RNA Aptamers

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# Plug and Play with RNA**

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RNA was traditionally thought to serve solely as a messenger between the genome and the proteome. Nowadays, it has become evident that RNA molecules play important roles in various cellular processes and in doing so they reveal properties almost as versatile as those known for proteins.[1] These developments have led to an increased understanding of RNA structure and function and, consequently, to efforts in constructing RNA-based tools. Noncoding RNA molecules can be found in almost every kingdom of life. The analysis of their biology has raised an increasing interest in RNA molecules and accordingly also in artificial, synthetic RNA building blocks. Although the chemical diversity of RNA molecules is rather limited—a circumstance that in turn facilitates straightforward synthesis—the secondary and tertiary structure of RNA molecules makes it possible to build surprisingly diverse recognition elements for a wide variety of compounds. Selective interactions of these RNA elements, so-called aptamers, with other RNA or DNA molecules as well as with proteins and small molecules have been described.[2] These interaction can be used for the spatial assembly of higher ordered structures and also for regulating biological processes inside cells.

Two very recent publications now demonstrate the high potential of synthetic RNA modules far beyond the current range of applications.[3,4] While Delebeque et al. designed multidimensional RNA structures in bacterial cells,[3] Paige et al. reported on RNA fluorophores which like green fluorescent protein (GFP) can be used as reporters to trace endogenous RNA molecules in eukaryotes.[4]

Delebeque and co-workers used RNA aptamers that recognize specific proteins and introduced them into defined RNA scaffolds. The expression of these multimeric RNA molecules results in the spontaneous assembly of defined three-dimensional architectures in prokaryotic cells. This assembly leads to the arrangement of protein targets of the individual RNA aptamers and the induction of distinct biological functions (Figure 1). The elaborate selection of adjacent base sequences made it possible to design RNA structures that can selectively self-assemble first into tiles, then into nanotubes, and finally into two-dimensional (2D) nanoarrays in the living host cell. Through the implementation of selective protein-binding RNA aptamers, the aptamers' target proteins can be localized in a controlled fashion on the surface of these nanoarrays. The aptamer domains on the nanostructures were hypothesized to serve as docking sites and templates for the controlled positioning of proteins in a certain arrangement.

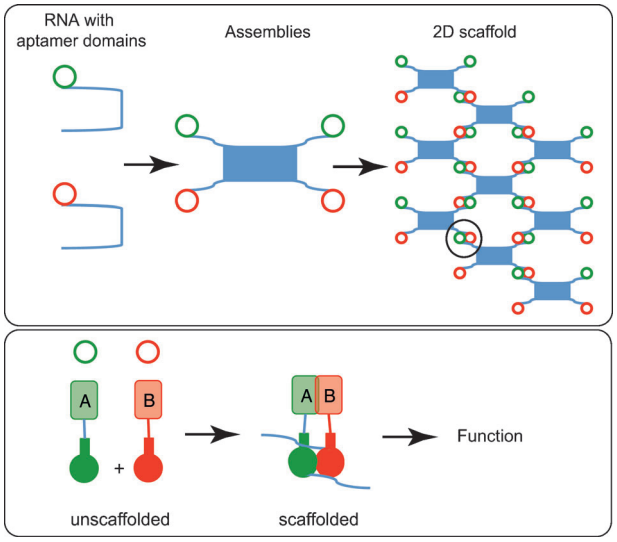


***Figure 1.*** Assembly of an RNA nanoarray for the controlled spatial arrangement of proteins inside prokaryotic cells. Top: RNAs harboring aptamer domains (green and red circles) are produced by the host cell's own transcription machinery and self-assemble into tiles by means of defined hybridization domains (blue lines). Several tiles further arrange into larger multidimensional scaffolds. Bottom: Enlargement of the area circled in block in the top scheme. The two fusion proteins A and B are bound by the aptamer domains on the scaffold such that they, and the biological functions they induce, are in close proximity.

Two impressive examples were reported that illustrate protein–protein interactions induced by the RNA scaffold. In the first, the fluorescing capabilities of a "split" green fluorescent protein were restored. In the second, two enzymes involved in the production of hydrogen, namely [FeFe]-hydrogenase and ferredoxin, were brought into close proximity. This RNA-scaffolded protein resulted in a more than 20-fold increase in hydrogen production compared to that of the reaction without the template. This demonstrates the

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successful RNA-mediated co-localization of the catalytic cascade.

Multienzymatic pathways are often highly spatially organized, such that the substrates are passed efficiently between the interacting proteins and the yield of sequential metabolic reactions is maximized. The research of Delebeque et al. opens the door to engineering synthetic pathways for the in vivo production of fuels, drugs, or chemicals that are otherwise difficult to tackle by traditional synthetic chemistry. Compared to DNA-based architectural approaches, RNA-related nanostructures can be generated inside living cells by the cells' own transcription machinery and, more importantly, they can autonomously self-assemble into functional nanostructures, as convincingly demonstrated by Delebeque and co-workers.

In the second study Paige et al. presented a simple, elegant strategy for introducing fluorescent tags for the live-cell imaging of RNA molecules. Starting with the fluorophore 4-hydroxybenzlidene imidazolinone (HBI), which is found in GFP, they synthesized an analogue to use in RNA aptamer identification. GFP gains its fluorescent properties as a consequence of correct protein folding, an autocatalytic intramolecular cyclization reaction, and coordination of the resultant fluorophore by hydrogen bonds.[5] By using the HBI analogue 3,5-difluoro-4-hydroxybenzylidene (DFHBI) as the target molecule for in vitro selection approaches, Paige et al. obtained RNA aptamers that not only recognize but also activate the fluorescence of DFHBI. Moreover, the successful candidate aptamer, named Spinach, was cloned into native 5S RNA molecules and expressed in mammalian cells (HEK293T), and fluorescence could be observed upon addition of DFHBI. Given the tremendous impact GFP and its derivatives have had in characterizing proteins in vitro and in vivo, thus, revolutionizing cell biology, one may imagine the impact these "green fluorescent RNAs" (GFRs) may have for the visualization of the dynamics of native RNA molecules in living cells. The fluorescence properties of the spinach–DFHBI complex are very similar to those of GFP but with negligible photobleaching. Spinach could already be used successfully as a tool to track the intracellular dynamics of endogenous RNAs in living mammalian cells, since the formation and translocation of 5S RNA was visualized through the fluorescence of the complex. Cloning the sequence of Spinach into an RNA molecule of interest may make it possible to track various endogenous RNAs, for example in living mammalian cells (Figure 2). Through the use of different GFRs, it may also be possible to track numerous endogenous RNAs simultaneously.

Although Paige et al. could generate RNA–fluorophore complexes spanning the entire visible-light spectrum, most identified RNA–fluorophore complexes exhibit low quantum yields, thus, currently limiting their use in sophisticated imaging applications. However, structural investigations might lead to a deeper understanding of these RNA–fluorophore complexes. A series of RNA–fluorophore pairs with emission maxima at different wavelengths, like that known for GFP and its derivatives, would make it possible to visualize the co-localization of different RNA molecules and proteins. Combined with other RNA-based tools, this system may be also useful for building allosteric or FRET sensors that light up once the aptamer recognizes its target in cells.[6] In this way not only the localization but also the quantitative analysis of target molecules might be possible.

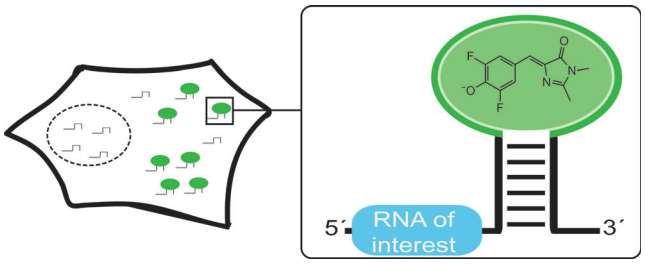


***Figure 2.*** Live-cell imaging of Spinach–DFHBI complexes. Spinach is an RNA aptamer that binds and also activates the fluorescence of DFHBI. The sequence of Spinach could be cloned into a RNA molecule of interest, thereby enabling the tracking of endogenous RNA in living mammalian cells.

Both publications demonstrate that RNA aptamers can serve as building blocks and powerful tools for engineering chemical reactions and tracking biological function in cells. The ease by which aptamers can be engineered in a modular, "plug and play" fashion should facilitate their broad applicability which is demonstrated here for the localization, visualization, and spatial organization of RNA and other biomolecules in cells; these are among the main challenges in biological assays. These reports demonstrate that RNA devices can be used to gain more knowledge about these processes and can be employed in a wide spectrum of applications. Once again, it has been shown that RNA devices, like proteins, can be used to construct sophisticated structural environments.

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