
Preface

Ribonucleic acids or RNAs are versatile. mRNAs serve as mediators of hereditary information from DNAs to proteins, tRNAs carry amino acids into the ribosome on mRNA, and rRNAs in the ribosome promote peptide bond formation between the amino acids. In addition, many diverse noncoding RNAs directly regulate gene expression in cis and/or trans. There are also many other types of RNAs known in nature, including RNA enzymes (ribozymes). It is considered that in an ancient RNA world (where proteins and DNAs did not still exist), a greater variety of functional RNAs would have flourished, though almost all of them are now defunct.

RNA functions are dependent on their sequences, which are composed of four kinds of nucleotides: A, G, U, and C. The sequences determine the higher order structures, which in turn make possible their unique functions. In other words, we could create new functional RNAs by appropriately lining up the nucleotides. Although RNAs are not as diverse as proteins, which have 20 amino acids as building block, RNAs have a large advantage in that they are easy to be amplified with the following three steps: reverse transcription, polymerase chain reaction (PCR), and transcription. We can therefore obtain functional RNAs from a randomized RNA pool by repeating selection–amplification cycles (in vitro selection). In fact, many types of functional RNAs have been selected via in vitro selection. The representative examples are aptamers that tightly bind to their specific ligand molecules and ribozymes that catalyze specific biochemical reactions. In vitro selection also enables us to obtain a ligand-responsive ribozyme called aptazyme, which is generally a conjugate between two foregoing functional RNAs, an aptamer and a ribozyme.

A riboswitch is also a functional RNA: a ligand-dependent and cis-acting gene regulator in mRNA. This regulatory RNA is composed of an aptamer domain, which binds to the specific ligand as described above, and an expression platform, which regulates transcription termination, translation initiation, self-cleavage, or splicing of its own mRNA. The interaction between the aptamer domain and the ligand induces conformational changes of the expression platform to up- or down-regulate gene expression. Natural riboswitches acting in response to endogenous metabolites have been identified mainly in bacteria and rarely in eukaryotes over the last decade. In parallel with these discoveries, a variety of methods have also been reported for artificially constructing arbitrary molecule-dependent riboswitches with the corresponding in vitro-selected aptamers or self-cleaving aptazymes.

This volume is mainly focused on the state-of-the-art methods developed in recent years for creating artificial riboswitches. Approximately half of the total chapters are devoted to screening or rational design methods for obtaining artificial riboswitches that function in either bacterial or eukaryotic translation systems. In these methods, an aptamer or an aptazyme is generally required for a gene regulator to acquire ligand responsiveness. Although an already identified aptamer or aptazyme is available, several chapters cover in vitro selection methods for obtaining a new aptamer or aptazyme for a ligand molecule that the reader might be interested in (a small molecule, protein, or photo-responsive molecule). In this context, one chapter is devoted to a computational method for designing a starting library of RNA sequences for in vitro selection. Protocols for evaluating the activities of the

resultant riboswitches are also presented. Some other chapters include protocols for construction of ligand-dependent, trans-acting gene regulators.

Artificial riboswitches and other ligand-responsive gene regulators that are obtainable through the cutting-edge methods described here make it possible to switch protein synthesis ON or OFF with arbitrary ligand molecules, which can be freely chosen when selecting the corresponding aptamers to be implemented in the gene regulators. Therefore, this book can be regarded as a collection of recipes for the gene circuit elements in synthetic biology and metabolic engineering. However, I would recommend this cookbook not only to bioengineers who aim to reprogram cell behaviors and molecular biologists who leverage these regulators for genetic studies but also to all researchers who just want to regulate the expression of a specific gene by an arbitrary molecule in various organisms, to detect a specific molecule with reporter protein expression in vitro or in vivo, or to design ligand-dependent RNA switches by using aptamers or aptazymes.

All chapters are written by experts from all around the world who are active in the front lines of the relevant research areas. I would like to express my gratitude to the contributors, all of whom were willing to write their lab protocols in an easily comprehensible manner, despite their busy research lives. I believe that the readers will be able to easily understand and follow the experimental procedures, thanks to the intelligible explanations and notes, and to construct their own artificial riboswitches. Last but not least, I am also grateful to Prof. John Walker for giving me the opportunity to edit this book.

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