

Chapter 5

Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future

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ABSTRACT

Systems and synthetic biology promise to develop new approaches for analysis and design of complex gene expression regulatory networks in living cells with many practical applications to the pharmaceutical and biotech industries. In this chapter the development of novel universal strategies for exogenous control of gene expression is discussed. They are based on designer allosteric ribozymes that can function in the cell. The synthetic riboswitches are obtained by a patented computational procedure that provides fast and accurate modular designs with various Boolean logic functions. The riboswitches can be designed to sense in the cell either the presence or the absence of disease indicative RNA(s) or small molecules, and to switch on or off the gene expression of any exogenous protein. In addition, the riboswitches can be engineered to induce RNA interference or microRNA pathways that can conditionally down regulate the expression of key proteins in the cell. That can prevent a disease's development. Therefore, the presented synthetic riboswitches can be used as truly universal cellular biosensors. Nowadays, disease indicative RNA(s) can be precisely identified by employing next-generation sequencing technologies with high accuracy. The methods can be employed not only for exogenous control of gene expression but also for re-programming the cell fate, anticancer, and antiviral gene therapies. Such approaches may be employed as potent molecular medicines of the future.

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INTRODUCTION

In the last several years sequencing technology has been improving dramatically (Reis-Filho, 2009). Nowadays, 200 million bases can be sequenced per day in the Human Genome Project (HGP) using the latest generation sequencing technology such as the Genome Analyzer IIe from Illumina and the Solid System IV from Applied Biosystems (Oetting, 2010). These machines offer ultra-throughput whole genome sequencing (Bentley et al., 2009), targeted re-sequencing, SNP analysis (Chan, 2009; Ramos et al., 2009), gene expression and small RNA analyses (Jung, Hansen, Makunin, Korbie, & Mattick, 2010), chromatin immune-precipitation and DNA methylation arrays. The next generation sequencing (NGS) technology promises to advance genomic research in the next years by producing whole genome and gene expression data in combination with epigenetic data (Liang et al., 2009). Such complex approach was not available only a few years ago. The huge quantity of experiential data produced by the NGS technology can be fully analyzed by parallel computing (Bateman & Quackenbush, 2009). The combination of NGS technology with high-performance computing (HPC) is set to make a breakthrough in genomics, and many have far-reaching applications in cancer research. This integrated approach promises to bring to light the complexity of factors that make one cell become cancerous (Morrissy et al., 2009; Wyman et al., 2009). Understanding the molecular mechanisms of cancerogenesis is an important step for finding a cure. However, knowing the molecular mechanisms of one disease doesn't lead immediately to its cure. In this chapter we describe two general approaches that can tackle various disorders, which are associated with expression of disease indicative RNA(s). (Teng & Xiao, 2009; Willenbrock et al., 2009).

The methods described in this chapter are based on allosteric hammerhead ribozymes (Porta & Lizardi, 1995), which can work as biosensors *in vitro* and as synthetic riboswitches and gene regula-

tory elements *in vivo* (R. Breaker & Penchovsky, 2008; Penchovsky & Breaker, 2005). Ribozymes are naturally occurring RNA molecules that possess a catalytic function similar to that of protein enzymes (Muller, 2009). Allosteric enzymes are biopolymers that can change their conformation on binding a specific effector molecule(s) to their allosteric domains (Penchovsky & Breaker, 2005). Effector binding domains are distant from the catalytic center in an allosteric enzyme. Allosteric protein enzymes are discovered to play an important role in biochemical pathways of many organisms. Unlike allosteric protein enzymes all allosteric ribozymes are synthetic molecules. The only naturally occurring ribozyme discovered up to now to sense the presence of an effector molecule, is known as the glmS ribozyme (Blount, Puskarz, Penchovsky, & Breaker, 2006). However, the glmS RNA is not an allosteric ribozyme. Its activator glucosamine-6-phosphate serves as a co-factor in the catalytic center of the ribozyme. The allosteric ribozymes are designed to sense the presence of small molecules or oligonucleotides and to catalyze certain chemical reactions by various engineering methods. Such methods include *in vitro* selection (Guryev & Cuppen, 2009), rational design (Tang & Breaker, 1997), and computational design procedures (Penchovsky & Breaker, 2005).

All allosteric ribozymes described in the current chapter are designed by a published computational procedure (Penchovsky & Breaker, 2005), which is also a subject of a pending patent application (R. Breaker & Penchovsky, 2008). The procedure yields a ribozyme sequence within minutes on an average personal computer with over 90% accuracy. It is advantageous in comparison to *in vitro* selection and rational design methods in terms of design accuracy and time spent. All presented ribozymes have a modular design that allows the allosteric domains to be easily altered. As a result, the ribozymes can be designed to sense any DNA and/or RNA oligonucleotide from 16 up to 22 nucleotides (nt) long. The ribozymes can be engineered to perform essential Boolean logic

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