RNA Technologies

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# Teaching Bacteria New Tricks—With RNA Switches**

*Jörg S. Hartig**

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If one reflects about future applications of genetically engineered bacteria, the degradation of environmental pollutants by appropriate organisms is a major target. A much more challenging scenario is the design of biological entities that carry out therapeutic interventions in human diseases. A currently unsolved task in these potential applications is the recruitment of such living scavengers at a specific location.

There have been attempts to alter the sensory system of bacteria that guides them along gradients of nutrition. By changing the specificity of the chemosensory receptors, bacteria could follow novel chemical attractants.[1] Although successfully demonstrated, the procedure of changing the receptor specificity is challenging and likely limited to compounds that are closely related to the naturally occurring stimulants of the chemosensory machinery. An elegant shortcut was introduced recently by Topp and Gallivan to guide bacteria along tracks of novel signaling molecules: they used RNAs instead of proteins to sense and follow a target compound.[2]

RNAs are extremely versatile tools for reprogramming cellular functions since they are critically involved in fundamental processes, such as gene expression and its regulation. Moreover, man-made RNA modules capable of binding specifically to target molecules (aptamers) as well as catalyzing certain reactions (ribozymes) are increasingly available, and can be integrated readily into existing functional RNAs in living systems. The properties of such engineered RNA systems are getting more and more predictable, for example, by increasing knowledge about the programmable and interchangeable character of certain RNA elements. Nature makes use of such a modular RNA toolbox as well: A variety of RNA-based mechanisms for the regulation of gene expression in response to small molecules such as metabolites have been discovered in recent years.[3,4]

Reminiscent of riboswitches, and even before the natural phenomenon was discovered, similar regulators were constructed artificially by incorporating aptamers into untranslated regions of messenger RNA.[5,6] Since then, several examples of artificial riboswitches have been introduced successfully (for example, theophylline-dependent activation of gene expression in *Bacillus subtilis*,[7] repression of eukaryotic expression,[5,8–10] and *trans*-acting switches in yeast[11,12]). Nevertheless, no general strategy for the discovery of artificial RNA switches that allows induction of gene expression in bacteria was available. Recently, Topp and Gallivan described a straightforward method to generate such switches.[13,14] In addition to their construction, they have set them into a very interesting context to teach bacteria to follow novel chemical tracks.

By using an in vivo screening protocol, switches were identified that respond to the xanthine analogue theophylline from partly randomized libraries of mRNAs containing a theophylline-binding aptamer.[13,14] These artificial switches follow a general principle found in a variety of naturally occurring riboswitches. Upon binding of the respective ligand, the accessibility of the ribosome binding site changes, thereby enabling enhanced gene expression (Figure 1). In a first example, Desai and Gallivan introduced a technique that allows selecting as well as screening for switches that respond

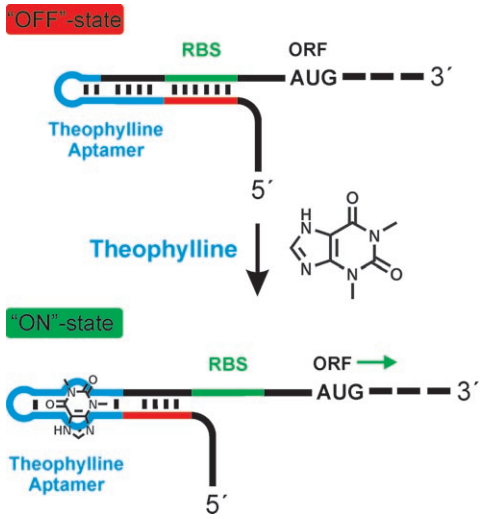


***Figure 1.*** An aptamer (blue) inserted into the 5′-untranslated sequence of a bacterial mRNA masks the ribosome binding site (RBS, green); ORF = open reading frame. Upon addition of a small molecule (theophylline) recognized by the aptamer, the RNA module reorganizes and renders the RBS accessible, thus resulting in increased gene expression. The green arrow denotes translation, the red region of the RNA denotes an anti-RBS sequence

[*] Prof. Dr. J. S. Hartig
Department of Chemistry
University of Konstanz
78457 Konstanz
Fax: (+49) 7531-88-5140
E-mail: joerg.hartig@uni-konstanz.de
Homepage: http://www.uni-konstanz.de/FuF/chemie/jhartig

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to theophylline.[13] They established a protocol that identifies clones exhibiting differential gene expression upon the presence of the effector theophylline by assaying β-lactamase as a reporter. This method allowed an artificial switch to be identified that increases gene expression upon the presence of theophylline. When the reporter β-lactamase gene was replaced by a sequence that encodes the enzyme chloramphenicol acetyl transferase, the transformed clones displayed theophylline- dependent resistance to the antibiotic. In a second study they optimized and characterized the theophylline-dependent RNA switches by identifying variants that displayed lower background expression. By doing so, they were able to identify clones that activate reporter gene expression by a factor of 35 upon addition of 1 mM theophylline.[14]

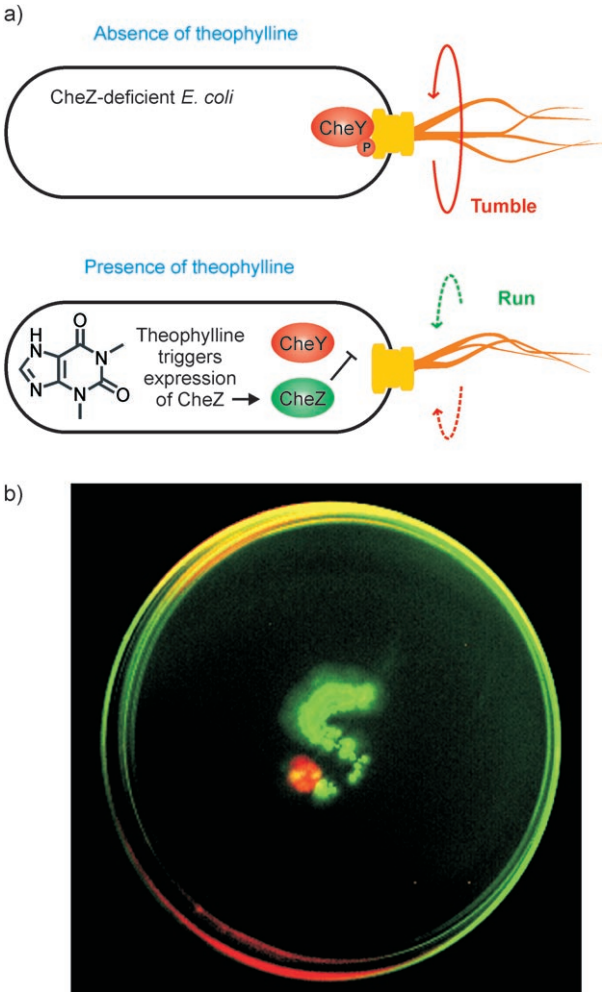


***Figure 2.*** a) Bacteria deficient in the protein CheZ are unable to move. If CheZ expression is placed under the control of a theophylline-dependent RNA switch, bacteria start moving if they encounter theophylline. b) Bacteria (visualized by GFP expression, green) containing a theophylline-responsive RNA switch that controls CheZ expression move along an S-shaped theophylline track (bacteria were inoculated at the top right end of the theophylline track). A different clone that lacks the theophylline-dependent cheZ expression stays put (visualized by RFP expression, red), inoculated at the bottom-left end of the S-shaped track). GFP/RFP: green/red fluorescing protein.

Next, one of the theophylline-responsive switches was used to address the aforementioned challenge of implementing artificial chemotaxis (control of the direction of motion) in *Escherichia coli*. Instead of switching the expression of a reporter gene, Topp and Gallivan used the theophylline-dependent device to control an essential regulatory protein of the chemotaxis machinery.[15] The protein CheZ controls the motility in *E. coli* by dephosphorylating CheY. In a CheZ-deficient strain, CheY remains phosphorylated, which results in a clockwise movement of the bacterial flagella, thereby resulting in tumbling bacteria (Figure 2a). If theophylline is present, it results in activation of CheZ expression followed by dephosphorylation of CheY and counterclockwise rotation of the flagella. As a consequence, the bacteria gain motility. Hence, by introducing a plasmid into a CheZ-deficient strain containing the theophylline switch in front of the coding sequence of the motility-controlling CheZ gene, bacteria start to move if they encounter theophylline. Bacteria engineered in this way move along tracks of theophylline in semisolid agar (see photograph in Figure 2b). The authors termed their artificial chemotaxis "pseudotaxis" since the two mechanisms differ in some aspects. For example, the natural chemotaxis system senses differences in the concentrations of chemoattractants irrespective of the absolute concentrations,[16] whereas the theophylline-sensing bacteria start moving if a certain threshold, defined by an absolute concentration, is exceeded.

The discussed reports have nicely demonstrated that RNA switches are well suited to engineer a system for the attraction of bacteria towards an artificial chemical stimulus. Since theophylline served as a proof of concept, it is now necessary to develop and implement aptamers that sense more relevant attractants such as pollutants or disease markers. The presented studies could be attributed to the emerging field of synthetic biology that is seeking to redesign existing living systems to fulfill novel tasks. Some important contributions from the RNA field have been summarized recently.[17] Reducing the complexity of the organisms as well as establishing standardized bioengineering rules will certainly simplify future endeavors in such directions.

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