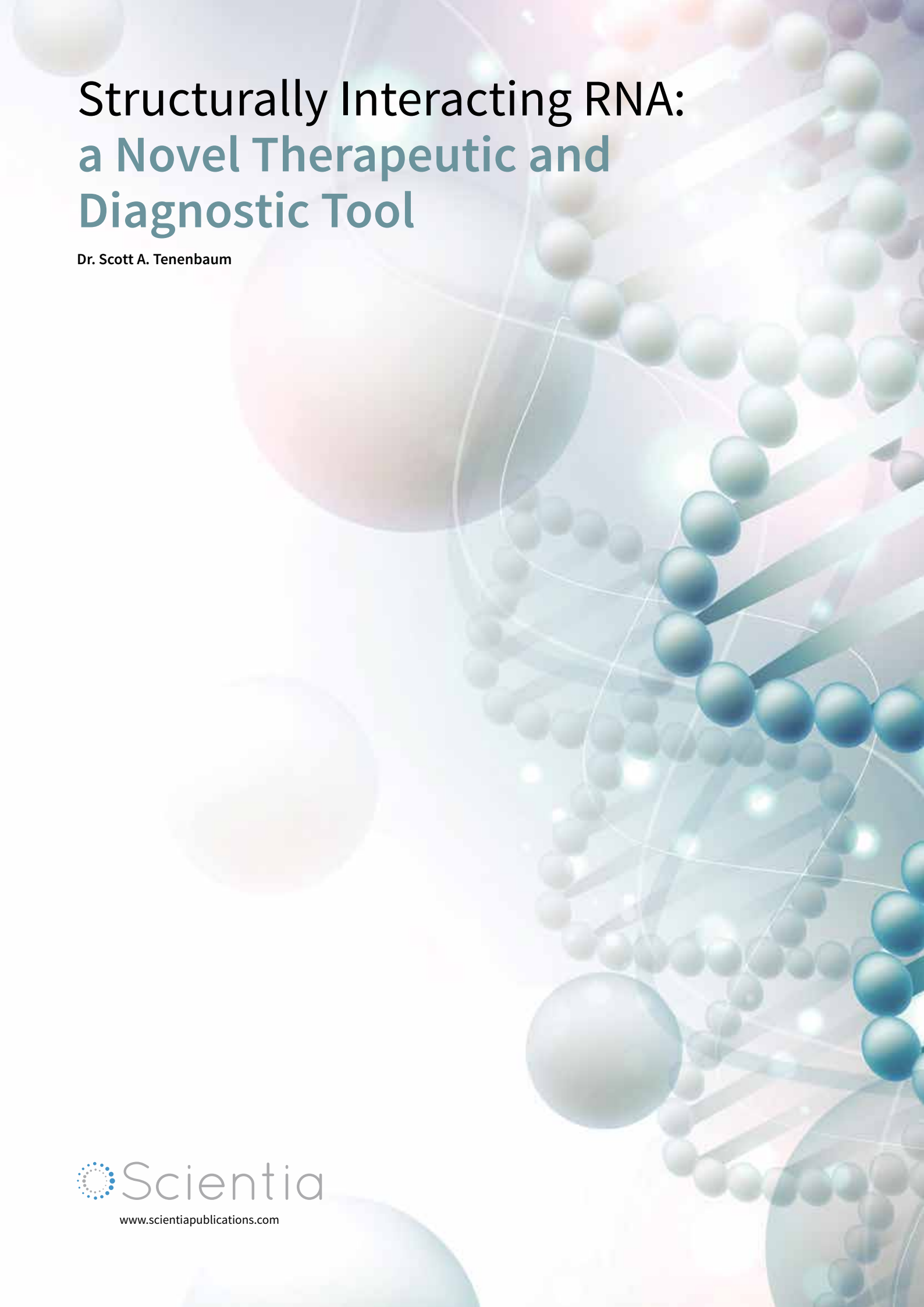
Structurally Interacting RNA: a Novel Therapeutic and Diagnostic Tool

Dr. Scott A. Tenenbaum



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Dr. Scott A. Tenenbaum is a molecular biologist who is interested in studying RNA biology, particularly RNA-protein interactions inside the cell. His work has led to the development of the structurally interacting RNA technology, which has important implication for the development of novel therapeutics.

To begin, what is your research background, and how did it lead you to your current position?

My PhD was in Microbiology and Immunology from Tulane University Medical Center, under the guidance of Dr. Robert Garry. In the process, we developed several new diagnostics and identified important interactions between RNA and proteins, which are major biological molecules of cells. We particularly identified some members of what we call 'RNA-binding proteins' (RBPs), which play an important role in regulation of gene activity in the cell. To further understand the function of these proteins, I joined the lab of Dr. Jack Keene at Duke Medical Center as a Post-doctoral fellow. Dr. Keene is one the leading RBPs researchers in the world, and has made great discoveries in this area. Together we developed and adopted new genomic-based techniques to identify and understand networks of RNA-protein interaction. This led to a whole new understanding of gene regulation and the birth of a new area of research.

I joined the University at Albany-SUNY as a faculty member in their new Genomic Cancer Center in 2003 and then moved to the College of Nanoscale Science and Engineering at SUNY-Polytechnic Institute in 2009 as an the Acting Vice President for Research and the Associate Director of Nanobioscience Constellation. My lab has continued to study RBP-RNA interactions, which have led us to the discovery of the structurally interacting RNA or what we have termed 'sxRNA'.

Whatis RNA and which biological functions is it responsible for?

RNA is one of the central building blocks of life. It plays a major role in protein synthesis going from DNA to RNA to Protein, the process known as the 'Central Dogma' of biology and genetics. Messenger RNA has traditionally been viewed as a molecule that simply transfers the coding information from the DNA to the final protein product. However, during the past several decades research has revealed many other cellular functions of RNA, including enhancement of enzymatic reactions, providing a structural scaffold for protein, RNA and DNA interactions, and regulation of protein synthesis from DNA (gene expression). Most importantly for our research, mRNA appears to not only contain the code for the 'what' but also has the regulatory code for the 'when, where and how much' a protein should be made.

As described in your publications, the sxRNA technology allows the control of expression of an ectopically delivered, RNA-based gene within a targeted cell type or tissue. What are the scientific principles underlying the sxRNA technology and how was it developed?

We first developed informatic and molecular tools that allowed us to identify naturally occurring sxRNA interactions, which appear to have the potential to interact and modify mRNA and are involved the regulation of generating the protein products produced from these mRNAs. This led us to commandeer the concept and try to design costum sxRNA molecules that can target chosen gene systems in order to switch their activity ON or OFF. There is a tremendous amount of informatic design that goes into the process followed by empirical testing of the switches for activity.

Are you planning to extend your research on sxRNA further? What might be the scope of the next step?

At the moment we are focused on three areas, anti-virals, biomanufacturing and advances in stem cell derived differentiated tissue. In the future we plan on developing sxRNAs for other disease targets and for gene therapy, as well as continuing to study the biology behind the natural occurrence of interacting RNA in the cell, and how they may be playing a role in regulation of gene expression.

Throughout your research on sxRNA, have you collaborated with other research groups or institutes? If so, what has been the role of your collaborators?

We have major collaborations with multiple groups including SUNY-Stony Brook and SUNY-Upstate Medical for development of anti-viral drugs, and The Neural Stem Cell Institute to develop sxRNA based stem cell therapeutics and The Austrian Centre of Industrial Biotechnology to improve biomanufacturing methods. Our approach has been to introduce the basic sxRNA technology and then provide the informatics and technical support to develop customized sxRNAs for their specific needs and applications.

Principles and Applications of Structurally Interacting RNA

Structurally interacting RNA (sxRNA) is a novel technology developed by Dr. Tenenbaum to control the expression of specific genes in the cell. Here we discuss the scientific principles of the technology and its potential applications for the development of therapeutics and biological research tools.

THE STORY OF RNA AND GENE EXPRESSION

RNA is a biological molecule that plays a central role in the generation of protein from DNA in the cell, a process that is essential for maintaining all forms of life. The genetic information required for protein synthesis is stored within the DNA in units known as the genes. During a process known as 'transcription', a particular gene is replicated into a homologous copy of RNA, called the messenger RNA (mRNA). Specific cellular machines called ribosomes then use the mRNA code to build the corresponding protein in a process known as 'translation.' The overall process of generating the protein product of a certain gene is referred to as 'gene expression'.

Eukaryotic cells carry a wide array of genes that encode a multitude of proteins of various structural and dynamic functions. However, throughout the developmental stages of the cell, and depending on the changes in the ambient physiological conditions, the expression of these genes might be either abundantly needed or undesired. Thus, the cell adopts a variety of regulatory mechanisms to control gene expression.

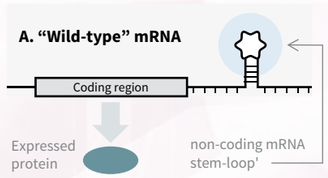
Besides being a core element in protein synthesis, RNA also plays a role in the regulation of gene expression. This regulatory role is mainly implemented by a group of RNAs collectively known as non-coding RNA, which modulate gene expression by controlling the process of mRNA translation. Examples of non-coding RNA include specific regions of the transcribed mRNA itself that do not undergo translation or what is referred to as the untranslated region (UTR), as well as other independently expressed small RNA molecules known as microRNA (miRNA). Much of the non-coding UTRs in mRNA is typically folded in numerous distinct conformational structures, that frequently include 'stem-loops', which can form in a manner that controls the availability of the adjacent coding-mRNA for translation into protein. This process is controlled through the effect of a large family of regulatory proteins known as RNA binding proteins (RBPs), which upon binding to the various stem-loop structures can trigger the induction or repression of translation as well as regulate the stability of the mRNA. Recently, it was discovered that the regulatory capacity of the stem-loop mRNA on gene expression can be further complemented by the interactions of miRNA, a second class of regulator non-coding RNA. miRNA molecules can bind to mRNA at specific regions, concealing or revealing binding sites for RBPs that can then modulate the translation process.

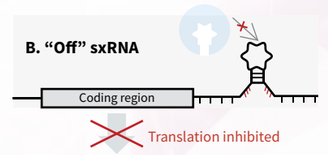
The structurally interacting RNA technology provides a basis for a novel class of therapeutics able to destroy virus-infected cells and cancer cells.

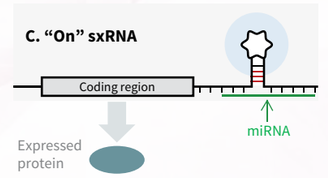
STRUCTURALLY INTERACTING RNA AS A MODULATOR OF GENE EXPRESSION

Using modern molecular technologies, Dr. Tenenbaum and his group have identified naturally occurring miRNA-mRNA interactions, which in contrast to the conventional linear binding of miRNA with mRNA, resulted in structural modification of stem-loops in the UTRs of mRNA targets. Inspired by the phenomenon, they have designed artificial RNA molecules that mimic miRNA in their capacity to modify mRNA structures, with the aim of developing a tool to control gene expression. These RNA molecules, which were designated "structurally interacting RNA (sxRNA), are designed so an embedded regulatory stem-loop is modified so its structural configuration can be regulated so that the binding of cellular RBPs that control mRNA translation is enabled or prevented. This mechanism provides a discrete 'ON-OFF' switch for the expression of the sxRNA target gene. Furthermore, the sxRNA technology can be engineered to take advantage of the cell's naturally occurring miRNA, which can be exploited to restore the optimal stem-loop structure and RBP binding, making an interchange of the ON-OFF gene expression statuses possible. This feature can be realized by designing sxRNA molecules that are engineered to structurally target a miRNA of interest. In such cases, after the integration of sxRNA with the regulatable stem-loop, a subsequent miRNA-sxRNA interaction will restore the optimum stem-loop structure, permitting the RBPs binding. Unlike the existing technologies that turn off gene expression, the

Structurally Interacting RNA (sxRNA)







Panel A. An RNA binding protein (RBP) binds to its naturally occurring "wild-type" stem-loop target sequence, which results in increased translation of an upstream gene by as much as an order of magnitude.
Panel B. We rationally design an sxRNA "switch" with a mutated stem-loop that prevents RBP binding and inhibits translation of the gene.
Panel C. In the presence of a targeted miRNA "trigger", a trans-acting, 3-way structure forms that stabilizes the stem-loop target structure. The RBP binds to the new sxRNA stem-loop target sequence resulting in increased translation of the upstream gene.

switchable gene expression control system offered by the sxRNA technology provides a means to turn ON gene expression, providing a solution for the transient control of the ectopic production of any protein product with a variety of amplitudes.

By coupling the translational regulation of gene expression with the unique microRNA signature patterns in the various cell types, the sxRNA technology can enable cell-specific expression of a desired protein or reporter gene for a diseased cells (e.g. virus-infected and cancerous cells) or even a specific developmental cell stage. The ability to target and control the expression of a desired gene at the level of mRNA rather than DNA opens up many new possibilities for vaccine, molecular and gene therapeutic tools. 'We have received very encouraging market feedback for this platform technology, which we believe has immediate commercial opportunities in important diagnostic and therapeutic areas', says Tenenbaum.

POTENTIAL APPLICATIONS OF SXRNA IN BIOMEDICINE

sxRNA technology represents a innovative way to control gene expression, with promising therapeutic applications such as the development of anti-viral therapies. 'Our sxRNA technology has advanced to the point that we are optimistic about targeting a number of significant and unique RNA molecules in infected cells in the hope of producing a new class of anti-viral therapeutics', says Tenenbaum. The technology has been recently proposed as a novel sxRNA-based antiviral drug against the Epstein-Barr virus. This virus is known to cause infectious mononucleosis (the kissing disease), and is commonly associated with the occurrence of various types of cancers such as lymphoma, nasopharyngeal and gastric carcinoma, and breast cancer. Currently, there are no specific antiviral agents to treat latent EBV infection, despite decades of research. However, Dr. Tenenbaum and his fellows have proposed promising sxRNA-based approaches to develop anti-EBV drugs. One of these approaches is based on the fact that some viruses such as EBV make their own miRNA in the cells. Here, these viral miRNA are exploited to interact with the sxRNA component of ectopically delivered custom-designed mRNA triggering its translation. In this case, the sxRNA-mRNA is designed to encode for a lethal protein that kills the cell when generated. Since the production of the lethal protein is limited to the presence of specific viral miRNA, the approach selectively kills infected cells, while sparing the healthy ones. A second approach to develop sxRNA-based anti-EBV drugs involves the 'anti-microRNA' technology, in which sxRNA is used to modify a stem-loop structure with which a specific cell-derived miRNA interacts to trigger the generation of an endogenous lethal protein. EBV typically use this miRNA to prevent the death of the host cells, which can be reversed by the anti-miRNA sxRNA, leading to death of the infected cells.

sxRNA is also of specific interest in stem cell research in which the desired homogeneity of cell populations is technically hindered by lengthy culturing periods and methods for selection of individual clones of interest. Dr. Tenenbaum and his team propose sxRNA as a tool to express antibiotic resistance or reporter genes, which serve as selection markers for the target clones during stem cell culture and differentiation. For such purpose, a mRNA-sxRNA complex encoding the reporter gene will be activated in response to interaction with complementary miRNA which is present only in the target cell population.

THE PRESENT AND FUTURE OF THE SXRNA TECHNOLOGY

As discussed, sxRNA is a well-developed technology that is close to commercial introduction, especially in areas of stem cell research and anti-viral therapy. However, according to Dr. Tenenbaum, some aspects of the technology are still being optimized. 'We are still adjusting the switching activity of our sxRNA constructs. We are also trying many new genes, including cell death genes and antibiotic resistance genes, so we can select for, or against, survival'', said Dr. Tenenbaum. The biggest current challenge for the therapeutic application of sxRNA is the issue of the delivery. Fortunately, several miRNA and mRNA therapeutic companies are currently putting efforts forth to develop strategies for robust delivery of RNA molecules.

The current focus of Dr. Tenenbaum and his group is mainly on anti-viral therapies and solutions for biomanufacturing and stem-cell production and homogeneity. In the future they will explore other therapeutic areas including gene therapy and will continue to study the natural occurrence of these sxRNA and how they might be playing a role in translational control of gene expression.

Researcher Profile

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Dr. Tenenbaum's research focuses on understanding some of the basic aspects of how the human genome works with an emphasis on post-transcriptional gene regulation. Along with his research team, Dr. Tenenbaum has helped to advance cutting-edge technology and computer-based informatic approaches to studying RNA biology, specifically RNA-binding proteins and how these molecules regulate information contained in RNA. Dr. Tenenbaum's research methods also include working on nano-based technology to make the research more robust and high-throughput. Dr. Tenenbaum is involved with HocusLocus Inc., a start-up biotechnology company that was spun-out of SUNY-Polytechnic Institute in Albany, NY. Hocus Locus is working to commercialize SUNY owned sxRNA intellectual property.

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