Twenty years of RNA: the discovery of microRNAs

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Almost exactly 20 years ago, a short commentary in Nature by Wickens highlighted the discovery a few weeks earlier by the Ambros and Ruvkun laboratories of what came to be known as the first microRNAs. In the course of investigating early development in *C. elegans*, genetic screens led to the very surprising observation that expression of a developmentally regulated gene, lin-14, was regulated post-transcriptionally not by a conventional protein-coding gene, but through short, 20–25 nucleotides long RNA species which appeared to base pair imperfectly with the untranslated region of the lin-14 mRNA. The title of the commentary, as tantalizing as it was insightful, was: "Deviants or emissaries."

It was by then widely accepted that non-coding regions of messenger RNAs, especially the 3′-untranslated regions (3′ UTRs), would constitute not just passive genomic left over, but hubs of regulation of RNA processing, stability, translation. Regulatory elements within 3′ UTRs were being discovered every week as were proteins that bound to them and regulated mRNA biogenesis. It had become clear that often-conserved sequences, for example AU-rich elements, provided landing pad for an ever growing collection of RNA-binding proteins that regulated the fate of an mRNA. However, what was reported by Ruvkun and Ambros was qualitatively different: the newly discovered very short RNA species were usurping the role of a *trans*-acting protein in regulating, as we now understand, mRNA stability and translation.

It is one thing to recognize that noncoding regions of mRNAs provide signals for targeting by the sequence-specific RNA-binding domains of regulatory proteins, quite another to discover that it is RNA itself that performs that function. RNA was after all supposed to be only a passive player in the transfer of genetic information from DNA to proteins. Of course ribozymes had been discovered about 10 years earlier and there was by then growing acceptance that the ribosomal RNA, and perhaps even the small nuclear RNAs, were not just scaffolds to position proteins for catalysis of protein synthesis or mRNA splicing, but might be the active center of the ribosome and splicesosome. Perhaps then, given the precedent of ribozymes, the discovery of microRNAs should have led us to ask why can RNA not act as a *trans*-acting factor to recognize regulatory sequences and assemble protein complexes that regulate mRNA biogenesis? After all, the rules of Watson–Crick base pairing ensure exquisite specificity which is difficult for proteins to match. The follow up was then inevitable: are these small RNAs the first members of a wide new class of regulatory molecules?

We now know that these molecules were even more wide spread than the most open minded RNA biologist would have anticipated, and that the discovery of the Lin-14 regulators had probably wider implications for biology than even the discovery of ribozymes. The enzymatic activity of ribozymes is relatively rare, perhaps the remnant of an ancient RNA world: most catalysts in contemporary biology are proteins. The small regulatory RNAs discovered by Ambros and Ruvkun did not turn out to be deviants, unique or rare examples of a world where post-transcriptional regulation of gene expression is performed by proteins and only occasionally by RNA. To the contrary, they would come to be known to play widespread, pervasive and important regulatory roles. MicroRNAs number in the thousands in vertebrate species, regulating essentially the entire proteome.

The discovery of microRNAs represented another landmark for RNA. Ever resistant to pigeonholing, RNA had come to invade yet another domain of biology previously believed to be the exclusive kingdom of proteins. It happened before with ribozymes and later on, with riboswitches, that bypass the need for small metabolite to bind intermediate proteins to regulate protein productions; and even more dramatically with long noncoding RNAs and various other noncoding transcripts that reach even further back in the genetic information flow to regulate transcription, the first examples of which were referred to in the same commentary by Wickens as "odd and enigmatic RNA species."

In the 20 years that followed, the scope and importance of RNA-based regulation of gene expression has grown to an extent that even the acceptance that the first microRNAs were not deviants but emissaries of a much larger regulatory world could not have anticipated. What still remain supremely interesting about microRNA is the sophistication, robustness and finesse of the regulatory logic. When up- or down-

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regulated, microRNAs tend to be elevated or repressed only a few fold, and in turn often repress target genes only a few fold as well. Yet the physiological effects of up or down regulation of microRNA expression can be very stunning, through the logic of combinatorial control. Might it be that the association of these emissaries with cancer, inflammation, and any other disease one can think of, will lead also to new therapeutic options to treat these diseases?