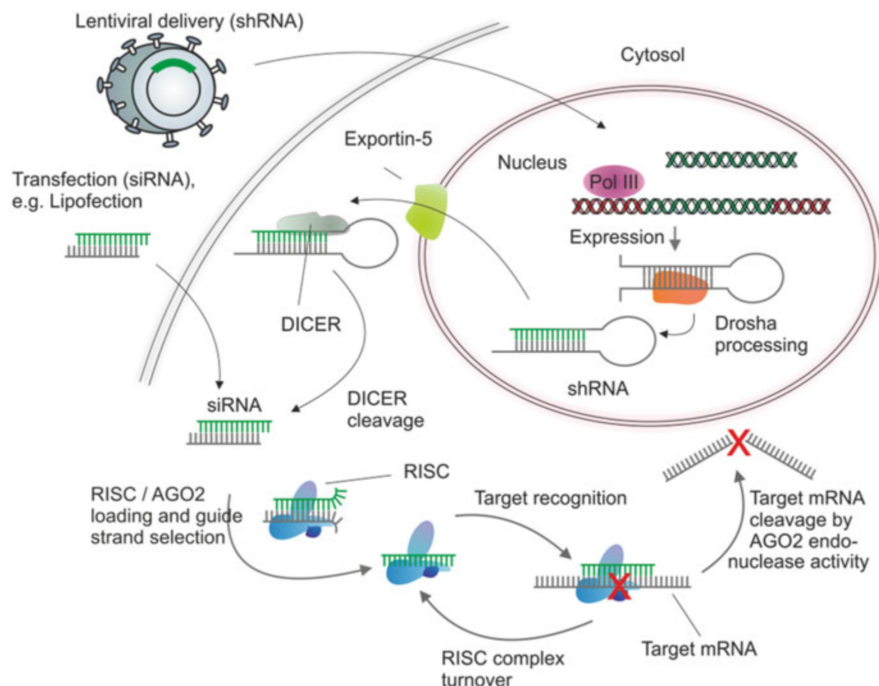

Fig. 2 The RNAi pathway offers different points of interacting with this endogenous regulatory mechanism. The two most common forms of inducing RNAi in cells are the transfection (most often by lipofection, i.e., the delivery of siRNA in lipid vesicles) of naked dsRNAs with 2-nt overhangs at the 3′ ends and the lentiviral delivery of shRNA expression plasmids. shRNAs are processed by Dicer into siRNAs. The guide RNA strand is then incorporated into the RISC (RNA-induced silencing complex), and the complementary strand is degraded. The guide strand then targets RISC to complementary mRNAs, and the Argonaute protein of RISC (Ago2 in mammals) cleaves the target mRNA, rendering it susceptible to degradation by exonucleases (Sigoillot and King 2011)

conditions have to be optimized for each cell line as they can result in toxicity due to high siRNA concentration or transfection reagent toxicity. Another disadvantage of siRNAs is that the siRNA response is limited by stability within the cell and therefore can generally only be used in short-term assays (<96 h). Despite these limitations, synthetic siRNA has been extremely useful in arrayed screening, due to its availability from commercial vendors in whole-genome libraries and its inherent safety as a nontoxic agent (Fennell et al. 2014).

The development of vector-based stem-loop shRNAs and their possible viral-mediated delivery has improved RNAi technology in many areas. shRNAs incorporate into the DNA and thereby exert long-term gene knockdown. The viral transduction allows targeting of hard-to-transfect and nondividing cells, and in vivo studies (Brummelkamp et al. 2002; McManus et al. 2002; Paddison et al. 2002). shRNAs are used in both arrayed and pooled libraries, the latter