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SnapShot: Posttranscriptional Gene Silencing

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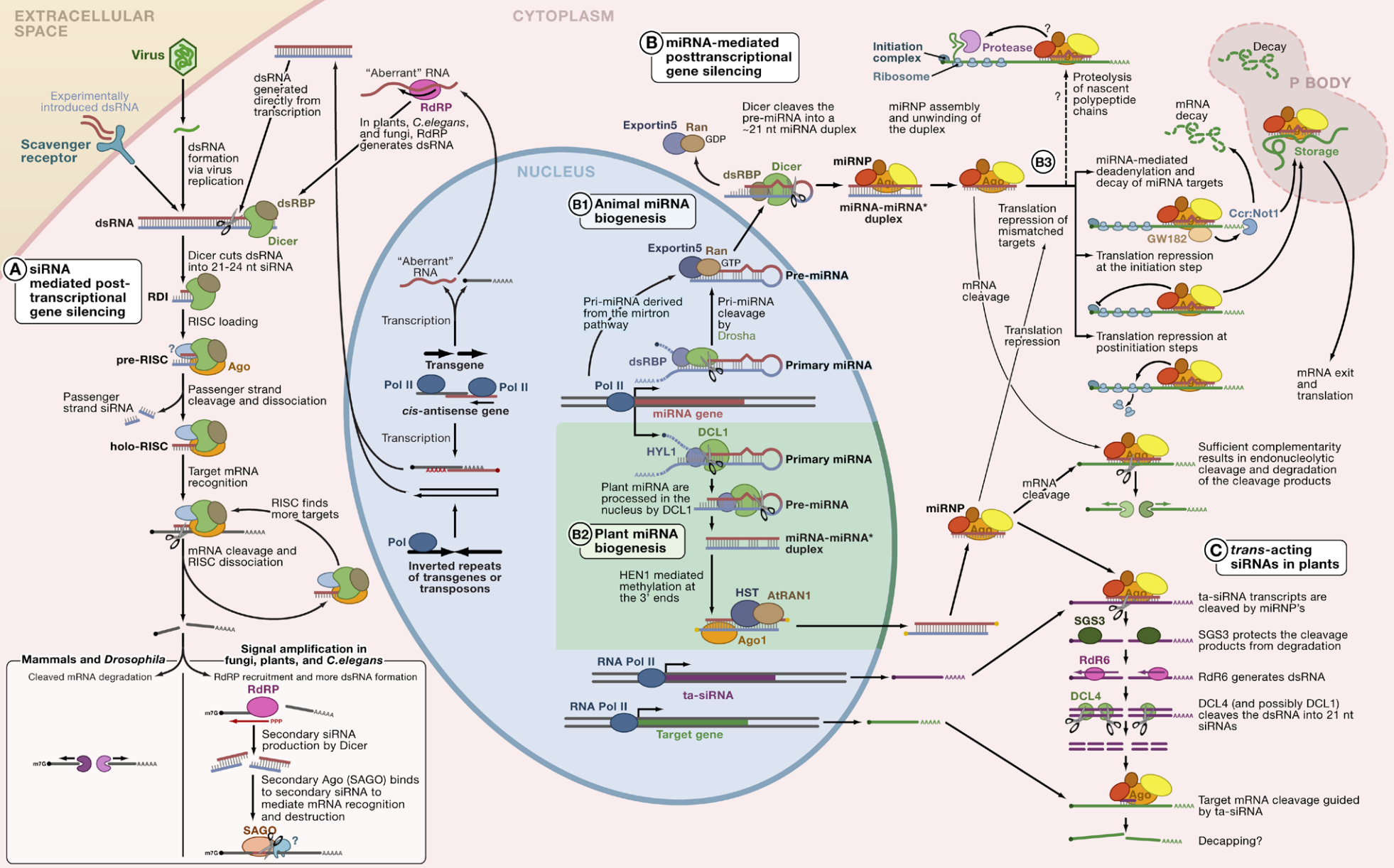
Cell



See online version for legend, abbreviations, and references.

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(A) siRNA-Mediated Posttranscriptional Gene Silencing

Double-strand (ds)RNAs that are taken up exogenously, formed during viral replication, or generated from aberrant cellular RNAs are cut into 21–23 nucleotide (nt) siRNAs by Dicer in association with dsRNA-binding (dsRBD) protein. The siRNAs are then loaded into RISC, a complex containing Argonaute (Ago) protein. After elimination of the siRNA passenger strand, RISC pairs with cognate RNA via the siRNA guide strand and cuts its target using the slicer activity of Ago. In mammals and *Drosophila* the cleaved target is then degraded. In plants, *C. elegans*, and fungi—whose genomes encode RNA-dependent RNA polymerase(s) (RdRP or RDR)—the cleaved target RNA serves as template for RdRP to generate more dsRNAs that will be diced into secondary siRNAs. These secondary siRNAs, which in *C. elegans* are recognized by SAGO (synthetic secondary-siRNA defective Ago), further downregulate target RNAs.

(B) miRNA-Mediated Posttranscriptional Gene Silencing

B1: Animal miRNA biogenesis. RNA polymerase II transcribes microRNA genes as primary miRNAs (pri-miRNAs) that fold back to form a stem-loop structure. Animal pri-miRNAs are cleaved in the nucleus by the RNase III enzyme Drosha and its dsRBD protein partner into 50–80 nt precursor miRNAs (pre-miRNAs). Pre-miRNAs are then actively exported by Exportin5 to the cytoplasm, where they undergo a second cleavage by the Dicer and its dsRBD protein partner. The resulting ~21 nt duplex, containing the mature miRNA strand and the passenger miRNA* strand, is unwound and the mature miRNA strand is incorporated into a microribonucleoprotein (miRNP) complex, containing an Ago protein. Recently, a second pathway for generating miRNAs was identified in *C. elegans* and *Drosophila*, where short introns are spliced and de-branched, yielding pre-miRNA-like hairpins termed mirtrons. Bypassing Drosha cleavage, the mirtron pathway is thought to merge with the canonical miRNA pathway during hairpin export.

B2: Plant miRNA biogenesis. Plant miRNA genes are transcribed by RNA polymerase II. In contrast to animal miRNA processing, the two cleavage steps that generate the miRNA-miRNA* duplex, occur in the nucleus by the same enzyme, DCL1, together with the dsRBD protein HYL1. Next, HEN1 methylates the 3′ ends of the duplex, and it is transported outside of the nucleus, presumably by the plant Exportin5 ortholog, Hasty. The miRNA duplex is loaded onto Ago1 protein within a miRNP complex, and after unwinding, the mature miRNA guides mRNA cleavage in most cases or represses translation in a few cases. It is still not clear whether Ago1 loading occurs only in the cytoplasm or within the nucleus as well.

B3: miRNA-mediated posttranscriptional gene regulation. A few mechanisms for miRNA posttranscriptional gene regulation have been suggested although many details are still missing. Partial base-pairing between an miRNA and the 3′ UTR of an mRNA target leads to translation repression of the mRNA at the initiation step, at a post-initiation step, or by decay of the mRNA following miRNA-mediated deadenylation. Prefect base-pairing between an miRNA and an mRNA can result in endonucleolytic cleavage and degradation of the cleavage products. Another mechanism that has been suggested, but has not been proven experimentally, is proteolysis of nascent polypeptide chains.

(C) *trans*-Acting-siRNAs in Plants

In plants, some miRNAs guide the cleavage of RNA transcripts derived from a *trans*-acting siRNA (ta-siRNA) gene. The cleavage products are protected from degradation by SGS3, and RdR6 converts either the 5′ or the 3′ fragment into dsRNA. DCL4 (and possibly DCL1) cleaves the dsRNA into 21 nt siRNAs that function as ta-siRNAs, guiding the cleavage of a target mRNA different from the transcript from which they were produced.

Abbreviations

Ago, Argonaute; DCL, Dicer like; DCR, Dicer; dsRBD, double-stranded RNA-binding domain-containing protein; HEN1, hua enhancer 1; HST, Hasty; HYL1, HYPONASTIC LEAVES 1; miRNA, microRNA; miRNP, microribonucleoprotein; Pol II, RNA polymerase II; Pol, RNA polymerase; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; RDI, R2D2-DCR2 initiator complex; RdRP/RDR, RNA-dependent RNA polymerase; RISC, RNA-induced silencing complex; SAGO, synthetic secondary-siRNA defective AGO; siRNA, small interfering RNA; ta-siRNAs, *trans*-acting siRNAs.

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