silencing of gene expression by miRNA is a fundamental and well-conserved mechanism of gene regulation present in all eukaryotes (plants and animals). Even microorganisms have a more primitive version of the same general machinery that they can use to protect themselves against foreign DNA (e.g., from phages and viruses). Because of the profound influence of miRNAs on gene regulation, these relatively short RNAs (22 nucleotides on average) have assumed central importance in the illumination of both normal developmental pathways, as well as pathologic conditions like cancer. Indeed, the Nobel Prize in Physiology or Medicine in 2006 was awarded for the discovery of miRNAs.

By current estimates, the human genome encodes approximately 1000 miRNA genes, some 20-fold less than the number of protein-coding genes. However, individual miRNAs appear to regulate multiple protein-coding genes, allowing each miRNA to co-regulate entire programs of gene expression. Transcription of miRNA genes produces a primary miRNA, which is progressively processed through various steps including trimming by the enzyme *DICER*. This generates mature single-stranded miRNAs of 21 to 30 nucleotides that are associated with a multiprotein aggregate called RNA-induced silencing complex (RISC; Fig. 1-3). Subsequent base pairing between the miRNA strand and its target mRNA directs the RISC to either induce mRNA cleavage or repress its translation. All mRNAs contain a so-called *seed sequence* in their 3′ untranslated region (UTR) that determines the specificity of miRNA binding and gene silencing. In this way, the target mRNA is *posttranscriptionally silenced*.

Small interfering RNAs (siRNAs) are short RNA sequences that can be introduced experimentally into cells. These serves as substrates for Dicer and interact with the RISC complex in a mannaer analogous to endogenous miRNAs. Synthetic siRNAs targeted against specific mRNA species have become useful laboratory tools to study gene function (so-called *knockdown* technology); they are also being developed as possible therapeutic agents to silence pathogenic genes, such as oncogenes involved in neoplastic transformation.

Long Noncoding RNA (lncRNA)

Recent studies have further identified an untapped universe of lncRNAs—by some calculations, the number of lncRNAs may exceed coding mRNAs by 10- to 20-fold. **lncRNAs modulate gene expression in many ways** (Fig. 1-4); for example, they can bind to regions of chromatin, restricting RNA polymerase access to coding genes within the region. The best known example of a repressive function involves XIST, which is transcribed from the X chromosome and plays an essential role in physiologic X chromosome inactivation. XIST itself escapes X inactivation, but forms a repressive "cloak" on the X chromosome from which it is transcribed, resulting in gene silencing. Conversely, it has recently been appreciated that many enhancers are sites of lncRNA synthesis, and these lncRNAs appear to often increase transcription from gene promoters through a variety of mechanisms (Fig. 1-4). Emerging studies are exploring the roles of lncRNAs in various human diseases, from atherosclerosis to cancer.

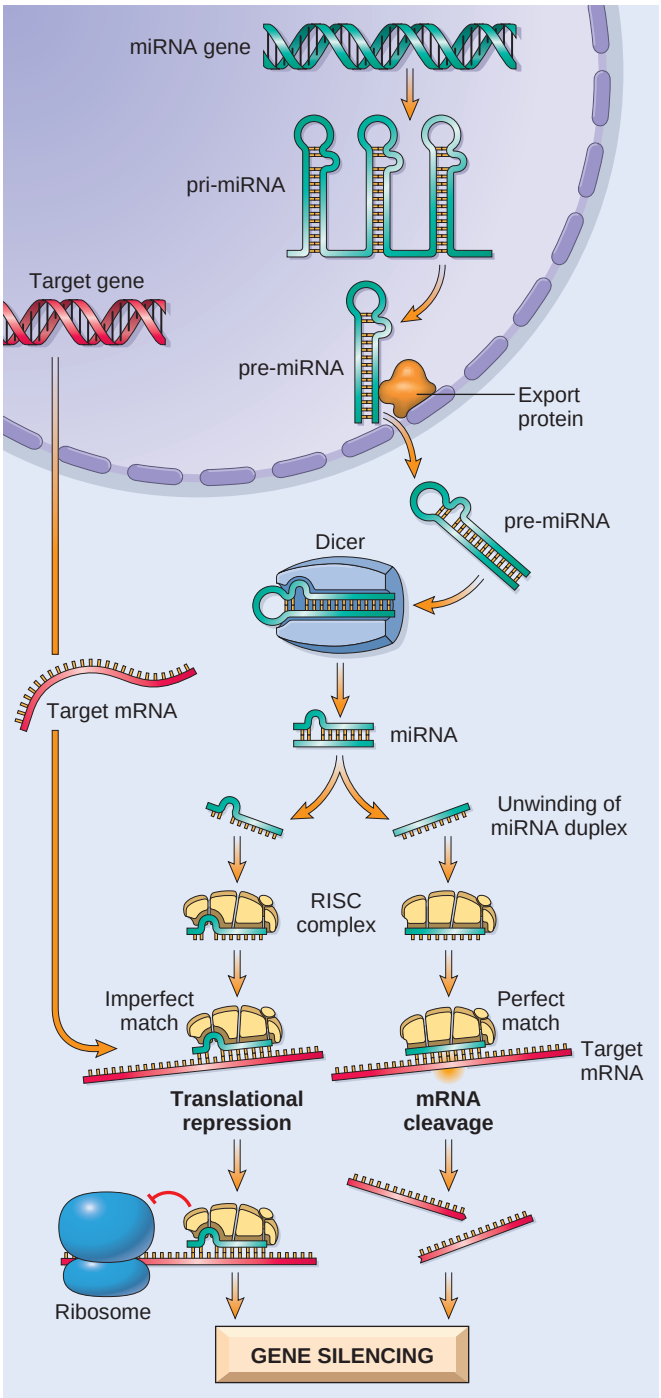

Figure 1-3 Generation of microRNAs (miRNA) and their mode of action in regulating gene function. miRNA genes are transcribed to produce a primary miRNA (*pri-miRNA*), which is processed within the nucleus to form *pre-miRNA* composed of a single RNA strand with secondary hairpin loop structures that form stretches of double-stranded RNA. After this pre-miRNA is exported out of the nucleus via specific transporter proteins, the cytoplasmic *Dicer enzyme* trims the pre-miRNA to generate mature double-stranded miRNAs of 21 to 30 nucleotides. The miRNA subsequently unwinds, and the resulting single strands are incorporated into the multiprotein *RNA-induced silencing complex (RISC)*. Base pairing between the single-stranded miRNA and its target mRNA directs RISC to either cleave the mRNA target or repress its translation. In either case, the target mRNA gene is silenced posttranscriptionally.