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RNA Science and its Applications—a look toward the future

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On November 3–4, 2011, the Symposium RNA Science and its Applications—a look toward the future was held at the University at Albany-SUNY in the capital of New York State. Unique to this Symposium's format were panel discussions following each of the four platform sessions: RNA Technological Innovation: Analysis, Delivery, Nanotechnologies, IT; Infectious and other diseases: The future of small molecule intervention; RNA Discovery and Innovation: Cell and Molecular Biology; and Cancer and Neurological Disease: The future of small RNAs as therapeutics and tools of investigation. The meeting was organized by Thomas Begley, Marlene Belfort, Daniele Fabris, Melinda Larsen, Pan T.X. Li, Albert Millis, Li Niu, David Shub, and Carla Theimer of The RNA Institute at University at Albany-SUNY, Paul F. Agris, Director, and Jennifer S. Montimurro, Program Manager.

We are now in an era where it is imperative to revolutionize healthcare for the treatment of difficult human diseases. This must be accomplished through a new paradigm of discovery, development and delivery of innovative medicines, diagnostics and vaccines. Pharmaceutical companies worldwide have made significant investments over the past 5–7 years in RNA as a novel therapeutic approach to human disease intervention. Three Nobel Prizes awarded for discoveries in RNA biology to eight RNA researchers since 2006 is testament to the excitement about the promise and power of RNA therapeutics. However, there are significant challenges that must be addressed to fully realize the diagnostic and therapeutic potential of RNA. Yet, US funding agencies lack a roadmap for there have been no comprehensive evaluations and advisement in RNA science and its applications for at least 10 years.

The RNA Institute, www.albany.edu/rna, hosted an international conference on November 3–4, 2011, in the capital of New York State with two days of scientific talks by the most forward-thinking and world-renown RNA scientists discussing the future of RNA research and the translational issues of RNA therapeutics and diagnostics that need to be overcome. The scientific talks covering the most significant contributions to RNA science to date were organized into four platform sessions each followed by a panel discussion addressing scientific and technological progress, the challenges and opportunities, investments made and needed by public and private sectors, and technological issues of RNA therapeutics.

This international conference with 200 participants was organized into four platform sessions with 24 scientific talks, including four keynote presentations by Eric Westhof (CNRS), Allan Jacobson (University of Massachusetts Medical School and PTC Therapeutics Inc.), David P. Bartel (Whitehead Institute/HHMI/Massachusetts Institute of Technology), and Paul F. Agris (The RNA Institute) followed by novel panel discussions highlighting each session, and 62 poster presentations. Participants included Merck, Pfizer, Medtronic, PTC Therapeutics, Pacific Biosciences, Albany Molecular Research, Inc., GE Global Research, SomaLogic, Inc., and the National Science Foundation (NSF). The four panel discussions were provocative in their exploration of the issues now facing the future of RNA science and its applications to technology and drug discovery.

RNA Technological Innovation: Analysis, Delivery, Nanotechnologies, IT

The first panel was chaired by Dieter Söll (Yale University) and included Laura Sepp-Lorenzino (Merck), Lothar Krinke (Medtronic, Inc.), and David Shub (University at Albany-SUNY). It is well-known that the delivery of RNA therapeutics is problematic. There are mechanical and biochemical possibilities for delivery. The panel addressed the present issues in delivery of RNA therapeutics and the approaches they felt would be most successful and feasible.

Presentations by Sepp-Lorenzino (Merck) and Krinke (Medtronic, Inc.) provided some answers. Delivery remains the main challenge with lipid and polymeric nano-particles (LNP and PNP) providing the greatest potency. However, there is limited applicability when biodistribution is restricted to liver/spleen. In addition, injectables (mostly intravenous) limit clinical and commercial use and significant toxicities arise from the delivery component. In particular, reproducibility is compromised with

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"empty" nanoparticles; the innate immune component is problematic; and there is direct liver toxicity plus secondary inflammation. However, siRNA conjugates may offer wider clinical applicability, but significant potency improvements are needed.

An alternative to oral and injectable RNA therapeutics is engineered (mechanical) delivery methods for naked siRNA. However, siRNAs have limited clinical and regulatory precedent. There are no approved siRNA products; formulation processes are complex; and chemistry, manufacturing and controls (CMC) may pose development challenges relative to manufacturability.

In summary, siRNA-based therapeutics promise to provide benefits to patients with unmet medical needs. Future advances in RNA chemistry, nanofabrication, and delivery systems should accelerate progress. Both siRNA and its delivery system are being optimized to maximize their pharmaceutical properties. An understanding of correlations, identifying structure-activity relationships (SAR), and exploiting mechanistic insights are keys to optimization of LNP for development. Also, an expanded applicability of therapeutic siRNA will require imparting siRNA molecules with drug-like properties that will allow for wider biodistribution and cell uptake selectivity while retaining potency and safety.

Delivery of RNA drugs is not only an important practical, but also a scientific, problem. Appointment of appropriately interested life scientists/engineers into a nurturing, multidisciplinary academic research environment is essential.

Infectious and Other Diseases: The Future of Small Molecule Intervention

The second panel was chaired by Ron Breaker (Yale University) and included Mathew Platz and David Berkowitz (NSF, Chemistry Division), Douglas Kitchen (Albany Molecular Research, Inc.) and Paul F. Agris (The RNA Institute). The panel discussion began with an introduction to the questions to be discussed and included phone-in comments by our NSF panelists.

Platz noted NSF support of Centers for Chemical Innovation and specifically described the Center for Chemical Evolution that is headed by Nicholas V. Hud at Georgia Tech, which perhaps most closely approaches the RNA interests of the symposium participants. Platz also called attention to the Chemistry of Life Processes NSF program that supports research projects at the interface between chemistry and biology. Berkowitz noted the existence of a website of awarded RNA research projects. He listed a series of projects supported and the investigators that are leading RNA-centric programs. These projects currently range from ribozyme engineering research and light-activated siRNAs to RNA structure research and the physics of nucleic acid packaging in viral capsids. Both NSF presenters stressed that they are very receptive to receiving proposals in the RNA sciences, but made it clear that more translational research to move RNA discoveries into more clinical or applied directions is beyond the scope of the NSF and therefore would be better suited for NIH support. They also noted that they do approximately 50 presentations at faculty meetings each year via Skype, and encouraged symposium attendees to consider this as a mechanism to learn more about NSF opportunities. When asked about the vision for research funding at the NSF, they made it clear that they are not a top-down organization, but rather consider funding the best ideas and initiatives put forth by the larger research community.

The question receiving the most attention from the panel related to the 100-year history of targeting proteins involved in disease, "What have we learned from protein targeting that can be applied to drug design against RNA targets?" Kitchen introduced the audience to the concepts of Lipinski's rules for drug development and noted that these will likely also be important when developing RNA drugs. He noted that proteins have a good mix of hydrophilic and hydrophobic moieties at ligand binding sites, whereas RNAs carry many charged phosphates, many polar and H-bonding groups, and that RNAs are composed of layered planar rings (more like graphite). These properties may demand different characteristics for compounds that bind RNA compared with the characteristics of protein-targeting drugs.

What are the functional and structural differences between RNA and protein that may change the rules for drug development between these two polymers? Agris noted that, in addition to the chemical differences between RNA and proteins, the structural mobility of RNA creates special challenges for drug developers. This issue was further highlighted by comments from Westhof. He noted that some antibiotics targeting ribosomes interact at regions of the RNA that undergo important switches in structure and that the drugs prevent this normal structural alteration. Therefore, a rational design approach for such target sites will not work well.

Breaker asked Westhof, Kitchen and the audience if they felt the current collections of compounds in chemical libraries is adequate for screening compounds that bind RNA. Agris and Kitchen noted that there are approximately 49 million compounds available in various chemical libraries (accessible through websites like PubChem) that may contain sufficiently drug-like compounds that are compatible with binding RNA targets. Screening these libraries in silico may be needed to focus valuable bench time and resources to test for activity against RNA targets of only those compounds that are most suitable.

Matthew Disney (speaker, The Scripps Research Institute, Scripps Florida) was asked his opinions on the challenge of targeting RNA given his experience in creating chemical fragments to target common RNA sequence/structure modules. He noted that ribosome targets are outliers (high concentration and very common in cells), and so drugs that hit ribosomes do not need to be very specific to work. Thus, compounds that target other more rare RNAs will need to be far more specific, which creates a greater challenge for developers of RNA-targeting drugs. Westhof brought up the notion of drugs that target riboswitches. It was noted that there are some examples of natural (roseoflavin) or synthetic (e.g., pyrithiamine, aminoethylcysteine) compounds that have been known for decades to kill bacteria, and that these compounds only recently have been determined to target riboswitches. Therefore, it is possible to make compounds that are selectively targeting more rare RNAs.

Some concern was voiced that riboswitches, like that for preQ^1, require a large number of H-bonds, but Joseph E. Wedekind

(presenter, University of Rochester) stated that the proteins that bind this compound also form numerous H-bond contacts with the compound, and so the principles of ligand binding appear to be similar between RNA and protein at least for this compound. Kitchen noted that one could find new druggable RNA targets by 'deorphaning' known drugs. Note that this is something that is beginning to be done in industry.

Since much attention was given to the first question, there was little discussion directed toward the topic of studying the complexes formed between small molecules and RNA posed by the following two questions: Assuming it is intellectually and commercially important, what investments need to be made in the area of RNA-small molecule interactions that will advance these interests? Can we harness knowledge of the evolutionary history of RNA by reverse engineering existing systems to assist in the design of new ligand-RNA interactions for useful applications? However, there are several key issues that are sure to challenge future academic researchers who wish to manipulate the functions and structures of RNAs:

(1) Large chemical libraries are now accessible to academic researcher, but screens for binding or RNA function modulation will need to be developed.

(2) Once hit compounds are found, the limiting factors for expanding on these will include expensive chemical synthesis of analogs and the need for quality medicinal chemistry expertise.

(3) The need for quality engineering of structured RNAs currently limits their utility for therapeutics and synthetic biology.

The institution that solves these challenges will be leading the effort to drug RNAs and harness the power of structured RNAs for various applications. Note that pharmaceutical companies (even with their great pressure for reducing drug discovery costs) were not able to effectively solve challenges 1 and 2.

RNA Discovery and Innovation: Cell and Molecular Biology

The third panel, chaired by Doug Conklin (University at Albany) with participants John Nelson (GE Global Research) and Neocles B. Leontis (NSF, Genes and Genome Clusters, BIO), discussed what we are missing with respect to RNA function and its role in the cell and how we might approach it. The premise for this discussion is that much of the history of RNA-based biology stems from its role in information transfer from DNA to protein. An enormous amount of work has gone into determining how RNA works in protein expression. Therefore, in many ways, we believe we know everything there is to know about RNA's role in translation now that the structure of the ribosome has been elucidated. Thus, the discussion was driven by the question posed to the panel, "What emerging technologies are on the horizon that may lead to a quantum advance in our understanding of RNA transport, localization, control of function and effect on cellular function in live cells and tissues?" Leontis felt that the structures responsible for protein expression are only the beginning and that the dynamics of the system are the main question stating, "We have the snapshots, and now we want the movie." He related recent changes in NSF's molecular and cellular biosciences section designed to accommodate in-depth comprehensive studies that might focus on RNA and RNA function. The familiar clusters of biomolecular dynamics, structure and function, cellular processes and genetic mechanisms remain. However, restructuring has now created a new cluster, networks and regulation, focusing on signaling and metabolic networks. It also encompasses the network theory work previously handled in the emerging frontiers advancing theory in biology program. He noted that this program seeks to fund studies focused on the integration of theory and modeling with experimental testing of the models. This program would be appropriate for proposals related to RNA theory and modeling coupled with experiments.

A major stumbling block in modern studies of RNA identified in the discussion is shared by many fields in biology today. The panel was asked to address the best way to learn about a single molecule or process now that cost-effective experimental approaches generate enormous data sets. For example, the past 15 years generated so much data that analysis has become a major challenge. In NSF proposals, a data management plan is now required such that data sets can be shared and information integrated between laboratories. Although it is generally realized that this is an important aspect of any proposal, we are still early enough in the "omics" revolution that creative approaches for disseminating and analyzing complex data sets are still being sought. Nelson related recent discussions he participated in at the National Human Genome Research Institute which views the computational analysis of enormous DNA sequencing projects as a major problem requiring an increase demand in the number of statisticians and bioinformaticians.

Do we have technologies to explore RNA molecules in-depth and on a large-scale, including the ability to monitor molecular changes such as covalent modifications in real time? An RNA 'omics' approach that could identify modifications in specific RNA molecules would be extremely useful in learning about the many small RNAs and their functions. Agris noted that there are currently 109 modifications known to exist in RNA molecules, but increases in our knowledge of these modifications has slowed since many were identified in abundant RNA species. Our lack of understanding of modifications in non-abundant RNAs represents an enormous black hole in our knowledge of RNA. Agris indicated that in one of its roles, The RNA Institute mass spectrometry facility is gearing up to carry out in-depth analyses on other RNA species. Nelson reminded the audience of recent advances in nano-sequencing technologies that may someday enable high throughput sequencing of individual nucleic acid molecules. The technology that differentiates one base from another may, in time, be tweaked to perform modified base calling. Such technologies would allow for RNAseq type experiments to be conducted on RNA molecules with embedded modified base information. The potential power of such a system is easy to see, although the panelists felt that it would probably take 5 to 15 years for this sort of approach to be useful.

Even with technologies that would reveal global changes in RNA modifications under various conditions, there was a general feeling that function may be difficult to ascertain. Breaker noted that a large number of researchers use "omics" approaches or at

least make use of these data sets. The functions of these RNAs are too diverse and difficult to access with a single method. Given the complexity of the problem, it is unlikely that shortcuts will be developed to determine system function and that more independent investigators are required to "smash through these challenges" one at a time using genetic and biochemical approaches that are tailor-made for each molecule or each molecular interaction. This is a common theme in biomedical research.

Solving these problems with a limited budget necessitates cooperation and prioritization. These remedies are as old as scientific budget shortages and have well-known limitations. Gold noted that focusing on any molecule a priori runs the risk of wasting effort if the investigator guesses incorrectly at which molecule should be prioritized. Still others felt that our current thinking may lead to the potential extinction of the independent investigator with the loss of the required insights needed to solve the problems related to single molecules. The advent of consortia that assemble large-scale data sets that are of limited use to solving questions related to function is one example. Others acknowledged that consortium science may be flawed, but that there is inherent value in the standardization of research subject and data collection that would not otherwise exist. This approach clearly yields better inter-laboratory integration. In the end, Leontis pointed out that like most problems this was a problem related to money and that communication with program officers is an excellent first step to channel funds toward rectification.

Cancer and Neurological Disease: The Future of Small RNAs as Therapeutics and Tools of Investigation

The fourth panel, chaired by Larry Gold (University of Colorado at Boulder and SomaLogic, Inc.), with participants Paul Agris (The RNA Institute), James Deshler (NSF, BIO/IOS) and Hua Shi (University at Albany-SUNY) summarized the Symposium and prospects for RNA funded research. Gold followed this by posing questions to contemplate in the near term to individual investigators and The RNA Institute as a research resource. What are the functions of nucleic acid therapeutics?

Gold voiced that we now have a measure of the abilities of nucleic acids as diagnostics in the form of microRNAs. He asked if we should "include aptamers in our future endeavors as measures of protein diagnostics" and the best way to "include informatics in our research and train graduate students in statistical analysis (of noise vs. signal) of RNA data."

Gold believes there is an authentic front edge to RNA science and its potential applications, and proffered the example of the posttranscriptional modification of RNA. Deshler offered that NSF is interested in integrated organismal systems, epigenetic and posttranscriptional controls, RNA trafficking, and posttranscriptional gene regulation that speak to adaptation. Integrated models run the gamut from chemistry to differentiation, development, nuclear export, modification, and targeting. Shi echoed these remarks by reminding everyone that it is best to think strategically than to address technical issues, and to address problems from new perspectives. Agris suggested that budget reductions require us to look at the research differently. Discussions of small focus groups composed of 4–6 individuals who would not normally collaborate could lead to new perspectives. The final word was that derived from a conversation between Larry Gold and Allan Jacobson at the symposium, "Omics [ribonomics] without biochemistry and genetics is ridiculomics."