MicroRNA (miRNA)

Regulation of transcription and translation in eukaryotes is complex. New layers of complexity are steadily found, such as a function for a part of the ‘junk’ DNA that is transcribed. MicroRNAs (miRNAs) were first discovered in 1993, when the miRNA lin-4 was determined to downregulate expression of the gene lin-14 in Caenorhabditis elegans [1][2]. However, since there is no homolog to lin-4 in other species, this discovery was considered to be unique. Specific and potent silencing of genes by double stranded RNA (RNAi) was discovered in 1998 [3], and the discovery of the miRNA let-7 in 2000 [4][5], with homologs in other species including humans, showed that miRNAs are quite common in eukaryotes. There are now known to be multiple types of small noncoding RNA (for review see [6]), with miRNAs being the largest family of noncoding RNAs involved in gene silencing.

What is miRNA?

MicroRNA (miRNA), 19-25 nucleotides in length, are typically encoded within introns, and have been discovered in metazoans, plants and viruses, as well as a few in protists and slime mold, with more being confirmed every day. In mammals, miRNAs are first transcribed as a long RNA transcript (between hundreds of nucleotides and tens of kilobases) [7], called primary miRNA (pri-miRNA), which contains imperfectly base-paired hairpin structures. These pri-miRNA, which may contain sequences encoding multiple miRNAs, are cleaved in the nucleus into shorter precursor miRNA (pre-miRNA). This reaction is performed by a protein complex called Microprocessor, (Figure 1, page 6), which involves Drosha, the RNase III enzyme, and DiGeorge Syndrome Critical Region 8 Protein (DGCR8), a double-stranded RNA-binding domain protein. Pre-miRNA is a short stem loop ~70 nucleotides in length with a 2-nucleotide 3’-overhang. This pre-miRNA is exported from the nucleus by Exportin-5, and cleaved in the cytoplasm by Dicer, another RNase III enzyme, into the mature 19-25 nucleotide miRNA:miRNA* duplex. The miRNA strand with lower base pairing stability (the guide strand) is loaded onto the RNA-induced silencing complex (RISC), composed of Dicer, TAR RNA binding protein (TRBP) and the Argonaute protein Ago2. The passenger guide strand, usually miRNA*, is sometimes functional, but is usually degraded.

In vertebrates the RISC complex is guided to its mRNA target by the miRNA strand, which typically base pairs imperfectly to its target in the 3' untranslated region, signaling the target for translational repression through unknown mechanisms. More than 500 miRNAs have been identified in humans [8][9], and each miRNA is proposed to have hundreds of mRNA targets due to the imperfect base pairing [10]. Therefore, the bioinformatic prediction that 30% of human genes are regulated by miRNA can be seen as a reasonable assumption [11]. Visit the following web address for a repository of all confirmed miRNA sequences:

http://www.SABiosciences.com/miRNAsearch.php

Small inhibitory RNA (siRNA) was discovered as a reagent that can be transfected into cells to transiently knockdown a specific protein. Many researchers are using this powerful tool to enhance their study of a gene of interest. Processing of siRNA is similar to miRNA, but varies from miRNA by its method of gene silencing; only 19-21 nucleotides in length, inhibition by siRNA requires an exact match to its single target miRNA, which differs from miRNA’s imperfect basepairing; in addition, siRNA inhibits this target by triggering miRNA degradation, whereas miRNA triggers translation inhibition (Table 1).

Relevance of miRNA to Human Biology

Before the discovery of miRNA, it had been known that a large part of the genome is not translated into proteins. This so called “junk” DNA was thought to be evolution’s debris with no function. We now realize that a portion of this coding DNA is highly relevant in the regulation of gene expression.

The importance of the miRNA regulatory pathways is underscored by the impressive list of diseases which have recently been found to be associated with abnormal miRNA expression (Table 2).

Cancer

miRNAs have been found to be downregulated in a number of tumors [10,25], and in some cases the reintroduction of these miRNAs has been shown to impair the viability of cancer cells. The value of miRNA profiles in tumor diagnostics is well established. For instance, strong up and down regulations of 16 miRNAs have been shown in primary breast tumors, and these markers may aid in the development of drug-resistance and treatment-selection tests [25]. Underlining the important role miRNA plays in oncology is the formation of several new companies which seek to expand development of miRNA-based therapeutics [25].
Age-Related Diseases

Evidence is accumulating that many age-related diseases are associated with a decreased control of cell signaling that occurs in mid-life [25]. The miRNA control of such systems as the cell cycle, DNA repair, oxidative stress responses and apoptosis, has been shown to become abnormally expressed in mid-life. It is highly likely that continued research will reveal important associations with the aging process, and may lead to therapeutics that can improve the quality of life.

Heart Disease

Two heart-specific miRNAs were deleted in mouse models resulting in abnormal heart development in a large proportion of the offspring [25]. While these lethal effects were expected, other studies show a more subtle role for miRNA in the heart. When miR-208 was eliminated, the mice appeared normal. Defects were revealed only when their hearts were stressed. These results show that comprehensive miRNA studies may be valuable in the diagnosis of heart disease.

Neurological Diseases

Numerous reports have demonstrated the role of miRNAs in neural development. Evidence for a role in Parkinsons disease comes from animal model studies published last year, showing that loss of miRNAs may be involved in the development and progression of the disease. In cell culture experiments, transfer of small RNA fragments partially preserved miRNA deficient nerve cells [25]. While these results and others point to an important role for miRNA in neurodegenerative disorders, much more work is needed to delineate the exact role of miRNAs in this important area.

Immune Function Disorders

Recent miRNA deletion studies have revealed a central role in the regulation of the immune response. The deletion of miRNA-155 impaired T and B cell differentiation in germinal centers, and greatly decreased antibody and cytokine production [24]. Two additional studies deleting miRNA-181 and 223 were found to control T cell response and granulocyte production, respectively [25]. As more roles for miRNAs in the immune response are found, the list of immune function disorders with a miRNA component is certain to expand also.

Future Directions for miRNA

miRNA may also be involved in other processes besides translational gene silencing. Currently there are hints of this, because mature mammalian miRNAs can be imported into the nucleus [17] and secreted from the cell [18]. These results suggest that miRNA may regulate transcription or paracrine signaling. Unlike siRNA, miRNA is endogenous, and therefore has the potential to enhance the understanding of the regulation of particular genes. In addition, miRNA is now touted as an additional layer of gene regulation, which can be dysregulated in diseases. Currently the study of miRNAs requires large scale arrays, since few miRNA targets are experimentally confirmed and individual miRNAs may have overlapping functions. The relative lack of attention devoted to miRNA will change in the future, as scientists realize that their favorite gene may have an additional layer of regulation never touched upon. While siRNA is merely an important tool, miRNA is evolving into a whole new field of research.

Table 1: Comparison of miRNA and siRNA.

<table>
<thead>
<tr>
<th></th>
<th>Length</th>
<th>Where Found?</th>
<th>Target Recog</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA</td>
<td>19-25 nt</td>
<td>Endogenous</td>
<td>Imperfect Match</td>
<td>Translational Repression</td>
</tr>
<tr>
<td>siRNA</td>
<td>19-21 nt</td>
<td>Exogenous</td>
<td>Exact Match</td>
<td>miRNA Cleavage</td>
</tr>
</tbody>
</table>

Table 2: Examples of miRNA Functions & Relevance of miRNA to Human Biology.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15/miR-16</td>
<td>Bcl2</td>
<td>Apoptosis</td>
<td>[19]</td>
</tr>
<tr>
<td>miR-1</td>
<td>GJA1/KCNJ2</td>
<td>Cardiac Arrhythmia</td>
<td>[20]</td>
</tr>
<tr>
<td>miR-146</td>
<td>IRAK1/TRAF6</td>
<td>Bacterial Infectious Response; TLR-NFκB</td>
<td>[21]</td>
</tr>
<tr>
<td>miR-520h</td>
<td>ABCG2</td>
<td>Stem Cell Differentiation</td>
<td>[22]</td>
</tr>
<tr>
<td>miR-106a</td>
<td>Rab1</td>
<td>Cancer Pathogenesis</td>
<td>[23]</td>
</tr>
<tr>
<td>miR-let7</td>
<td>Multiple</td>
<td>Cell Cycle Regulation</td>
<td>[25]</td>
</tr>
<tr>
<td>miR-155</td>
<td>-</td>
<td>Adaptive Immunity</td>
<td>[26]</td>
</tr>
<tr>
<td>miR-223</td>
<td>-</td>
<td>Granulocyte Regulation</td>
<td>[27]</td>
</tr>
<tr>
<td>miR-208</td>
<td>-</td>
<td>Stress Response (Heart)</td>
<td>[24]</td>
</tr>
</tbody>
</table>

Complete System for miRNA Research from SABiosciences

SA Biosciences’ RT2 miRNA PCR Arrays & qPCR Assays generate high-quality and genome-wide miRNA expression data with nothing more than a simple RT-PCR protocol. Our patented miRNA technology ingeniously integrates a universal tailing & reverse transcription reaction specific for miRNA with the accurate expression level measurement of distinct miRNA sequences that may only differ by a single nucleotide base. With this technology, you can easily get a comprehensive survey of miRNA expression in your cell line or tissue of interest.

SA Biosciences’ complete miRNA PCR System includes:

- RT2 miRNA Arrays and Assays
- RT2 miRNA First Strand Kit
- RT2 SYBR Green PCR Master Mix

What the System Offers:

Detecting every miRNA across the entire genome in a specific and sensitive way is a very challenging technology task. Many miRNA family members and otherwise distinct miRNA species have very similar sequences. Moreover, other RNA species such as snRNA, tRNA, mRNA, and rRNA can cause non-specific amplification, making the specific analysis of mature miRNA even more problematic. With SA Biosciences’ complete miRNA PCR System & expression analysis system, these problems are solved.

RT2 miRNA PCR Arrays and Assays dramatically improve the specificity through patent pending primer design & proprietary reverse transcription chemistry. Our miRNA PCR Arrays include built-in control elements to insure the quality of your experimental data. The free data analysis software takes your raw threshold cycle data and automatically generates figures and tables ready for publication. With the RT2 miRNA PCR Assay and Arrays, you can expect:

- Sensitivity: As little as 0.5 µg total RNA needed
- Multi-Sequence Flexibility: Analyze one to 376 sequences simultaneously
- Simplicity: As easy as a real-time PCR experiment
References