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The development of electrochemical assays for microRNAs

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ABSTRACT

Twenty years has passed since the first discovery of microRNA (miRNA) *lin-4* in *Caenorhabditis elegans*. Over the last decade, the study of miRNA biology has attracted tremendous attention. Increasing evidence has demonstrated that miRNA dysregulation could lead to a large number of genetic diseases and miRNA expressions are closely associated with the pathogenesis of most human malignancies. Recent studies have further suggested that miRNA expression profiles may serve as reliable biomarkers for cancer diagnosis, prognosis, and therapy. This provides powerful impetus and a growing demand for researchers to develop a simple analytical methodology which will allow an accurate, sensitive, selective, and cost effective miRNA analysis at point-of-care. In this regard, electrochemical methods offer many attractive characteristics and could play a leading role in future miRNA detection and quantification. This review revisits the discovery of miRNA, examines the possibility of miRNA biomarkers, discusses the conventional methods and emerging techniques for miRNA detection and quantification, and postulates future prospects in connection to electrochemical detection and quantification of miRNAs. It is hoped that this review will open new perspectives toward the development of new and novel electrochemical strategies for miRNA detection and quantification.

1. Introduction

When microRNA (miRNA) *lin-4* was first discovered in soil nematode *Caenorhabditis elegans* (*C. elegans*) by Lee and his colleagues in 1993 [1], many believed that was an anomaly and would not occur frequently in nature. It was not until 2000 (the year Reinhart et al. discovered yet another miRNA *let-7* which could regulate the transcription of gene *lin-14* in *C. elegans*) [2] before it became clear to the academics that a new door to research has opened. Just two years later, Calin and his colleagues reported that two miRNAs, miR-15a and miR-16-1, were found in the mutated region of chromosome 13, and the loss of this chromosomal region was consistently associated with chronic lymphocytic leukemia (CLL; the most prevalent human leukemia) [3]. After these seminal findings which sparked the recent boom and interest in miRNA research, it became evident that these small (~22 nucleotides (nt)) endogenous noncoding RNAs [4] regulate gene expression via one of the four mechanisms (messenger RNA (mRNA) cleavage, translational repression, translational enhancement, or deadenylation) [5,6]. While the complete functional role of miRNAs remains an area of intensive research, miRNAs are believed to be involved in several biological processes such as development, differentiation, cell proliferation, cell metabolism, and apoptosis in many life forms, including *C. elegans*, plants, *Drosophila melanogaster* and even humans [6,7].

As of today, 1600 pre-miRNAs and over 2000 mature miRNAs in the *Homo sapiens* genome have been registered in the miRBase (http://microrna.sanger.ac.uk), a collective registry of currently known miRNA sequences and targets hosted by the Sanger Institute [8]. Accumulated studies have indicated that miRNAs target and regulate more than 60% of all protein-encoding genes in humans [9]. In addition, the aberrant expression of miRNAs has been demonstrated to cause several human malignancies and disorders, including cancer [10], heart diseases [11], neurological disorders [12], and diabetes [13]. For example, the global down-regulation of miRNA expression is a unique signature in cancer since specific dysregulations of certain miRNAs are consistently found in specific cancer types [10,14]. It is, therefore, possible to use miRNA expression profiles as biomarkers in cancer diagnosis, prognosis, and therapy. This is especially true for the stable circulating miRNAs, which circulate in human body fluids such as blood, urine, and saliva, as they can be detected from easily attainable blood, urine, or saliva samples [15–18]. However, it is of paramount importance that such miRNA expression profiling methods are highly sensitive, specific, and afford the possibility of taking these technologies from the laboratory to point-of-care. The inherent miniaturization of electrochemical biosensors and their compatibility with standard microfabrication and semiconductor technologies provide attractive features such as simple, accurate, and inexpensive platforms for miRNA expression profiling. These fascinating features, coupled

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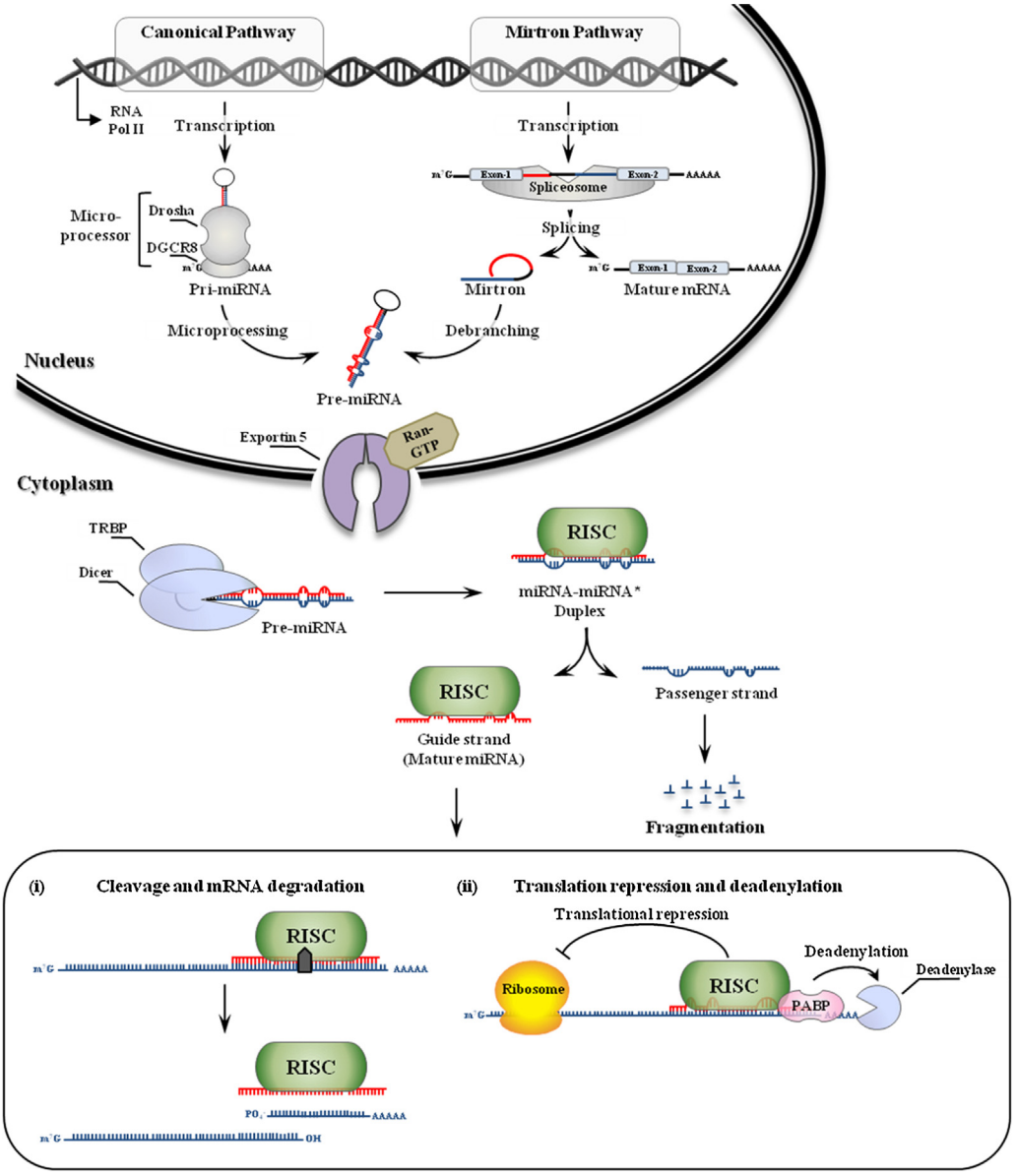

Fig. 1. Schematic illustration of miRNA biogenesis and functions.

with the ultrahigh sensitivity and selectivity assured by electrochemical measurements, provide tremendous opportunities for electrochemical detection to play a leading role in future miRNA detection and quantification. While further exploring the possibility of miRNA biomarkers, this review also aims to benchmark such emerging miRNA detection techniques with conventional methods with particular emphasis placed on the role electrochemistry can play in miRNA detection, quantification, and expression profiling.

1.1. MicroRNA biogenesis and function

Given that the coding sequences for miRNAs are located in the non-protein coding (intergenic) genes or the intronic regions of the annotated protein-coding genes, maturation of miRNAs could, therefore, follow either of the two pathways: canonical or mirtron (Fig. 1). In the canonical pathway, RNA polymerase II (RNA Pol II), or in some rare cases polymerase III, directs transcription in the non-protein coding regions to produce the primary miRNAs (pri-miRNAs). These pri-miRNAs possess stem-loop structures and measure up to 1000 nt in length. Microprocessing begins when the double-stranded RNA binding protein, DiGeorge Syndrome Critical Region 8 (DGCR8), recognizes and binds to the terminal loop structure of pri-miRNA. Consequently, Ribonuclease (RNase) III Drosha, which is associated with DGCR8, will excise the proximal and distal of the stem of pri-miRNA to generate an ~70 nt precursor miRNA (pre-miRNA) with 2 nt overhang at the 3′ hydroxyl. On the other hand, the bypassing of the microprocessor to produce pre-miRNAs directly as a result of splicing and debranching of the very short introns (mirtrons) from protein-coding genes is the key characteristic of the mirtron pathway. For subsequent enzymatic processing, pre-miRNAs are exported out of the nucleus by the nucleocytoplasmic shutter Exportin-5 with GTP-bound Ran protein being responsible for driving this process. Once in the cytoplasm, the RNase III enzyme, Dicer, along with its cofactor transactivating response RNA-binding protein (TRBP), cleaves off the terminal loop of pre-miRNAs to yield an ~20 bp miRNA–miRNA* duplex. Following the Dicer cleavage, the mature miRNA (guide strand of the duplex) is identified on the basis of its weaker base-pairing and thermodynamic instability relative to the other (passenger strand) and integrates with the miRNA-induced silencing complex