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Part A—MicroRNAs: Discovery, analyses, targets

Gary Brewer

Guest Editor, Department of Molecular Genetics, Microbiology & Immunology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA, Fax: 732-235-5223, E-mail address: brewerga@umdnj.edu

Discoveries of small, non-coding RNAs that control gene expression have revolutionized molecular biology. The search term ‘microRNA’ yielded 575 hits in PubMed for the past year alone. And microRNAs are but one class of non-coding RNAs, which also include small interfering (si)RNAs and Piwi-interacting (pi)RNAs, among others. Not surprisingly, with the explosion of small-RNA research has come an explosion of commercially available reagents, kits, protocols, and services for small-RNA discovery, function, and expression analyses. For example, there are a number of commercially available systems for small-RNA quantitation using real-time PCR-based methods. As well, reagents are available for overexpression or inactivation of many small RNAs to permit dissection of their functions. Undoubtedly, excellent commercial resources for small-RNA research will continue to arrive on the scene. There is still, however, much room for the do-it-yourself enthusiast. We thus present the first (Part A) of two issues devoted to microRNA research, with Part B, guest edited by Thomas Schmittgen, to follow in the coming months. In both issues we have strived to include articles describing methods and tools that, not always but for the most part, are unique and not necessarily available commercially, just yet. The focal point of the articles in both issues is microRNAs; however, they should be readily applicable to most other small-RNA classes as well. Finally, the articles in both issues encompass four areas: microRNA function and biogenesis; discovery; expression profiling; and target identification/validation.

MicroRNAs regulate and are regulated. They suppress translation of their target mRNAs and/or promote mRNA degradation to posttranscriptionally silence gene expression. Little is known about the mechanistic details of the processes involved. However, B. Wang et al. have developed a cell-free system that recapitulates microRNA-directed translational suppression. As there is some controversy over whether suppression occurs at translation initiation or elongation (or protein degradation for that matter), their biochemical system should prove invaluable for elucidating the suppression mechanism(s). The targets affected in a given cell of course depend upon the repertoire of microRNAs expressed in that cell. P. Leuschner and J. Martinez earlier observed specific microRNA precursors that did not yield mature microRNAs. Their conclusion: precursor processing may posttranscriptionally regulate mature microRNA biosynthesis. In their article they report a cell-free system that faithfully reproduces cell-type-specific precursor processing.

As of this writing, there are ~500 known microRNAs for humans; estimates are that more await discovery in most organisms. To address these needs, we have selected for Part A three articles, by C. Lu et al., H. Mano and S. Takada, and J. Yin and R. Zhao, utilizing cDNA library construction and novel microarray-based approaches for small-RNA discovery. R. Kulkarni

and P. Kulkarni present a computational approach for discovery of bacterial small RNAs. As well, articles by T. Tuschl and M. Zavolon in Part B will provide additional approaches for microRNA cloning and sequence analyses.

An essential component in the toolkit for the molecular biology of gene expression is the ability to measure levels of gene products. Part A includes three articles that describe complementary approaches for expression analyses. É. Várallyay et al. illustrate use of locked nucleic acid (LNA)-based probes for northern analyses of microRNA expression, while M. Castoldi et al. utilize LNA probes for microarray-based analyses. LNA probes increase the sensitivity and specificity of nucleic acid hybridization. To complete the Part A troika, R. Thompson et al. utilize RNA oligonucleotide probes for *in situ* analyses of microRNA expression. Of course, the advantage of *in situ* analysis is that it provides spatial information within a cell or tissue. Part B will contain additional articles on microRNA expression profiling by cDNA microarrays (C. Croce et al.), real-time PCR quantification (T. Schmittgen and C. Chen), and *in situ* hybridization (G. Nuovo).

The last article in the Part A series addresses an age-old problem in small-RNA research: identification of specific posttranscriptional gene targets. This is a problem due to partial complementarity of many small RNAs with their mRNA targets. Computational approaches developed by a number of laboratories have proven invaluable. However, U. Ørom and A. Lund utilize transfection of biotinylated microRNA into cells and its subsequent purification under native conditions to identify associated mRNA(s). The success of their proof-of-principle experiment indicates this strategy could revolutionize target identification. Two articles in Part B will provide the requisite *in vivo*, follow-up approaches: experimental validation of a microRNA target(s) (T. Elton) and genetic analysis of microRNA function using modified antisense oligonucleotides to inhibit activity of a specific microRNA (C. Esau).

In this age of “-omics” and “-omes,” it is important to remember that microRNAs, just like transcription factors and RNA-binding proteins, serve as the tie that binds expression of genes into transcriptome subsets that constitute biochemical pathways. As such, an article by Y. Gusev in Part B describes computational analyses of biological functions and pathways united by microRNAs in cancer.

Finally, I thank each of the authors for their excellent contributions. It is now up to the rest of us to utilize, build upon, and, if necessary, improve upon what the authors have begun.