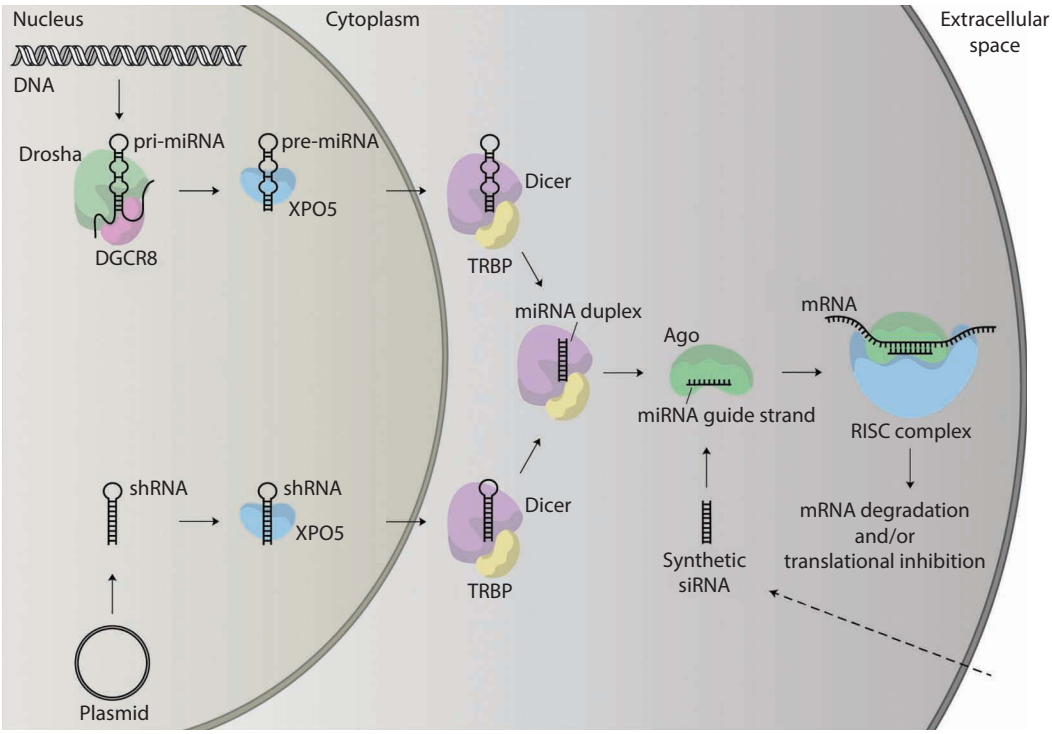

FIGURE 6.11 RNA interference (RNAi) is a cellular pathway of gene silencing. In eukaryotic cells, miRNA is generated from genome-encoded long single-stranded RNA that is folded into a dsRNA structure (pri-miRNA). The pri-miRNA is processed in the nucleus by the RNase III ribonuclease Drosha, and its cofactor DGCR8, into a shorter imperfectly base-paired hairpin structure called pre-miRNA (60–80 nucleotides). The pre-miRNA is then exported to the cytoplasm by exportin 5 (XPO5). In the cytoplasm, the pre-miRNA is further processed by Dicer, another RNase III ribonuclease, acting in association with Tar RNA-binding protein (TRBP) into the mature miRNA duplex (19–25 nucleotides). The guide strand is loaded into an Argonaute (Ago) protein that together with other proteins and co-factors form the RNA-induced silencing complex (RISC) which is guided to its mRNA target by the miRNA strand. This results in gene silencing through either translational repression or site-specific cleavage and degradation of the mRNA. Artificially designed siRNA duplexes which are used to target disease-related genes, can be delivered to the cells via small gene cassettes like plasmids that are transcribed into a short hairpin RNA (shRNA) capable of entering the RNAi pathway. Alternatively, the siRNA duplex is synthesized in vitro and delivered directly into the cytoplasm by a suitable method such as transfection, nanocarrier encapsulation, or viral gene transfer.

strategy has resulted in the development of RNAi therapeutics that has recently entered late-stage clinical trials for the treatment of transthyretin (TTR)-mediated amyloidosis, specifically TTR-mediated cardiomyopathy and TTR-mediated polyneuropathy.

Although RNAi-based therapeutics has shown great promise in recent years, a number of important challenges remain in order for widespread application of this therapeutic principle. These challenges include improvements in nuclease resistance, reduced immune activation, and development of efficient delivery methodologies.

6.5 CONCLUDING REMARKS

In this chapter, we have discussed how small molecules can be generated and can be used to probe and discover biology, both in an academic setting, but also in the initial drug design and development process. We also discussed the application of chemical biology technologies in studies of proteins