Twenty years of *RNA*: then and now

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This issue marks the 20th anniversary of publication of *RNA* (actually one month late). To celebrate this occasion, more than 200 RNA researchers (some of whom are pictured on the cover) were invited to submit short pieces describing their personal reflections of how the field has evolved over the past 20 years as well as outline what they believe to be the major questions left to be answered. A number of respondents questioned the value of having an issue filled with redundancy; many anticipated everyone would write about RNAi, microRNAs, lncRNAs, RNAseq, riboswitches, etc. Certainly, these fields are mentioned by several people, but I am amazed at the diversity of topics discussed in depth by over 130 contributors. Indeed, a thorough reading of this issue will provide the uninitiated with a scholarly introduction to almost every corner of the ever expanding universe of RNA research.

Before providing my own perspectives, I must issue three disclaimers. First, although I attempted to be as inclusive as possible, I know that many RNA researchers were inadvertently overlooked when invitations to participate were sent out. To those scientists, I extend my deepest apology. Second, because of production constraints, neither figures nor references were permitted. For this, I apologize both to the authors of the pieces and to those whose work was not formally cited. I do note, however, that many authors indicate that they will provide completely referenced pieces upon request.

While acknowledging the spectacular advances in the multiple areas enumerated above over the past two decades, I will use some of my space to focus on what I believe are a few of the most important and challenging areas in RNA research going forward. The list is by no means complete. Finally, it was difficult to determine the order in which the pieces would appear. Because this is a Society journal I decided that pieces written by past presidents would appear first in chronological order, followed by current associate editors in alphabetical order, followed by editorial board members also in alphabetical order. Contributions from others then follow in alphabetical order.

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Article and publication date are at <http://www.rnajournal.org/cgi/doi/10.1261/rna.051284.115>. Freely available online through the RNA Open Access option.

Alternative splicing

When *RNA* the journal began, it was still interesting to document specific cases of alternative splicing. With the advent of RNAseq, it has become clear that the vast majority of RNAs are processed to yield two or more isoforms. Some alternative splicing events are clearly regulatory in that self-regulation produces an isoform that elicits nonsense mediated decay. Other examples of functionally relevant alternative splicing stand out, e.g., PTB-nPTP, Calcitonin-CGRP, membrane bound and secreted antibodies, pro-apoptotic-anti-apoptotic, Dscam, etc. However, barring some technical breakthrough, the function, if any, of the plethora of other alternative splicing events is likely to remain enigmatic. Nevertheless, it might prove interesting and informative to parse alternative splicing events into subcategories. In particular, cataloging those events that include or remove sites of post-translational modifications (e.g., phosphorylation, glycosylation, etc.) or localization signals (e.g., membrane association) might provide insight into the integration of alternative splicing programs with other cellular processes such as signaling pathways. I suspect that there exist significant intersections between alternative splicing programs and both post-translational protein modifications as well as signaling pathways.

Finally, with regard to alternative splicing, the rise of whole genome approaches such as RNAseq and HITS CLIP has led to a corresponding decline in biochemical analyses of the mechanism(s) of alternative splicing. I believe this is unfortunate because detailed mechanistic analysis has the potential to continue to reveal not only novel RNA–protein interactions but also the roles of the over 1000 uncharacterized RNA binding proteins (see below). Of course this is my bias. I for one would like to know why and how Nova has opposite effects when bound upstream or downstream of splice sites.

Long noncoding RNAs

The current feeding frenzy surrounding lncRNAs is unlikely to abate soon. Nevertheless, this field is desperate for

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mechanistic insight. It is not at all clear, at least to me, how those RNAs that are thought to act in *trans* find their genomic targets, and even less clear what they do when they do find them. The view that these RNAs represent a heretofore unrecognized and sophisticated layer of gene regulation is both appealing but also somewhat unsettling. I am a bit skeptical regarding at least some of the functions attributed to lncRNAs. Moreover the lack of evolutionary conservation of these RNAs is puzzling, to say the least. I cannot think of any other example where functionally important molecules do not contain conserved regions.

RNA modifications

This old area has re-emerged in recent years and is again a vibrant area of research. Particularly intriguing to me are modifications at internal sites in mRNAs. It has been known for some time that certain mRNAs contain m⁶A, m⁵C, and inosine. More recently the Gilbert lab has shown that mRNAs can also contain pseudouridine. m⁶A has gone from having no discernable function to too many functions. m⁵C to my knowledge has no known functions. Yi-Tao Yu, a former student of mine and a postdoc with Joan Steitz, has shown that U containing codons causes remarkable changes in how the genetic code is read. It will be of high interest to determine if pseudouridines in mRNA cause functionally relevant miscoding. While all of these modifications are intriguing, several significant questions remain to be answered: How are sites of modification selected; what is the stoichiometry of modification; and in cases where modification is clearly substoichiometric, how does it exert a biologically significant effect.

mRNP biology

Finally, and in my mind, most importantly is the elucidation of mRNP composition and dynamics. When I began graduate school, it was already known that mRNAs were packaged into mRNPs. Although some progress has been made in cataloging mRNP constituents, we still do not know the composition of any mRNP in any organism. It is known that higher eukaryotic genomes encode over 1000 RNA binding proteins; however, only a tiny fraction of these have been studied. Certain proteins, e.g., SR and hnRNP proteins, have been extensively studied, and they clearly play multiple important roles in cellular RNA metabolism, but we simply do not know what the vast majority of RNA binding proteins do. Undoubtedly, many are constituents of mRNPs. A major challenge going forward is to determine how specific mRNP proteins and constellations of proteins contribute to mRNA half-life, translatability, and subcellular localization. mRNA localization is clearly important in *Drosophila*, but has been barely touched upon in higher eukaryotic cells. I suspect that localization is going to be a major overlooked determinant in gene expression, and I really look forward to once

and for all seeing the mRNP code decrypted, assuming of course that such a code exists.

Elucidating mRNP composition and dynamics will also illuminate how specific proteins can apparently have multiple and sometimes opposite functions. One example is Hur which has been reported to enhance or repress translation of certain mRNAs. It also has been reported to do many other things. Clearly, the context and content of the RNPs which have as one component Hur must be important in determining the functional readout of Hur knockdowns.

Finally, although many (most?) mRNAs are subject to translational control, we know very little about the mechanisms by which this control is exerted. In our lab, we have observed orders of magnitude differences in protein production from mRNAs containing identical 5' UTRs and coding sequences but different 3' UTRs. We do not have a clue as to the mechanisms underlying these remarkable differences.

About the journal

When RNA was founded in 1994, the internet and electronic communications were in their infancy. We relied heavily on faxes and FedEx. The remarkable improvements in communication (I finally broke down and bought a smartphone) certainly have been welcome and have made running the journal much easier and much more efficient. Less welcome, at least to me, is the advent and overwhelming expansion of supplementary material. I firmly believe that data necessary to support the conclusions of a manuscript should be contained within the main text of the manuscript. Only large data sets, tables, movies, etc. belong in supplementary materials. I long for the days when "data not shown" was a perfectly reasonable way to describe nonessential data. Regrettably, these days "data not shown" is interpreted by many reviewers as "data not done." This attitude is incongruous to me since scientific communication is based on the implicit assumption that authors are, if nothing else, honest. Any breakdown in this trust will inevitably lead to a breakdown in the publishing system.

Having said that, another big change that occurred over the past 20 years makes me, for one, quite uncomfortable. As more and more studies have become "genome-wide" there has been an onslaught of bioinformatics. Indeed, papers now often include as data, scatter plots, box and whisker plots, heat maps, principal component analyses projections, and other graphical devices. Expansive conclusions are often drawn from these "data." I quite frankly cannot evaluate the quality of these "data" and I suspect that most biochemists and molecular biologists cannot either. Accordingly, we are forced to trust the authors' interpretations even though we largely do not know how they were derived. One could assume that there are standard statistical methods that all bioinformaticians use, but anyone who has seen two informatics experts argue at length about what tools are appropriate for a

given question knows that is not true. Should statistical analyses get a pass during review? I don't think so. However, at present I do not see how to rectify this situation. Trained bioinformaticians are in short supply and even they cannot be expected to go back to raw data sets to validate conclusions. I view the evaluation of statistical treatment of large data sets as a major long term problem in biomedical publishing; also see Tom Cech's comments in this issue.

In the early seventies I drove used cars around the country for wholesale car dealers in the Bronx and supplemented that meager income by gambling on board games and cards. When it dawned on me that this was not a viable long term career, I went to graduate school. There, the very first things I learned were that in molecular biology or biochemistry, anything less than tenfold was not an effect and that if you need statistics to prove a point, you did not have a point to make. Given that background, I cringe when I see bar graphs where differences of 20% or even less are presented as significant, at least when bars are topped by one or two asterisks.

To conclude my screed, I will just mention a few other pet peeves. First, it drives me crazy when crosslinking data, be it CLIP, CLASH, CHIP, or any other approach, is interpreted quantitatively. Second, I am frustrated by rampant overinterpretation of RNAi experiments. Just because one can rescue a phenotype with an RNAi immune construct, does not mean observed effects are direct. Typical RNAi knockdowns are carried out for days and the cell has ample time to attempt

to compensate for loss of a specific protein by doing any number of things. Finally, on a nonscientific note, I find it incredibly annoying that a tenfold effect must, at referees' insistence, be documented with error bars.

Lighter things

The past 20 years have passed incredibly fast. When the journal was born, I was a nervous wreck hoping against hope that it would survive. Now I am a nervous wreck knowing that I will have to pass it on to someone else at a future date. It would be nice if interested individuals would make themselves known now and that they could assure me that they will love the journal as much as I do.

The journal has been good to me in many ways, not least of which is the fact that it keeps me at least somewhat current on most areas of RNA biology. I am grateful to all Associate Editors past and present for their help and friendship. I am also grateful for the authors who submit and the referees who evaluate and make in most cases helpful suggestions. Without this community the journal would not exist. I thank Cold Spring Harbor Press, especially John Inglis, Linda Sussman, Denise Weiss, and Michael Henigman for making our job much easier. Finally, I am most grateful to Ann Marie Micenmacher who has been an indispensable asset to the journal since the very beginning. I greatly value her dedication and especially her patience in dealing with me.

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A PUBLICATION OF THE RNA SOCIETY

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RNA 2015 21: 471-473

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