-Regulatory Activities

The ribozyme-based device platform allows for predictable tuning of basal expression levels by supporting the implementation of multiple copies of ribozyme switches within the 3’ UTR of each target gene (Fig. 3A). Within a ribozyme device cluster, each switch acts independently and a single switch in a ribozyme-active conformation will cleave and inactivate the entire transcript (Chen et al., 2010; Win and Smolke, 2008). Therefore, multi-copy switch devices increase the probability of ribozyme-mediated transcript cleavage, resulting in more stringent gene-control systems.

We built multi-copy devices for each ON switch (up to three copies) and characterized these extended control systems in HEK 293 cells. Increasing the number of ribozyme switches within a single target transcript increases the stringency (i.e., lowers the basal expression level) and the dynamic range of the mammalian control system in terms of fold change between the ON and OFF states (i.e., activation ratio; Fig. 3B–E). The observed trends in gene-regulatory activity as a function of switch copy number in mammalian cells are consistent with previous studies in yeast examining two-copy switch devices (Win and Smolke, 2008). The basal level of the L2b8 control system drops from 26.0% (L2b8(1x)) to 7.88% (L2b8(3x)) when two additional copies of L2b8 are incorporated, representing a 3.3-fold increase in stringency (Fig. 3C). Incorporation of two additional copies of L2b9 increases the activation ratio of the control system from 1.45 (L2b9(1x)) to 3.50 (L2b9(3x)), representing a 2.40-fold increase in switch activity (Fig. 3D).

The gene-regulatory activity of a multi-switch control system can be predicted from the activity exhibited by the switch in single copy. Under conditions in which all ribozyme switches in a device cluster act independently, each additional copy of a switch results in a fixed fold-decrease in basal expression levels from the control system. That is, predicted 2x basal level = (measured 1x basal level)² and predicted 3x basal level = (measured 1x basal level)³. For example, the predicted 2x level of L2b1 is (0.704)² = 0.495, or 49.5%, which is consistent with the experimentally measured level of 43.7% (Table S3). The data indicate that the observed gene-regulatory activities of 2x and 3x switch control systems can be accurately predicted based on measured activities from the corresponding 1x switch control systems (Fig. 3F; R² = 0.956).

Taken together, the data support that multi-copy ribozyme switch control systems exhibit similar trends in mammalian and yeast cells. The data further support the model that ribozyme switches exhibit independent cleavage activities when simultaneously integrated within the 3’ UTR of a target gene. Minor discrepancies between the measured and predicted values of these higher-order gene-control devices may be explained by kinetic limitations in ribozyme cleavage and the use of protein expression levels as a surrogate for in vivo ribozyme cleavage activities. Importantly, the conservation of modularity and predictability of multi-copy ribozyme switches from yeast to mammalian cells implies an RPP capable of developing multi-input and higher-order processing devices.

Ribozyme Switches Provide Titratable Gene Regulation and Conserved Switching Properties When Either Transiently Expressed or Stably Integrated in Mammalian Cells

The ability to adjust and stably maintain gene expression output as a function of the concentration of the molecular input is critical for the fine-tuning of protein levels across genetic devices and the proper functioning of complex genetic systems. Therefore, the dose-dependent
response and long-term stability of ribozyme-based devices in mammalian cells are important properties of these control systems.

We examined the dose response curves for a series of theophylline-responsive (L2b9(1x), L2b9(3x), L2bOFF1(1x)) and tetracycline-responsive (L2b1tc(1x), L2b18tc(1x)) ribozyme-based control systems by transient transfection and compared them against the wild-type hammerhead ribozyme non-switch control (sTRSV) in HEK 293 cells. The data illustrate that the gene-regulatory activity of a given device can be titrated across a range of molecular input concentrations for both ON and OFF switches (Fig. 4A and B). In contrast, the non-switch control (sTRSV) does not respond to either theophylline or tetracycline, confirming the specificity of the response to active switch systems.

Importantly, the maximum ligand concentrations used were dictated by toxicity levels (Fig. S3) and do not reflect the theoretical maximum switch response.

We next examined the long-term robustness of ribozyme-based devices when stably integrated into a mammalian cell line. Since downstream applications generally require stable expression of transgenic products, it is important to demonstrate performance consistency across transient and stable expression systems if a rapid development platform based on transient transfection assays were to be used. We performed site-specific integration of ribozyme-based device construct L2b9(3x) and non-cleaving control (sTRSV Ctrl) into HEK Flp-In 293 cells (Life Technologies, Grand Island, NY) to allow direct comparison in stably integrated cell lines. The dose response activities of the resulting cell lines were characterized in the presence of 0, 1, and 5 mM theophylline over 5 days (Fig. 4C). At an early time point (Day 1), the stably integrated ribozyme switch exhibits a dampened dynamic range compared to the eventual peak (Day 3), indicating a time lag before the device becomes fully induced. Importantly, the peak performance of the stably integrated RNA-based control system closely matches that of transiently expressed constructs (stable: 22.1–47.0–70.6%, transient: 18.8–41.9–66.0%, at 0, 1, and 5 mM theophylline, respectively; Fig. 4C–D). Moreover, both basal and induced expression levels in stable cells remain consistent through Day 5, affirming the long-term robustness of the devices.

In summary, the data indicate that ribozyme-based device performance as a function of input concentration is consistent under transient transfection and stable integration conditions. Taking advantage of this performance consistency, an RPP workflow in which new ribozyme-based device functions are prototyped and optimized in yeast, verified in the desired mammalian cell line through transient transfection assays,
We demonstrate a ribozyme-based device platform that can be directly transported from yeast to mammalian cells in a “plug-and-play” manner. Specifically, a variety of ribozyme switches encoding different functions (i.e., ON, OFF) and responsive to different small-molecule inputs (i.e., theophylline, tetracycline) are directly translatable to diverse mammalian cell lines, including human HEK 293, HeLa, and subsequently integrated into the cell line of interest can expedite the development of downstream applications.

**Gene-Regulatory Activities for Ribozyme-Based Devices Measured in a Microbial Host Are Highly Predictive of Activities Exhibited in Various Mammalian Cell Lines**

The ability of a gene-control system to maintain consistent function across various mammalian cell lines is important for the versatility of the genetic device and its effective implementation across diverse applications. Different mammalian cell types can have vastly different transcript processing rates, capabilities, and machinery, which could interfere with the ability to reliably and rapidly prototype gene-regulatory devices. Therefore, the predictable behavior of a device platform across diverse cell types is an important property in establishing an RPP.

We examined the ability of the ribozyme-based device platform to maintain function across different model human cell lines—HEK 293 (embryonic kidney), HeLa (cervical cancer), and U2OS (osteosarcoma)—by transient transfection (Figs. S2–S4). While all switches are functional in the three cell lines tested, differences exist in the quantitative performance of the devices across cell lines (Table S2). For example, the average activation ratios of the examined theophylline-responsive ON switches are similar in HEK 293, HeLa, and U2OS cells (1.9, 1.7, and 1.9, respectively). However, the range of basal expression levels from the ribozyme switches is the largest in HEK 293 cells, such that both the smallest (1.13 for L2b1(1x)) and largest (3.53 for L2b8(2x)) activation ratios are observed in this cell line. Importantly, despite the differences, a correlation analysis on the gene-regulatory activities of all theophylline-responsive ON switches demonstrates a strong linear correlation in the activities between yeast and each of the human cell lines tested ($R^2 = 0.630–0.969$; Fig. 5).

Collectively, the results indicate that ribozyme switches maintain their activity in a variety of mammalian cell lines. The results also highlight a strategy by which ribozyme switches can be efficiently designed and optimized to exhibit desired quantitative activities in mammalian cell lines of interest. Namely, a small set of ribozyme switch constructs can first be characterized in both yeast and the mammalian cell line of choice to establish the linear regression model. Then, additional ribozyme-based devices that are rapidly generated and prototyped in yeast can be easily mapped to predicted mammalian cell levels via the regression. Thus, large ribozyme-based device libraries can be rapidly screened in yeast for new or optimized activities, and only a few of these prototyped devices will need to be tested subsequently in the lower-throughput mammalian cell lines. Under situations in which the desired gene-regulatory levels are known and must be precisely established, this RPP approach will yield the desired mammalian gene-control system with greatly reduced development effort.

**Discussion**

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and U2OS, without any change to the device sequence and without optimizing device placement in the 3’ UTR. Select ribozyme switches have previously been used in mouse CTLL-2 and primary human central memory T cells to regulate cell proliferation (Chen et al., 2010). Here, we show that the basal and induced gene expression levels associated with a given ribozyme switch in a mammalian cell can be predicted from its performance in yeast cells. We establish that the gene-regulatory activity associated with a single-copy switch device can be used to quantitatively predict the activity of multi-copy ribozyme switch constructs, providing a method for rationally tuning the stringency of an RNA control system to meet application-specific requirements. In addition, the activity of a ribozyme switch can be titrated with the level of input molecule added to the cells, thus offering another method for fine-tuning the activity of the control system.

Our results highlight a genetic device platform that allows rapid prototyping and optimization in a microbial host followed by straightforward transfer of select devices exhibiting desired activities to mammalian cell lines in a predictable manner, thus supporting an RPP for advanced mammalian gene-control devices. Typical strategies for generating new genetic devices exhibiting desired activities require screening of large sequence libraries (Bartel and Szostak, 1993; Esvelt et al., 2011; Liang et al., 2012; Sinha et al., 2010). However, the transfection efficiency of mammalian cells limits the diversity that can be practically searched to $\sim 10^3$ to $10^5$. In contrast, the searchable diversity in yeast can be up to three to four orders of magnitude higher than that achievable in mammalian cell culture ($\sim 10^6$ to $10^7$), and yeast presents additional advantages in terms of ease of culturing, rapid doubling times, and availability of genetic tools (Gietz and Woods, 2001). Therefore, a device platform that can be translated between yeast and mammalian cell lines will leverage high-throughput, yeast-specific methods (Botstein and Fink, 2011; Liang et al., 2011) to efficiently tailor devices for mammalian cell applications.

RNA-based devices provide a compelling platform for genetic controllers in mammalian cells; they are adaptable to diverse genetic outputs and inputs, have tunable regulatory properties, and possess a compact, non-immunogenic, RNA-only footprint (Chen et al., 2010, 2012). Ribozyme-based cleavage is independent of cell-specific machinery and thus presents a mechanism that can work across cell types. However, not all ribozyme-based devices have been implemented with gene-control architectures that support direct transfer across diverse organisms (Ogawa and Maeda, 2008; Wieland and Hartig, 2008; Wieland et al., 2009). While these devices can be redesigned to function in higher organisms, the process is non-trivial and the resulting dynamics do not generally reflect properties originally observed in the microbial host. For example, a recently reported ribozyme-based OFF switch for gene expression control in mammalian cells (Auslander et al., 2010) was

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**Figure 5.** Ribozyme switch activity in yeast is predictive of activity in mammalian cells across different cell lines. The activity of theophylline-responsive ON switches in (A) HEK, (B) HeLa, and (C) U2OS are reported as a function of activity of the corresponding switch in yeast. Basal expression level correlations are shown in the top row and induced expression level correlations (at 5 mM theophylline) are shown in the bottom row.
based on a theophylline-responsive ribozyme originally generated through library screening in E. coli (Wieland and Hartig, 2008). The bacterially optimized switch sequence was functional in mammalian cells only after a combination of rational design and sequence randomization and screening in the mammalian system. Furthermore, the gene-regulatory mechanism required fundamental changes when moving from the bacterial system (i.e., control of ribosome binding site accessibility) to the mammalian system (i.e., control of mRNA stability by cleavage in the 5′ UTR). As a result, the ribozyme switch originally designed as an ON switch in bacteria only functions as an OFF switch in mammalian cells. In contrast, our platform supports engineering of diverse single-output functions in a manner that is directly translatable across organisms.

One potential limitation associated with genetic devices that are multi-purpose, generalizable, and portable is that their performance can be less efficient for a particular task compared to a less universal device that has been specifically optimized for that task in the desired host organism. However, gene-regulatory activities exhibited by the portable ribozyme-based device platform described here are similar to the activities of several previously reported devices specifically optimized for activity in mammalian cells. For example, the best ribozyme ON switch presented here is L2b8(2x) with a dynamic range of 10.2–36.0% and activation ratio of 3.53. In comparison, the extensively optimized OFF ribozyme switch described above exhibits a dynamic range of ~100% to 22% (activation ratio of 4.5) at 5 mM theophylline from an enzymatic reporter (Auslander et al., 2010). Several RNA switches that incorporate shRNA silencing mechanisms have been described (An et al., 2006; Beisel et al., 2008), and the best switches from each study exhibit dynamic ranges of approximately 30–95% and 21–85% (activation ratios of 3.2 and 4.0) at 10 and 5 mM theophylline, respectively. In addition, several RNA switches that incorporate miRNA silencing mechanisms have been described (Beisel et al., 2011; Kumar et al., 2009). The best switches from each study exhibit dynamic ranges of approximately 95–22% and 4–16.4% (activation ratios of 4.3 and 4.1) at 3 and 1.5 mM theophylline, respectively. It should also be noted that no other RNA-based device platform supports both ON and OFF switches in the same platform.

We used three distinct mammalian cell lines, ribozyme switches responsive to two different inputs encoding different functions, and transient as well as stable expression to validate a uniquely flexible platform that supports rapid prototyping of genetic devices in yeast followed by direct transfer of devices to mammalian cells. This RPP enables the high-throughput characterization of genetic devices and tuning of regulatory parameters that are preserved in a predictable manner when moved into mammalian cells, including input molecule, response curve, and relative dynamic ranges. Our RPP will support faster and more efficient development of mammalian devices for applications that require precise performance control, including production of complex pharmaceuticals, cellular diagnostics, and cellular therapeutics. Future work may extend the concepts presented here to develop other genetic devices that can transfer directly between yeast and mammalian cells. For example, portable genetic devices may be designed that direct protein binding to the 5′ UTR of a target gene to repress translation (Stripecke et al., 1994), modify splice site accessibility or group I introns (Long and Sullenger, 1999), or control post-translational modifications such as phosphorylation (Waskiewicz and Cooper, 1995) and ubiquitination (Kerscher et al., 2006). Establishing RPPs will transform our ability to design sophisticated genetic functions in organisms for which tools for precise genetic manipulation remain elusive, leading to new paradigms in biological system design and advancing synthetic biology into non-model organisms.

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