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摘要:The recently discovered microRNAs (miRNAs) are a large family of small regulatory RNAs that have been implicated in controlling diverse pathways in a variety of organisms (1, 2). For posttranscriptional gene silencing, one strand of the miRNA is used to guide components of the RNA interference machinery, including Argonaute 2, to messenger RNAs (mRNAs) with complementary sequences (3, 4). Thus, targeted mRNAs are either cleaved by the endonuclease Argonaute 2 (5, 6), or protein synthesis is blocked by an as yet uncharacterized mechanism (7, 8). Genes encoding miRNAs are transcribed as long primary miRNAs (pri-miRNAs) that are sequentially processed by components of the nucleus and cytoplasm to yield a mature, approx 22-nucleotide (nt)-long miRNA (9). Two members of the ribonuclease (RNase) III endonuclease protein family, Drosha and Dicer, have been implicated in this two-step processing (10-13). To further our understanding of miRNA biogenesis and function it will be essential to identify the protein complexes involved. We were interested in defining the proteins required for the initial nuclear processing of pri-miRNAs to the approx 60- to 70-nt stem-loop intermediates known as precursor miRNAs (pre-miRNAs) (9, 10). This led to our identification of a protein complex we termed Microprocessor, which is necessary and sufficient for processing pri-miRNA to premiRNAs (14). The Microprocessor complex comprises Drosha and the double-stranded RNAbinding protein DiGeorge syndrome critical region 8 gene (DGCR8), which is deleted in DiGeorge syndrome (15, 16). In this chapter, we detail the methods used for the biochemical

isolation and identification of the Microprocessor complex from human cells. We include a protocol for the in vitro analysis of pri-miRNA processing activity of the purified Microprocessor complex.