

Review Article

Molecular mechanisms of Dicer: endonuclease and enzymatic activity

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The enzyme Dicer is best known for its role as a riboendonuclease in the small RNA pathway. In this canonical role, Dicer is a critical regulator of the biogenesis of microRNA and small interfering RNA, as well as a growing number of additional small RNAs derived from various sources. Emerging evidence demonstrates that Dicer's endonuclease role extends beyond the generation of small RNAs; it is also involved in processing additional endogenous and exogenous substrates, and is becoming increasingly implicated in regulating a variety of other cellular processes, outside of its endonuclease function. This review will describe the canonical and newly identified functions of Dicer.

Introduction

The Dicer enzyme is a member of the ribonuclease (RNase) III family. It is most well known as the endonuclease that functions in the RNA interference (RNAi) pathway to cleave long double-stranded RNA (dsRNA) molecules into short dsRNA molecules, known as small RNAs, including microRNA (miRNA) and small interfering RNA (siRNA). Indeed, Dicer is considered a key factor in the biogenesis of most small regulatory RNAs, and the majority of Dicer studies have focused on this role. However, increasing evidence shows that Dicer also has functions outside of the small RNA pathway. Dicer's endonuclease function is not only involved in small RNA biogenesis, but also in the processing of other endogenous and exogenous substrates. Furthermore, its function is not limited to cleavage, but may regulate other cellular processes. This review will describe the canonical and newly identified functions of Dicer.

Dicer structure and domains

Although a full-length mammalian Dicer has not been crystallized, many studies have predicted its structure and the positions of its domains and interacting partners [1–5]. The main functional domains of Dicer are ordered from the N- to the C-terminus as follows: helicase domain included with DExD/H, TRBP-BD and HELICc, DUF283, PAZ (Piwi/Argonau/Zwille) domains, RNase IIIa and IIIb domains, and dsRNA-binding domain (RBD) (Figure 1). Cryo-electron microscopy and crystallography showed that Dicer resembles the shape of the letter L, with a head, a body, and a base [6,7]. At the head is the PAZ domain, which contains binding pockets for the 3' overhang of a dsRNA substrate. The PAZ domain of Dicer is unique in that it has an extra loop enriched in basic amino acids, which changes the electrostatic potential and molecular surface of the pocket. These differences may affect Dicer RNA binding and handing off the substrate to other protein complexes [8]. The PAZ domain also has a phosphate-binding pocket that recognizes the phosphorylated 5' end of small RNAs [3]. On the lower half of the Dicer body are the RNase IIIa and IIIb domains, which form the catalytic core of Dicer; each domain is thought to be responsible for the cleavage of one strand of the dsRNA substrate [9]. The DExD/H domain is located in the base of the L and forms a clamp near the RNase III domain active site [1,10]. A recent study showed that the DUF283 domain of Dicer is capable of binding single-stranded nucleic acid [11].

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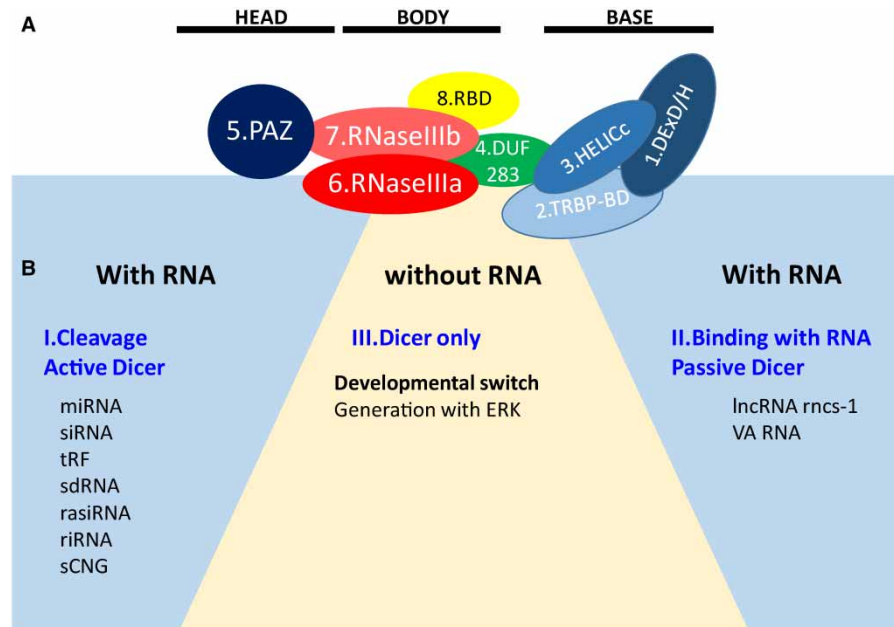


Figure 1. Dicer structure and functions.

(A) The structure of Dicer. The number one indicates the amino-terminal. The amino-terminal helicase domain forms a clamp-like structure in the base of the L shape and is thought to reorganize and wrap around dsRNA. DEXD/H, DEXD/H box helicase domain; TRBP-BD, trans-activation response RNA-binding protein-binding domain; HELICc, helicase conserved carboxy-terminal domain; DUF283, domain of unknown function; PAZ, Piwi/Argonaute/Zwille domain; two RNase III domains; RBD, dsRNA-binding domain. (B) Multifaceted Dicer function. I. Active Dicer recognizes many types of RNA and can cleave small RNAs. II. Passive Dicer can be stably bound to RNA without endonuclease activity. Dicer does not efficiently process RNA if no free ends are available [33,177] which explains the resistance of the long non-coding RNA rncs-1 against dicing [178]. III. Dicer alone can function as a binding protein. For example, Ras/Erk signaling and Dicer regulate oogenesis, and Dicer was identified as a putative ERK substrate [166].

Evolutionary relationships among Dicer homologs

Various numbers of Dicer family proteins can be found in different organisms. Dicer probably arose from an early eukaryotic origin, as it is absent from archaeobacteria, but can be found in many eukaryotic organisms, including plants, fungi, and metazoans (Figure 2A) [12,13]. The ancestral RNase III domains are found in eubacteria [14]; but these capabilities were not conserved in eukaryotic Dicer [15]. Dicer has been lost from many parasitic protozoa [16,17] as well as from fungi lacking RNAi, including the model budding yeast *Sccharomyces cerevisiae* [18]. The evolutionary phylogenetic tree of animal Dicers shows that an ancient duplication gave rise to Dicer1 and Dicer2 genes very early in metazoan evolution [19]. Both the miRNA and siRNA pathways rely on a single Dicer protein in vertebrates, and in the Nematoda phylum of invertebrates [9,20]. However, other invertebrates, including the fly *Drosophila melanogaster* and the prawn *Litopenaeus vannamei* of the Arthropoda phylum, and the fluke *Clonorchis sinensis* and the planarian *Schmidtea mediterranea* of the Platyhelminthes phylum retain both the Dicer1 and Dicer2 genes [21]. In *Drosophila*, Dicer1 is essential in the miRNA pathway, while Dicer2 facilitates the siRNA pathway [22]. Consistent with a role in immune defense, Dicer2, the siRNA-dedicated Dicer in *Drosophila*, is more closely related to the common ancestral Dicer protein than the miRNA pathway-dedicated Dicer1 [23]. This evidence suggests one common Dicer design evolved during metazoan evolution, from a universal factor for the miRNA and siRNA pathways, into a factor specifically adapted for either pathway.

Selective Dicer-mediated cleavage of various RNAs is driven by the evolutionarily conserved helicase domain (Figure 2B) [21,24,25]. Definitive functions for eukaryotic Dicer can be ascribed to different activities within the helicase domain, which is thought to facilitate movement of the protein along long dsRNA molecules [26]. This domain is analogous to the retinoic acid-inducible gene I-like family in the helicase superfamily 2 [27,28].

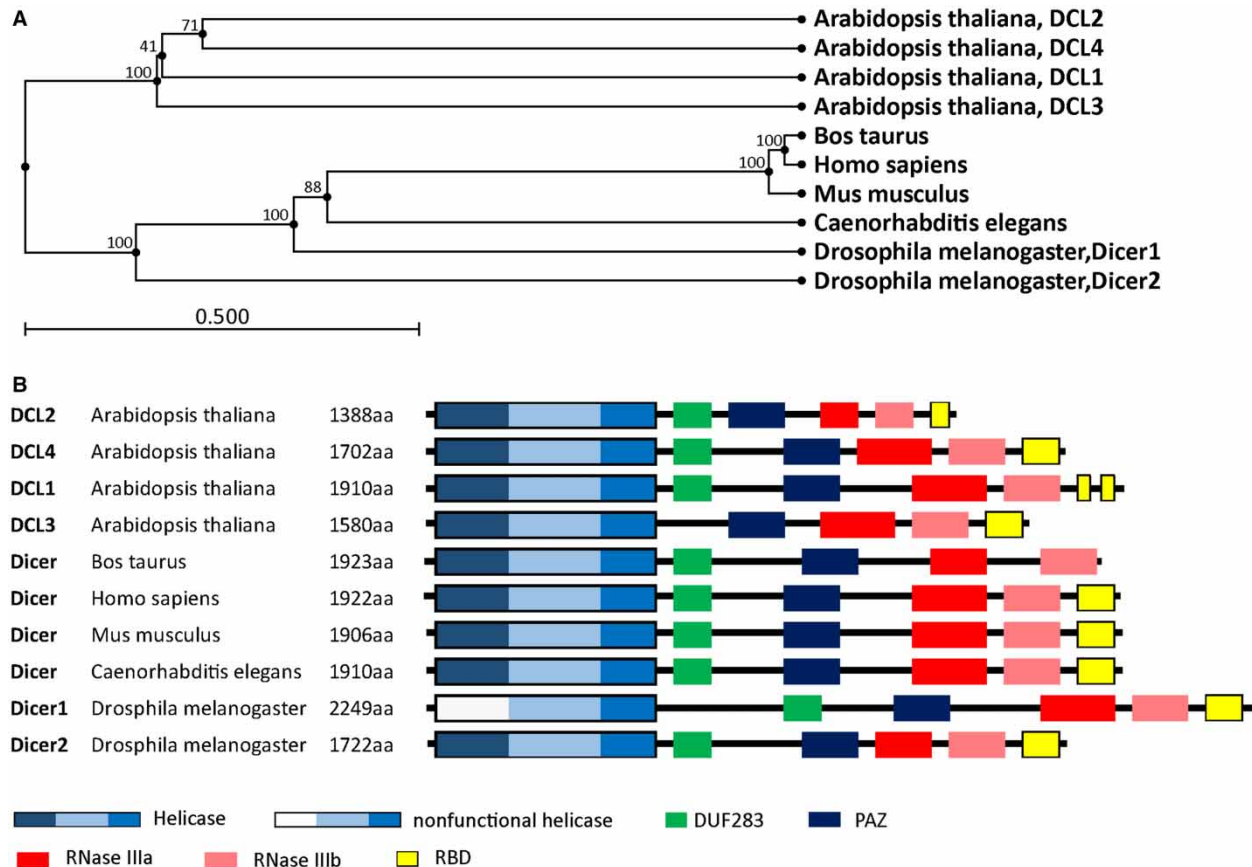


Figure 2. Phylogenetic tree of Dicer and domain analysis.

(A) Phylogenetic diversity of eukaryotic Dicer proteins. We inferred the Dicer family phylogeny using the maximum likelihood method. The number has been indicated in bootstrap value. (B) Complex Dicer protein domain composition. DCL, Dicer-like.

In *Drosophila*, the helicase domain of Dicer1, which cannot hydrolyze ATP, selectively interacts with the loops of precursor miRNAs (pre-miRNAs) and inhibits cleavage of long dsRNAs [25,29]. In contrast, the helicase domain of Dicer2 requires ATP and processes long dsRNAs to produce siRNAs. In *Caenorhabditis elegans*, Dicer also needs ATP and the helicase domain to process long dsRNAs with blunt or 5' overhanging termini; mutation of this domain results in weak production of endogenous siRNAs but maintains the ability to dice pre-miRNAs [26,30]. In *Arabidopsis thaliana*, DCL1 mainly facilitates the biogenesis of imperfect stem-loop RNAs into 21 nt miRNAs [31] and also requires ATP [32]. However, although the helicase domains of mammalian Dicer proteins are strongly conserved with *Drosophila* Dicer2 [25], human Dicer does not require ATP [33,34], and cleaves both pre-miRNAs and long dsRNAs [35,36].

Canonical endonuclease function of Dicer

In the canonical pathway, the biogenesis of most small RNA classes, including miRNAs and many siRNAs, occurs through a stepwise process in the cytoplasm. This process involves association with Dicer and specific members of the large family of Argonaute (AGO) proteins, and assembly into various effector complexes, including the RNA-induced silencing complex (RISC). First, RNAs form a pre-RISC with Dicer [37], aided by the Hsc70/Hsp90 chaperone machinery [38–40]. The RNA is then cleaved by Dicer, in concert with two different dsRNA-binding proteins, the TRBP (HIV trans-activating response RNA-binding protein) and PACT (protein activator of PKR) [41]. After the double-stranded small RNA is captured, one of the two strands is selected as the guide, or active strand, and incorporated into an AGO protein to form the RISC. The duplex RNA is unwound and AGO2 degrades the other strand, known as the passenger strand. In general, the thermodynamic asymmetry of the end of the small RNA duplex is a major factor in determining which strand will be the guide

strand; the guide strand is typically the strand with the less stable 5' end [42,43]. Notably, the guide strand is already determined by the polarity of small RNA duplexes upon loading into the RISC, before unwinding occurs [44–48]. The activated RISC then recognizes a specific target site by complementary intermolecular base pairing throughout the single-stranded guide RNA molecule [49]. The resulting RISC-bound miRNAs or siRNAs either guide the sequence-specific degradation of complementary RNAs or inhibit the translation of partly complementary target messenger RNAs by post-transcriptional gene silencing in the cytoplasm, depending in part on the nature and degree of guide/target complementarity [50,51].

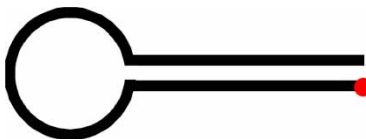
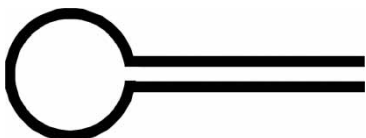
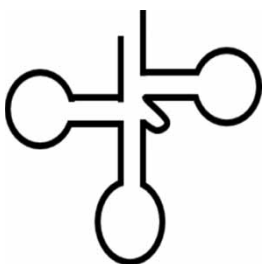
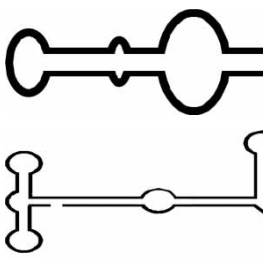
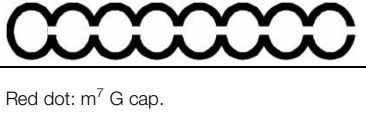
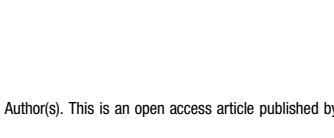

Additional endonuclease functions of Dicer

In addition, Dicer controls the fate of many other RNA species (Table 1). Dicer-derived small RNAs share several characteristic features. Small RNAs are typically ~20–30 nucleotides (nt) long; have a characteristic and highly specific structure (i.e. 2-nt 3' overhangs, and 5' phosphate and 3' hydroxyl groups); and contain both passenger and guide strands [24]. This section will describe the role of Dicer in processing a variety of RNA substrates into small RNAs, which together may form a very complex regulatory network.

Dicer-derived small RNAs from tRNA

Dicer is important for the production of transfer RNA (tRNA)-related fragments (tRFs), a heterogeneous class of small RNAs. tRFs are generated from tRNAs, and high-throughput sequencing and analysis show that they

Table 1 Types of endogenous small RNAs generated by Dicer

Origin of small RNA		Small RNA	Mechanism of action
	Pre-miRNA	miR320a-3p miR484-3p miR3615-3p miR7706-3p	miRNA biogenesis [176]
	Pre-miRNA	Let-7b,d,g, miR-98, etc.	miRNA biogenesis [176]
	tRNA	5' tRFs 3' CCA tRFs	RNAi regulation Translation regulation [53,55–59]
	tRNA	3' U tRFs	RNAi RNA metabolism [52,54]
	snoRNA	sdRNA	sdRNA RNA metabolism [61–69]
	LINE1 SINEs	rasiRNA riRNA	Degradation/repeat-induced small RNA [72–77]
	Triplet repeat RNA	sCNG CAG sRNA	Degradation /siRNA [78–81]

Red dot: m⁷ G cap.

map to known tRNA genes [52–54]. The most abundant forms of tRFs can be classified into several groups. Three types of tRFs are derived from mature tRNA or pre-tRNA: 3' U tRFs (tRF-1), 5' tRFs (tRF-5), and 3' CCA tRFs (tRF-3). 3' U tRFs are generated by tRNA 3'-endonuclease ELAC2 (RNaseZ) [52]. 5' tRFs are produced from the 5' end of the tRNA generated at any point of tRNA processing, provided the 5' leader sequence is removed by RNaseP, and are formed by a cleavage in the D loop. 3' CCA tRFs are derived from the 3' ends of mature tRNA by cleavage at the T loop and carry the trinucleotide CCA at the acceptor stem. Both 5' tRFs and 3' CCA tRFs have 5' phosphate and 3' hydroxyl ends, similar to miRNAs, and can be cleaved by Dicer protein complexes [53,55–58].

3' CCA tRFs are processed in a Dicer-dependent manner [53]. Both *in vitro* and *in vivo* studies show that tRFs derived from human tRNA(Gln) are dependent on Dicer [53,55]. The tRNA(Gly)-derived tRF has been suggested to have miRNA-like functions. Dicer is implicated in the generation of CU1276, a 22-nt tRNA (Gly)-derived small RNA expressed in mature B cells, which is physically associated with AGO and represses an endogenous target mRNA. It inhibits the mRNA of the single-stranded DNA-binding protein RPA1, which suppresses cell proliferation and modulates the DNA damage response [58]. The miRNA biogenesis pathway utilizes tRNA processing enzymes and is found in specific herpesviruses. Murine γ -herpesvirus 68 miRNA encodes a viral tRNA-like sequence transcribed by RNA polymerase III to generate tRNA-like primary miRNA precursors (pri-miRNAs). Pri-miRNAs carry a 5' tRNA moiety and are cleaved by a cellular tRNaseZ. Dicer processes the 3' tRNA moiety to generate mature viral small RNAs [59].

The lupus autoantigen La is an RNA chaperone associated with the miRNA pathway. La functions as a gatekeeper that ensures correct tRNA maturation and protects the miRNA pathway from tRF. In the absence of La (La knockdown cells), various pre-tRNAs bind to Dicer, and the tRFs derived from the 3' ends of tRNAs are bound to AGO [60].

Dicer-derived small RNAs from non-coding RNA

Dicer plays a role in processing several types of non-coding RNAs, which has implications for various regulatory mechanisms.

Dicer and snoRNA

Processing of non-coding small nucleolar RNA (snoRNA) to shorter, more stable snoRNA-derived RNAs (sdrRNAs) is widespread, with sdrRNAs identified in vertebrates, including humans [61–68], plants [63], fission yeast [63], and protozoa [69]. A high-throughput sequencing study of human small RNAs demonstrated that specific processing led to accumulation of small RNAs emerging from well-characterized non-coding RNAs, including snoRNAs [61]. sdrRNAs are associated with AGO proteins and show miRNA capabilities, which may have implications for control of mRNA processing and translation [62,69]. Association with Dicer varies by type; many sdrRNAs derived from box H/ACA snoRNAs are processed by Dicer, while processing of box C/D snoRNAs can be Dicer-independent [57,62]. In contrast, knockdown of Dicer and AGO in *Giardia lamblia* (protozoa) led to identification of small fragments with miRNA characteristics and showed that Dicer is required for miR2 production from box C/D snoRNA [69]. The identification of small fragments derives from the scaRNA of ACA45 (SCARNA15) by deep sequencing of human small RNAs associated with AGO proteins. The scaRNAs are a specific class of snoRNA that localize to Cajal bodies and guide the modification of RNA polymerase II transcribed spliceosomal small nuclear RNAs (snRNAs) U1, U2, U4, and U5 [70]. ACA45 is a 108-nt double-hairpin RNA that can be processed by Dicer to generate 20- to 22-nt ACA45 small RNA products [62].

A lot of data support the hypothesis that Dicer has direct roles in post-transcriptional gene-silencing activity of many miRNA-like sdrRNAs, but the mechanism underlying their biogenesis remains unknown. It has been shown that processing of box C/D RNAs into sdrRNAs can be Dicer-independent, while production of box H/ACA-derived sdrRNAs requires Dicer [57,62]. Dicer is able to process dsRNA from snoRNAs [54,62,63,71]. Thus, snoRNAs can serve as a source of short regulatory RNA species generated by Dicer, which may be involved in the control of processing and translation of various mRNAs.

Dicer and long interspersed nuclear element-1

Another example of non-coding RNA processing by Dicer involves LINE-1 (long interspersed nuclear element-1), a retrotransposon. The LINE-1 promoter has a bidirectional orientation, containing sense and antisense transcripts that form double-stranded long hairpin RNAs, which are then processed by Dicer into

repeat-associated siRNAs (rasiRNAs). rasiRNAs are loaded into AGO proteins to silence LINE-1 by RNA-directed DNA methylation. Inhibition of biogenesis of rasiRNAs has been suggested to increase the processing of LINE-1 transcription via Dicer inhibition [72]. Other experiments using engineered LINE-1 reporters have shown that mutation of Dicer increases LINE-1 transcripts. The rasiRNAs generated by Dicer negatively regulate LINE-1 transcription at the post-transcriptional level [73,74].

Dicer and short interspersed element

Geographic atrophy (GA), an age-related macular degeneration disease of the retinal pigment epithelium, is associated with reduced expression of Dicer. Inhibition of Dicer leads to accumulation of Alu repeat RNAs of ~300-nt derived from short interspersed elements (SINEs). This accumulation is cytotoxic and triggers interferon-mediated, caspase 8-dependent apoptosis. Injection of Alu repeat RNA into mouse eyes generates GA [75]. This condition is rescued by Dicer cleavage of Alu repeat RNAs, suggesting that Dicer-dependent degradation of the RNAs is critical for detoxification [75]. Dicer processing of Alu repeat RNA prevents activation of the host inflammasome [76]. The DR2 Alu repeat RNAs are processed into 28–65-nt repeat-induced small RNAs (riRNAs) under DCIER-dependent conditions. These riRNAs are stabilized by binding with AGO3 and recruit mRNA-decapping complexes, which block translation and degrade key stem cell transcripts like Nanog and Tdgl1 mRNAs [77].


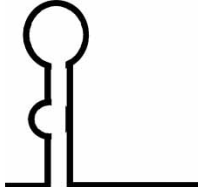
Dicer-derived small RNAs from triplet repeat RNA

Dicer has direct and indirect roles in curtailing expression of toxic triplet repeat RNAs. Triplet repeat expansion disorders include myotonic dystrophy type 1 (DM-1), fragile X-associated tremor ataxia syndrome, Huntington’s disease (HD), and spinocerebellar ataxia (SCA), which frequently encode RNAs with long internal triplet repeat structures [78]. *In vitro* studies showed that single-stranded CGG-RNA is cleaved by Dicer to generate short CGG-RNAs [79]. Dicer recognizes and processes the hairpin structure of expanded CNG repeats, producing small CNG-repeated RNAs (sCNG) [78,80]. The Dicer-dependence of sCNG biogenesis has been demonstrated in fibroblasts of patients with DM-1 (sCUG), HD (sCAG), and SCA (sCAG) [78]. The relevance of sCNG has been reported in HD, which is caused by an abnormal CAG expansion within the first exon of the Huntingtin (HTT) gene. The sCAGs from HTT RNA stimulate neurotoxic activity [81]. Importantly, the toxic activity of sCAG is dependent on Dicer.

Dicer-derived small RNAs from exogenous RNA

In addition to its role in processing endogenous RNA, Dicer is also important in processing exogenous RNA, including viral-associated (VA) RNA (Table 2). Viral infection can produce virus-derived small RNAs. For example, VA RNA I and II are 160-nt long non-coding RNAs from the adenovirus genome, which are processed similarly to miRNAs, resulting in the production of 22-nt VA-RNA-derived miRNAs (mivaRNAs). These mivaRNAs are processed by Dicer [82–84].

Table 2 Types of exogenous small RNAs generated by Dicer

Origin of small RNA	Small RNA	Mechanism of action	
	Adenovirus VA RNA	mivaRNA	Antiviral response [82–84]
	HIV TAR RNA	miR-N367	Antiviral response [88–91]

RNA genome viruses do not express small RNA in infected cells; this phenomenon is believed to be a mechanism of host immune evasion [85,86]. However, the bovine leukemia virus, a retrovirus with an RNA genome, encodes a conserved cluster of small RNAs that are produced as shorter RNA polymerase III-transcribed hairpins that are directly cleaved by Dicer [87].

Recent studies show that RNA viruses also process miRNA-like RNAs [88,89]. A small RNA within the Nef (negative regulatory factor) region of the human immunodeficiency virus type 1 (HIV-1) genome is proposed to play a role in inhibition of viral transcription [90]. A 19-nt small RNA is derived from hairpin structures, including TAR (trans-activation response element) RNA [91]. Dicer processes these structures into functional small RNAs that target the HIV-1 genome. The Nef-derived miRNA (miR-N367) reduces Nef expression and suppresses replication by reducing HIV-promoter activity.

Additional functions of Dicer

In addition to the critical role of Dicer in small RNA biogenesis, it also has important roles in genome regulation and surveillance, unconnected to small RNA production. For example, although its RNAi role occurs in the cytoplasm, Dicer can also localize to the nucleus [92,93] and interact with nuclear pore components [94]. Dicer is present in autophagy and is required for autophagosome formation [95–97]. Dicer is found in the exosomes that are tiny vesicles with RNAs and proteins that are secreted from cells [98,99]. Dicer can also bind to 'passive' RNA-binding sites without cleavage or small RNA generation [100]. Such interactions act as a buffering system to stabilize passive-site RNAs and control the catalytic activity of the enzyme by sequestering it from other targets. Dicer can also process viral sources of dsRNAs to produce viral siRNAs (viral small interfering RNAs) involved in various antiviral silencing responses [101].

Dicer in the nucleus

Several lines of evidence suggest that Dicer plays important roles in the nucleus. The C-terminal dsRNA-binding domain of Dicer contains a nuclear localization signal, which allows it to shuttle between the nucleus and the cytoplasm [93]. Human Dicer is associated with a nuclear pore complex component, nucleoporin 153, which assists Dicer in transport and localization to the nucleus [94]. Recent studies have shown that mammalian Dicer can also function in the nucleus [102]. Dicer inhibition leads to altered nucleolar structure; a significant increase in nucleolar size was observed in cells depleted of Dicer [103]. Some studies show that Dicer is required for cleavage and processing of pre-ribosomal RNA (rRNA) [104,105], which occurs mainly in the nucleolus [106], suggesting a connection between nuclear Dicer, rRNA synthesis, and ribosome biogenesis. However, another study shows that Dicer is associated with rDNA, but did not process pre-rRNA [7]. This suggests Dicer may affect pre-rRNA processing in different ways, such as transcriptional regulation.

Nuclear siRNAs corresponding to heterochromatic loci are associated with silencing; Dicer and AGO1 mutations destroy the production of these endo-siRNAs and the assembly of heterochromatin in the fission yeast *Schizosaccharomyces pombe* [107,108]. These siRNAs produce sequence-dependent silencing by recruiting histone-modifying enzymes that initiate heterochromatin formation and correlate with AGO1 and other silencing components [107,109]. Such siRNA-mediated transcriptional silencing has been reported in *Arabidopsis* [110,111], *Drosophila* [112], and mammalian cells [113]. In mammalian cells, nuclear siRNA-mediated transcriptional gene silencing was induced by histone deacetylases and DNA methyltransferases [113]. Another study showed heterochromatin defects at centromeres in Dicer-inhibited cells [114], suggesting that the Dicer-related RNAi machinery is implicated in the formation of the heterochromatin structure. Recent studies show that mammalian Dicer can be located in the nucleus [102]. The nuclear localization and function of mammalian Dicer remains one of the least understood aspects of Dicer biology.

Dicer in exosomes

Exosomes are small extracellular vesicles with an average size of 30–100 nm, which were simply considered cell debris for many years. Today, exosomes are known to be rich in lipids, proteins, and RNAs, including protein-coding transcripts (i.e. mRNAs) as well as many types of non-coding RNAs (e.g. miRNAs, long non-coding RNAs, circular RNAs, snoRNAs, snRNAs, tRNAs, rRNAs, and piwi-interacting RNAs) [115]. Exosomes allow these RNAs to be transferred from parent cells to recipient cells, where they can regulate or serve as templates for protein production [116,117].

In addition to recognition of a novel form of cell–cell communication, the discovery of miRNAs in exosomes has suggested new applications for exosomes, such as potentially easy-access biomarkers, or, given that

exosomes are non-immunogenic, possibly as novel therapeutics [118–124]. miRNAs transported via exosomes could function as gene expression regulators in recipient cells, and growing evidence from exosomes in cancer and other processes has expanded the known subsets of exosome functions [121,125–128]. The discovery of miRNAs in exosomes also suggests a mechanism through which dysregulation of endogenous miRNAs may affect disease pathogenesis.

Dicer, AGO2, and TRBP proteins are detected in exosomes derived from breast cancer cells, but not from non-tumorigenic breast cells [98]. Exosomes derived from cancer cells, but not normal cells, were enriched in mature miRNAs, suggesting that pre-miRNAs can convert into mature miRNA in exosomes from cancer but not from non-tumorigenic breast cells. Tumor-derived exosomes isolated from cells or fluids of patients with breast cancer can be transferred to non-cancer cells in a Dicer-dependent manner. Mice form tumors when injected with cancer-derived exosomes, except in the presence of Dicer inhibition. Thus, Dicer is important for the transformation of normal cells to cancer cells, following exposure to exosomes. Exosomes carrying Dicer-derived miRNAs can affect cell–cell communication. Dicer-sufficient macrophage-derived exosomes carry miRNAs into Dicer-deficient endothelial cells to measurably reduce targeted sequences [99]. Thus, exosomes released by cancer cells may bioengineer miRNAs with the assistance of Dicer, resulting in tumor boosters.

Exosomes derived from HIV-1-infected cells contain TAR RNA and Dicer, as well as components of the host miRNA machinery [129]. The inclusion of TAR RNA and these components requires CRM1 transport receptor-mediated export from the nucleus; after packaging, the viral exosomes are released into the extracellular space. In recipient cells, exosomal TAR RNA down-regulates apoptosis by reducing expression of pro-apoptotic protein Bim and transcriptional regulator cyclin-dependent kinase 9. Thus, viral exosomes may provide a mechanism for intercellular viral spread in HIV-infected hosts.

Dicer DNase function

Dicer also plays a role in the recognition and processing of DNA in apoptosis [130]. The caspase CED-3 cleaves the two RNase III domains of Dicer in *C. elegans*, leaving a truncated though catalytically active protein consisting only of the C-terminal RNase III and dsRNA-binding domains. This truncated Dicer inactivates its RNase function, gains DNase function, and initiates breaks on chromosomal DNA. The DNA fragments are metabolized to complete apoptotic DNA degradation.

Two faces of Dicer viral interactions

Dicer antiviral function

Antiviral RNAi is a powerful mechanism to protect against viral infections. Specific features of viral RNA are sensed as foreign, and Dicer cleaves the double-stranded viral RNA into viral siRNA [10,131–134]. As described above, VA RNAs are processed by Dicer into small RNAs (mivaRNAs). These are incorporated into the RISC and may play an important role in suppressing RNAi or miRNA regulation during viral infection. VA RNA I is quickly generated after infection of host cells and amasses high copy numbers to support adenovirus survival [135–138]. VA RNA I has RNA-silencing properties, in addition to its well-established role in blocking dsRNA-dependent protein kinase PKR activation [139–141]. Activated PKR is designed to stop translation in virus-infected cells by phosphorylating a translation initiation factor [84]. VA RNA is a highly structured RNA that is bound by the nuclear export protein Exportin 5. It is rapidly transcribed after infection and produces very high copy numbers. VA RNA I saturates the Exportin 5 pathway, thereby inhibiting the nuclear export of pre-miRNAs. Additionally, due to its pre-miRNA-like secondary structure, VA RNA I suppresses Dicer mRNA movement to the cytoplasm by saturating the Exportin 5-dependent export pathway [139,142]. On the other hand, VA RNA I plays an important role in blocking antiviral function, thereby inhibiting Dicer mRNA. This suggests that Dicer has roles in antiviral function.

Although VA RNA is a poor substrate for Dicer cleavage, a few VA RNAs are cleaved to ~22-nt mivaRNAs. mivaRNA association with the RISC is crucial for adenovirus replication [143]. A recent study showed that mivaRNAs down-regulate adenovirus replication, and knockdown of Dicer significantly promotes adenovirus replication by inhibiting production of mivaRNAs [82]. This suggests that Dicer performs its antiviral function against adenovirus via cleavage of VA RNAs.

Vaccinia virus (VACV) infection shows that inhibition of Dicer protein is associated with reduction in pre-miRNA processing [144]. Flaviviruses produce a small pathogenic RNA called subgenomic flavivirus RNA

(sRNA), which suppresses RNA silencing in insect and mammalian cells. The sRNAs are associated with Dicer in infected cells and compete with endogenous small RNAs, resulting in decreased Dicer activity [145–148]. The human herpesvirus Epstein–Barr virus (EBV)-derived miRNA miR-BART6-5p targets human Dicer mRNA [149]. This suggests that viral miRNA influences Dicer transcription via a more subtle mechanism. This phenomenon shows the competition between Dicer of host cells and viral miRNAs of exogenous RNA.

EBV miRNA can be edited by adenosine deaminase acting on RNA to convert adenosine into inosine. A-to-I-edited miR-BART6-3p decreases the efficiency with which the miRNA encoded on the opposite strand, miR-BART6-5p, is loaded into the RISC in human cells. miR-BART6-5p targets human Dicer through four binding sites in its 3'UTR. Therefore, editing of miR-BART6-3p has a positive effect on Dicer expression. In turn, Dicer levels affect the expression levels of multiple genes that regulate the infectious and lytic states of EBV. Thus it is postulated that editing of miR-BART6-3p could be an indirect way to modulate miRNA biogenesis and thereby the viral life cycle [149].

Viruses encode proteins that suppress RNAi in mammalian cells, e.g. E3L (VACV), B2 (Nodamura virus), NSs (La Crosse virus), Tat (HIV), and VP30, VP35, and VP40 (Ebola virus) [142,150–154]. In particular, B2 of the Nodamura virus binds exogenous short hairpin RNAs and endogenous pre-let-7d and inhibits Dicer small RNA biogenesis [152]. The helicase domain of Dicer interacts with the lysine 51 residue of HIV Tat. The interaction between Tat and Dicer is dependent on the presence of small RNA, as RNase treatment inhibits this interaction. Tat interferes with Dicer processing of miRNA biogenesis [91]. VP30 and VP35 can function through direct interaction with Dicer or Dicer-associated factors TRBP and PACT, and inhibit the production of functional small RNAs [153,154]. These results demonstrate that viral RNA-binding proteins have the potential to interfere with miRNA biogenesis through RNA–Dicer interactions.

Dicer proviral function

Virus-derived small RNAs that direct specific antiviral defenses through biogenesis of a small RNA may be dependent on the cleavage activity of Dicer. However, it is still under debate whether infection by virus induces or suppresses antiviral small RNA.

A. thaliana encodes multiple Dicer proteins (DCL 1–4) involved in distinct endogenous RNA-silencing pathways and antiviral defenses, which show functional redundancy and specificity [155,156]. Inactivation of DCL4 but not DCL2 or DCL3 induces a high level of viral replication, indicating that DCL4 is essential for intracellular antiviral silencing. While DCL2 can produce abundant 22-nt viral siRNAs when DCL4 is absent, these siRNAs are less efficient in mediating an antiviral defense [157,158]. As described above, Dicer has two types in *Drosophila*. Dicer1 produces mature miRNAs in an ATP-dependent manner. Dicer2 cleaves cell-derived dsRNA precursors to produce endogenous siRNAs [159] and exogenous siRNAs [160,161], and mediates host defenses against viruses by activating Toll immune signaling [160]. The specific type of Dicer that processes virus-derived dsRNA into small RNAs in *A. thaliana* and *D. melanogaster* suggests an antiviral strategy, but other Dicer types do not produce antiviral small RNAs from viruses. In contrast, humans have one type of Dicer that processes both miRNAs and siRNAs. These various roles of Dicer led us to hypothesize that Dicer might regulate both antiviral and proviral mechanisms.

Infection of human cells with a wide range of viruses, including yellow fever virus, Sindbis virus, Venezuelan equine encephalitis virus, measles virus, influenza A virus, reovirus, vesicular stomatitis virus, HIV-1, or herpes simplex virus 1, failed to reveal any enhancement in viral replication in Dicer-inhibited human cells (human cells with the absence of Dicer) [162]. miRNAs derived from the Dengue virus genome include six different miRNA-like molecules (viral small RNA: vsRNA 1–6) [163]. Inhibition of vsRNA-5 leads to significant increases in viral replication. However, inhibition of the other vsRNAs has no direct effect on viral replication. Importantly, viral replication proceeded more slowly in human Dicer-deficient cells. KUN-miR-1 is derived from the terminal 3' stem loop of West Nile virus (WNV) [164], and its production reduces inhibition of Dicer, with a significant reduction in WNV replication.

Dicer as a developmental switch

The Dicer protein can command specific interactions with other proteins. For example, Ras/ERK signaling and Dicer regulate *C. elegans* oogenesis; Dicer is a putative ERK substrate [165]. Phosphorylation of Dicer by ERK localizes it to the nucleus; it remains present during most of oogenesis but is rapidly lost from the terminal oocyte shortly before fertilization [166]. An ERK-mediated switch in Dicer activity could regulate genes during oogenesis and in the oocyte–embryo transition.

Dicer and cancer

In addition to other diseases described throughout this review, many groups have reported a correlation between Dicer expression and cancer. For example, Dicer expression shows significant changes in different stages of lung adenocarcinoma [167]. Reduced expression of Dicer is associated with poor prognosis in some types of lung, breast, skin, endometrial, and ovarian cancer [168–172]. In contrast, Dicer is overexpressed in metastatic lesions of prostate cancer [173], and is increased in Burkitt lymphoma [174]. There is no clear correlation between Dicer expression, cancer type, and disease progression; whether these inconsistencies can be attributed to technical artifacts, to tissue specificity, or to some other biological process remains unknown. Thus, whether Dicer acts as a tumor suppressor or an oncogene remains controversial [175]. However, given Dicer's dual roles in other biological processes, one can speculate that Dicer may function as both a tumor suppressor and an oncogene.

Conclusions

Accumulating evidence continues to demonstrate the critical role of the Dicer enzyme in not only the biogenesis of miRNA and siRNA in the canonical RNAi pathway, but also in controlling the fate of many other small RNA species. Furthermore, the list of non-endonuclease roles of Dicer continues to grow. Future directions in Dicer research should include: expanding our understanding of Dicer in the biogenesis and regulation of a growing network of endogenous small RNAs, which have interrelated implications for the regulation of complex physiological processes, including cancer; delineating the regulation of Dicer itself, including the causes and consequences of Dicer subcellular localization; and determining the role of Dicer in response to exogenous RNAs, and how this connects to the immune system.

Abbreviations

AGO, Argonaute; CRM, chromosome region maintenance; DCL, Dicer-like; DExD/H, DEAD-like and helicase domain; DM-1, myotonic dystrophy type 1; dsRNA, double-stranded RNA; EBV, Epstein–Barr virus; ERK, extracellular signal-regulated kinase; GA, geographic atrophy; HD, Huntington's disease; HIV-1, human immunodeficiency virus type 1; HSV, herpes simplex virus; HTT, Huntingtin; LINE-1, long interspersed nuclear element-1; miRNA, microRNA; mivaRNAs, VA-RNA-derived miRNA; Nef, negative regulatory factor; PACT, protein activator of PKR; PAZ, Piwi/Argonaut/Zwille; PKR, RNA-dependent protein kinase; pre-miRNAs, precursor miRNAs; pri-miRNAs, primary miRNA precursors; rasiRNA, repeat-associated siRNA; RBD, dsRNA-binding domain; riRNA, repeat-induced small RNA; RISC, RNA-induced silencing complex; RNAi, RNA interference; RNase, ribonuclease; rRNA, ribosomal RNA; SCA, spinocerebellar ataxia; scaRNA, Cajal body-specific RNA; sCNG, small CNG; sdrRNA, snoRNA-derived RNAs; sflRNA, subgenomic flavivirus; sflRNA, subgenomic flavivirus RNA; SINE, short interspersed elements; snoRNA, small nucleolar RNA; snRNA, small nuclear RNAs; TAR, trans-activation response element; TRBP, HIV trans-activating response RNA-binding protein; tRF, transfer RNA-related fragments; tRNA, transfer RNA; VA RNA, viral-associated RNA; VACV, vaccinia virus; viral siRNA, viral small interfering RNA; vsRNA, viral small RNA; WNV, West Nile virus.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

- Lau, P.-W., Guiley, K.Z., De, N., Potter, C.S., Carragher, B. and MacRae, I.J. (2012) The molecular architecture of human Dicer. *Nat. Struct. Mol. Biol.* **19**, 436–440 doi:10.1038/nsmb.2268
- Wilson, R.C., Tambe, A., Kidwell, M.A., Noland, C.L., Schneider, C.P. and Doudna, J.A. (2015) Dicer-TRBP complex formation ensures accurate mammalian microRNA biogenesis. *Mol. Cell* **57**, 397–407 doi:10.1016/j.molcel.2014.11.030

- 3 Tian, Y., Simanshu, D.K., Ma, J.-B., Park, J.-E., Heo, I., Kim, V.N. et al. (2014) A phosphate-binding pocket within the platform-PAZ-connector helix cassette of human Dicer. *Mol. Cell* **53**, 606–616 doi:10.1016/j.molcel.2014.01.003
- 4 Taylor, D.W., Ma, E., Shigematsu, H., Cianfrocco, M.A., Noland, C.L., Nagayama, K. et al. (2013) Substrate-specific structural rearrangements of human Dicer. *Nat. Struct. Mol. Biol.* **20**, 662–670 doi:10.1038/nsmb.2564
- 5 Wang, H.-W., Noland, C., Siridechadilok, B., Taylor, D.W., Ma, E., Felderer, K. et al. (2009) Structural insights into RNA processing by the human RISC-loading complex. *Nat. Struct. Mol. Biol.* **16**, 1148–1153 doi:10.1038/nsmb.1673
- 6 Svobodova, E., Kubikova, J. and Svoboda, P. (2016) Production of small RNAs by mammalian Dicer. *Pflugers Archiv* **468**, 1089–1102 doi:10.1007/s00424-016-1817-6
- 7 Sinkkonen, L., Hugenschmidt, T., Filipowicz, W., Svoboda, P. and Aramayo, R. (2010) Dicer is associated with ribosomal DNA chromatin in mammalian cells. *PLoS ONE* **5**, e12175 doi:10.1371/journal.pone.0012175
- 8 Macrae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.Z. et al. (2006) Structural basis for double-stranded RNA processing by Dicer. *Science* **311**, 195–198 doi:10.1126/science.1121638
- 9 Zhang, H., Kolb, F.A., Jaskiewicz, L., Westhof, E. and Filipowicz, W. (2004) Single processing center models for human Dicer and bacterial RNase III. *Cell* **118**, 57–68 doi:10.1016/j.cell.2004.06.017
- 10 Flemr, M., Malik, R., Franke, V., Nejeplinska, J., Sedlacek, R., Vlahovicek, K. et al. (2013) A retrotransposon-driven dicer isoform directs endogenous small interfering RNA production in mouse oocytes. *Cell* **155**, 807–816 doi:10.1016/j.cell.2013.10.001
- 11 Kurzynska-Kokorniak, A., Pokornowska, M., Koralewska, N., Hoffmann, W., Bienkowska-Szewczyk, K. and Figlerowicz, M. (2016) Revealing a new activity of the human Dicer DUF283 domain in vitro. *Sci. Rep.* **6**, 23989 doi:10.1038/srep23989
- 12 Cerutti, H. and Casas-Mollano, J.A. (2006) On the origin and functions of RNA-mediated silencing: from protists to man. *Curr. Genet.* **50**, 81–99 doi:10.1007/s00294-006-0078-x
- 13 Shabalina, S.A. and Koonin, E.V. (2008) Origins and evolution of eukaryotic RNA interference. *Trends Ecol. Evol.* **23**, 578–587 doi:10.1016/j.tree.2008.06.005
- 14 Lamontagne, B., Larose, S., Boulanger, J. and Elela, S.A. (2001) The RNase III family: a conserved structure and expanding functions in eukaryotic dsRNA metabolism. *Curr. Issues Mol. Biol.* **3**, 71–78 PMID:11719970
- 15 Johanson, T.M., Lew, A.M. and Chong, M.M.W. (2013) MicroRNA-independent roles of the RNase III enzymes Drosha and Dicer. **3**, doi:10.1098/rsob.130144
- 16 Ullu, E., Tschudi, C. and Chakraborty, T. (2004) RNA interference in protozoan parasites. *Cell. Microbiol.* **6**, 509–519 doi:10.1111/j.1462-5822.2004.00399.x
- 17 Baum, J., Papenfuss, A.T., Mair, G.R., Janse, C.J., Vlachou, D., Waters, A.P. et al. (2009) Molecular genetics and comparative genomics reveal RNAi is not functional in malaria parasites. *Nucleic Acids Res.* **37**, 3788–3798 doi:10.1093/nar/gkp239
- 18 Drinnenberg, I.A., Weinberg, D.E., Xie, K.T., Mower, J.P., Wolfe, K.H., Fink, G.R. et al. (2009) RNAi in budding yeast. *Science* **326**, 544–550 doi:10.1126/science.1176945
- 19 Mukherjee, K., Campos, H. and Kolaczowski, B. (2013) Evolution of animal and plant Dicers: early parallel duplications and recurrent adaptation of antiviral RNA binding in plants. *Mol. Biol. Evol.* **30**, 627–641 doi:10.1093/molbev/mss263
- 20 Kim, J.K., Gabel, H.W., Kamath, R.S., Tewari, M., Pasquinelli, A., Rual, J.-F. et al. (2005) Functional genomic analysis of RNA interference in *C. elegans*. *Science* **308**, 1164–1167 doi:10.1126/science.1109267
- 21 Gao, Z., Wang, M., Blair, D., Zheng, Y., Dou, Y. and Rossi, J. J. (2014) Phylogenetic analysis of the endoribonuclease Dicer family. *PLOS ONE* **9**, e95350 doi:10.1371/journal.pone.0095350
- 22 Lee, Y.S., Nakahara, K., Pham, J.W., Kim, K., He, Z., Sontheimer, E.J. et al. (2004) Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* **117**, 69–81 doi:10.1016/S0092-8674(04)00261-2
- 23 Murphy, D., Dancis, B. and Brown, J.R. (2008) The evolution of core proteins involved in microRNA biogenesis. *BMC Evol. Biol.* **8**, 92 doi:10.1186/1471-2148-8-92
- 24 Kidwell, M.A., Chan, J.M. and Doudna, J.A. (2014) Evolutionarily conserved roles of the Dicer helicase domain in regulating RNA interference processing. *J. Biol. Chem.* **289**, 28352–28362 doi:10.1074/jbc.M114.589051
- 25 Tsutsumi, A., Kawamata, T., Izumi, N., Seitz, H. and Tomari, Y. (2011) Recognition of the pre-miRNA structure by Drosophila Dicer-1. *Nat. Struct. Mol. Biol.* **18**, 1153–1158 doi:10.1038/nsmb.2125
- 26 Welker, N.C., Maity, T.S., Ye, X., Aruscavage, P.J., Krauchuk, A.A., Liu, Q. et al. (2011) Dicer's helicase domain discriminates dsRNA termini to promote an altered reaction mode. *Mol. Cell* **41**, 589–599 doi:10.1016/j.molcel.2011.02.005
- 27 Jankowsky, E. (2011) RNA helicases at work: binding and rearranging. *Trends Biochem. Sci.* **36**, 19–29 doi:10.1016/j.tibs.2010.07.008
- 28 Fairman-Williams, M.E., Guenther, U.-P. and Jankowsky, E. (2010) SF1 and SF2 helicases: family matters. *Curr. Opin. Struct. Biol.* **20**, 313–324 doi:10.1016/j.sbi.2010.03.011
- 29 Ma, E., Zhou, K., Kidwell, M.A. and Doudna, J.A. (2012) Coordinated activities of human dicer domains in regulatory RNA processing. *J. Mol. Biol.* **422**, 466–476 doi:10.1016/j.jmb.2012.06.009
- 30 Welker, N.C., Pavelec, D.M., Nix, D.A., Duchaine, T.F., Kennedy, S. and Bass, B.L. (2010) Dicer's helicase domain is required for accumulation of some, but not all, *C. elegans* endogenous siRNAs. *RNA* **16**, 893–903 doi:10.1261/ma.2122010
- 31 Kurihara, Y. and Watanabe, Y. (2004) Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proc. Natl Acad. Sci. U.S.A.* **101**, 12753–12758 doi:10.1073/pnas.0403115101
- 32 Dong, Z., Han, M.-H. and Fedoroff, N. (2008) The RNA-binding proteins HYL1 and SE promote accurate in vitro processing of pri-miRNA by DCL1. *Proc. Natl Acad. Sci. U.S.A.* **105**, 9970–9975 doi:10.1073/pnas.0803356105
- 33 Zhang, H., Kolb, F.A., Brondani, V., Billy, E. and Filipowicz, W. (2002) Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. *EMBO J.* **21**, 5875–5885 doi:10.1093/emboj/cdf582
- 34 Provost, P., Dishart, D., Doucet, J., Frendewey, D., Samuelsson, B. and Rådmark, O. (2002) Ribonuclease activity and RNA binding of recombinant human Dicer. *EMBO J.* **21**, 5864–5874 doi:10.1093/emboj/cdf578

- 35 Ma, E., MacRae, I.J., Kirsch, J.F. and Doudna, J.A. (2008) Autoinhibition of human dicer by its internal helicase domain. *J. Mol. Biol.* **380**, 237–243 doi:10.1016/j.jmb.2008.05.005
- 36 Soifer, H.S., Sano, M., Sakurai, K., Chomchan, P., Saetrom, P., Sherman, M.A. et al. (2008) A role for the Dicer helicase domain in the processing of thermodynamically unstable hairpin RNAs. *Nucleic Acids Res.* **36**, 6511–6522 doi:10.1093/nar/gkn687
- 37 Betancur, J.G. and Tomari, Y. (2012) Dicer is dispensable for asymmetric RISC loading in mammals. *RNA* **18**, 24–30 doi:10.1261/ma.029785.111
- 38 Miyoshi, T., Takeuchi, A., Siomi, H. and Siomi, M.C. (2010) A direct role for Hsp90 in pre-RISC formation in *Drosophila*. *Nat. Struct. Mol. Biol.* **17**, 1024–1026 doi:10.1038/nsmb.1875
- 39 Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T. et al. (2010) Hsc70/Hsp90 chaperone machinery mediates ATP-Dependent RISC loading of small RNA duplexes. *Mol. Cell* **39**, 292–299 doi:10.1016/j.molcel.2010.05.015
- 40 Iki, T., Yoshikawa, M., Nishikiori, M., Jaudal, M.C., Matsumoto-Yokoyama, E., Mitsuhashi, I. et al. (2010) In vitro assembly of plant RNA-induced silencing complexes facilitated by molecular chaperone HSP90. *Mol. Cell* **39**, 282–291 doi:10.1016/j.molcel.2010.05.014
- 41 Fukunaga, R., Han, B.W., Hung, J.-H., Xu, J., Weng, Z. and Zamore, P.D. (2012) Dicer partner proteins tune the length of mature miRNAs in flies and mammals. *Cell* **151**, 533–546 doi:10.1016/j.cell.2012.09.027
- 42 Khvorovova, A., Reynolds, A. and Jayasena, S.D. (2003) Functional siRNAs and miRNAs exhibit strand bias. *Cell* **115**, 209–216 doi:10.1016/S0092-8674(03)00801-8
- 43 Schwarz, D.S., Hutvagner, G., Du, T., Xu, Z., Aronin, N. and Zamore, P.D. (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell* **115**, 199–208 doi:10.1016/S0092-8674(03)00759-1
- 44 Miyoshi, K., Tsukumo, H., Nagami, T., Siomi, H. and Siomi, M.C. (2005) Slicer function of *Drosophila* Argonautes and its involvement in RISC formation. *Genes Dev.* **19**, 2837–2848 doi:10.1101/gad.1370605
- 45 Matranga, C., Tomari, Y., Shin, C., Bartel, D.P. and Zamore, P.D. (2005) Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* **123**, 607–620 doi:10.1016/j.cell.2005.08.044
- 46 Rand, T.A., Petersen, S., Du, F. and Wang, X. (2005) Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell* **123**, 621–629 doi:10.1016/j.cell.2005.10.020
- 47 Kawamata, T., Seitz, H. and Tomari, Y. (2009) Structural determinants of miRNAs for RISC loading and slicer-independent unwinding. *Nat. Struct. Mol. Biol.* **16**, 953–960 doi:10.1038/nsmb.1630
- 48 Yoda, M., Kawamata, T., Paroo, Z., Ye, X., Iwasaki, S., Liu, Q. et al. (2010) ATP-dependent human RISC assembly pathways. *Nat. Struct. Mol. Biol.* **17**, 17–23 doi:10.1038/nsmb.1733
- 49 Tolia, N.H. and Joshua-Tor, L. (2007) Slicer and the Argonautes. *Nat. Chem. Biol.* **3**, 36–43 doi:10.1038/nchembio848
- 50 Pillai, R.S., Bhattacharyya, S.N. and Filipowicz, W. (2007) Repression of protein synthesis by miRNAs: how many mechanisms? *Trends Cell Biol.* **17**, 118–126 doi:10.1016/j.tcb.2006.12.007
- 51 Hock, J., Weinmann, L., Ender, C., Rudel, S., Kremmer, E., Raabe, M. et al. (2007) Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. *EMBO Rep.* **8**, 1052–1060 doi:10.1038/sj.embor.7401088
- 52 Lee, Y.S., Shibata, Y., Malhotra, A. and Dutta, A. (2009) A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Genes Dev.* **23**, 2639–2649 doi:10.1101/gad.1837609
- 53 Cole, C., Sobala, A., Lu, C., Thatcher, S.R., Bowman, A., Brown, J.W. S. et al. (2009) Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. *RNA* **15**, 2147–2160 doi:10.1261/ma.1738409
- 54 Li, Z., Ender, C., Meister, G., Moore, P.S., Chang, Y. and John, B. (2012) Extensive terminal and asymmetric processing of small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. *Nucleic Acids Res.* **40**, 6787–6799 doi:10.1093/nar/gks307
- 55 Babiarz, J.E., Ruby, J.G., Wang, Y., Bartel, D.P. and Blelloch, R. (2008) Mouse ES cells express endogenous shRNAs, siRNAs, and other microprocessor-independent, Dicer-dependent small RNAs. *Genes Dev.* **22**, 2773–2785 doi:10.1101/gad.1705308
- 56 Schopman, N.C. T., Heynen, S., Haasnoot, J. and Berkhout, B. (2010) A miRNA-tRNA mix-up: tRNA origin of proposed miRNA. *RNA Biol.* **7**, 573–576 doi:10.4161/ma.7.5.13141
- 57 Langenberger, D., Cakir, M.V., Hoffmann, S. and Stadler, P.F. (2013) Dicer-processed small RNAs: rules and exceptions. *J. Exp. Zool. B: Mol. Dev. Evol.* **320**, 35–46 doi:10.1002/jez.b.22481
- 58 Maute, R.L., Schneider, C., Sumazin, P., Holmes, A., Califano, A., Basso, K. et al. (2013) tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. *Proc. Natl Acad. Sci. U.S.A.* **110**, 1404–1409 doi:10.1073/pnas.1206761110
- 59 Bogerd, H.P., Karnowski, H.W., Cai, X., Shin, J., Pohlars, M. and Cullen, B.R. (2010) A mammalian herpesvirus uses non-canonical expression and processing mechanisms to generate viral microRNAs. *Mol. Cell* **37**, 135–142 doi:10.1016/j.molcel.2009.12.016
- 60 Hasler, D., Lehmann, G., Murakawa, Y., Klironomos, F., Jakob, L., Grässer, F.A. et al. (2016) The lupus autoantigen La prevents Mis-channeling of tRNA fragments into the human MicroRNA pathway. *Mol. Cell* **63**, 110–124 doi:10.1016/j.molcel.2016.05.026
- 61 Kawaji, H., Nakamura, M., Takahashi, Y., Sandelin, A., Katayama, S., Fukuda, S. et al. (2008) Hidden layers of human small RNAs. *BMC Genomics* **9**, 1–57 doi:10.1186/1471-2164-9-157
- 62 Ender, C., Krek, A., Friedlander, M.R., Beitzinger, M., Weinmann, L., Chen, W. et al. (2008) A human snoRNA with microRNA-like functions. *Mol. Cell* **32**, 519–528 doi:10.1016/j.molcel.2008.10.017
- 63 Taft, R.J., Glazov, E.A., Lassmann, T., Hayashizaki, Y., Carninci, P. and Mattick, J.S. (2009) Small RNAs derived from snoRNAs. *RNA* **15**, 1233–1240 doi:10.1261/ma.1528909
- 64 Brameier, M., Herwig, A., Reinhardt, R., Walter, L. and Gruber, J. (2011) Human box C/D snoRNAs with miRNA like functions: expanding the range of regulatory RNAs. *Nucleic Acids Res.* **39**, 675–686 doi:10.1093/nar/gkq776
- 65 Langenberger, D., Bermudez-Santana, C.I., Stadler, P.F. and Hoffmann, S. (2010) Identification and classification of small RNAs in transcriptome sequence data. *Pac. Symp. Biocomput.* 80–87 PMID:19908360
- 66 Ono, M., Scott, M.S., Yamada, K., Avolio, F., Barton, G.J. and Lamond, A.I. (2011) Identification of human miRNA precursors that resemble box C/D snoRNAs. *Nucleic Acids Res.* **39**, 3879–3891 doi:10.1093/nar/gkq1355
- 67 Scott, M.S., Avolio, F., Ono, M., Lamond, A.I. and Barton, G.J. (2009) Human miRNA precursors with box H/ACA snoRNA features. *PLoS Comput. Biol.* **5**, e1000507 doi:10.1371/journal.pcbi.1000507

- 68 Politz, J.C., Hogan, E.M. and Pederson, T. (2009) MicroRNAs with a nucleolar location. *RNA* **15**, 1705–1715 doi:10.1261/rna.1470409
- 69 Saraiya, A.A. and Wang, C.C. (2008) snoRNA, a novel precursor of microRNA in *Giardia lamblia*. *PLoS Pathog.* **4**, e1000224 doi:10.1371/journal.ppat.1000224
- 70 Darzacq, X., Jády, B.E., Verheggen, C., Kiss, A.M., Bertrand, E. and Kiss, T. (2002) Cajal body-specific small nuclear RNAs: a novel class of 2'-O-methylation and pseudouridylation guide RNAs. *EMBO J.* **21**, 2746–2756 doi:10.1093/emboj/21.11.2746
- 71 Taft, R.J., Simons, C., Nahkuri, S., Oey, H., Korbje, D.J., Mercer, T.R. et al. (2010) Nuclear-localized tiny RNAs are associated with transcription initiation and splice sites in metazoans. *Nat. Struct. Mol. Biol.* **17**, 1030–1034 doi:10.1038/nsmb.1841
- 72 Faulkner, G.J. (2013) Retrotransposon silencing during embryogenesis: dicer cuts in LINE. *PLoS Genet.* **9**, e10003944 doi:10.1371/journal.pgen.1003944
- 73 Heras, S.R., Macias, S., Plass, M., Fernandez, N., Cano, D., Eyraes, E. et al. (2013) The microprocessor controls the activity of mammalian retrotransposons. *Nat. Struct. Mol. Biol.* **20**, 1173–1181 doi:10.1038/nsmb.2658
- 74 Yang, N. and Kazazian, Jr, H.H. (2006) L1 retrotransposition is suppressed by endogenously encoded small interfering RNAs in human cultured cells. *Nat. Struct. Mol. Biol.* **13**, 763–771 doi:10.1038/nsmb1141
- 75 Kaneko, H., Dridi, S., Tarallo, V., Gelfand, B.D., Fowler, B.J., Cho, W.G. et al. (2011) DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. *Nature* **471**, 325–330 doi:10.1038/nature09830
- 76 Tarallo, V., Hirano, Y., Gelfand, B.D., Dridi, S., Kerur, N., Kim, Y. et al. (2012) DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. *Cell* **149**, 847–859 doi:10.1016/j.cell.2012.03.036
- 77 Hu, Q.D., Tanasa, B., Trabucchi, M., Li, W., Zhang, J., Ohgi, K.A. et al. (2012) DICER- and AGO3-dependent generation of retinoic acid-induced DR2 Alu RNAs regulates human stem cell proliferation. *Nat. Struct. Mol. Biol.* **19**, 1168–1175 doi:10.1038/nsmb.2400
- 78 Krol, J., Fiszler, A., Mykowska, A., Sobczak, K., de Mezer, M. and Krzyzosiak, W.J. (2007) Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. *Mol. Cell* **25**, 575–586 doi:10.1016/j.molcel.2007.01.031
- 79 Handa, V., Saha, T. and Usdin, K. (2003) The fragile X syndrome repeats form RNA hairpins that do not activate the interferon-inducible protein kinase, PKR, but are cut by Dicer. *Nucleic Acids Res.* **31**, 6243–6248 doi:10.1093/nar/gkg818
- 80 Galka-Marciniak, P., Urbanek, M.O. and Krzyzosiak, W.J. (2012) Triplet repeats in transcripts: structural insights into RNA toxicity. *Biol. Chem.* **393**, 1299–1315 doi:10.1515/hsz-2012-0218
- 81 Bañez-Coronel, M., Porta, S., Kagerbauer, B., Mateu-Huertas, E., Pantano, L., Ferrer, I. et al. (2012) A pathogenic mechanism in Huntington's disease involves small CAG-repeated RNAs with neurotoxic activity. *PLoS Genet.* **8**, e1002481 doi:10.1371/journal.pgen.1002481
- 82 Machitani, M., Sakurai, F., Wakabayashi, K., Tomita, K., Tachibana, M. and Mizuguchi, H. (2016) Dicer functions as an antiviral system against human adenoviruses via cleavage of adenovirus-encoded noncoding RNA. *Sci. Rep.* **6**, 27598 doi:10.1038/srep27598
- 83 Machitani, M., Yamaguchi, T., Shimizu, K., Sakurai, F., Katayama, K., Kawabata, K. et al. (2011) Adenovirus Vector-Derived VA-RNA-Mediated innate immune responses. *Pharmaceutics* **3**, 338–353 doi:10.3390/pharmaceutics3030338
- 84 Mathews, M.B. and Shenk, T. (1991) Adenovirus virus-associated RNA and translation control. *J. Virol.* **65**, 5657–5662 PMID:PMC250225
- 85 Houzet, L. and Jeang, K.-T. (2011) MicroRNAs and human retroviruses. *Biochim. Biophys. Acta* **1809**, 686–693 doi:10.1016/j.bbaggm.2011.05.009
- 86 Cullen, B.R. (2010) Five questions about viruses and microRNAs. *PLoS Pathog.* **6**, e1000787 doi:10.1371/journal.ppat.1000787
- 87 Kincaid, R.P., Burke, J.M. and Sullivan, C.S. (2012) RNA virus microRNA that mimics a B-cell oncomiR. *Proc. Natl Acad. Sci. U.S.A.* **109**, 3077–3082 doi:10.1073/pnas.1116107109
- 88 Langlois, R.A., Shapiro, J.S., Pham, A.M. and Tenover, B.R. (2012) In vivo delivery of cytoplasmic RNA virus-derived miRNAs. *Mol. Ther.* **20**, 367–375 doi:10.1038/mt.2011.244
- 89 Shapiro, J.S., Varble, A., Pham, A.M. and Tenover, B.R. (2010) Noncanonical cytoplasmic processing of viral microRNAs. *RNA* **16**, 2068–2074 doi:10.1261/rna.2303610
- 90 Omoto, S., Ito, M., Tsutsumi, Y., Ichikawa, Y., Okuyama, H., Brisibe, E.A. et al. (2004) HIV-1 nef suppression by virally encoded microRNA. *Retrovirology* **1**, 44 doi:10.1186/1742-4690-1-44
- 91 Bennasser, Y. and Jeang, K.-T. (2006) HIV-1 Tat interaction with Dicer: requirement for RNA. *Retrovirology* **3**, 95 doi:10.1186/1742-4690-3-95
- 92 Matsui, M., Li, L., Janowski, B.A. and Corey, D.R. (2015) Reduced expression of Argonaute 1, Argonaute 2, and TRBP changes levels and intracellular distribution of RNAi factors. *Sci. Rep.* **5**, 12855 doi:10.1038/srep12855
- 93 Doyle, M., Badertscher, L., Jaskiewicz, L., Güttinger, S., Jurado, S., Hugen Schmidt, T. et al. (2013) The double-stranded RNA binding domain of human Dicer functions as a nuclear localization signal. *RNA* **19**, 1238–1252 doi:10.1261/rna.039255.113
- 94 Ando, Y., Tomaru, Y., Morinaga, A., Burroughs, A.M., Kawaji, H., Kubosaki, A. et al. (2011) Nuclear pore complex protein mediated nuclear localization of Dicer protein in human cells. *PLoS ONE* **6**, e23385 doi:10.1371/journal.pone.0023385
- 95 Zhang, P. and Zhang, H. (2013) Autophagy modulates miRNA-mediated gene silencing and selectively degrades AIN-1/GW182 in *C. elegans*. *EMBO Rep.* **14**, 568–576 doi:10.1038/embor.2013.53
- 96 Kovaleva, V., Mora, R., Park, Y.J., Plass, C., Chiramel, A.I., Bartenschlager, R. et al. (2012) miRNA-130a targets ATG2B and DICER1 to inhibit autophagy and trigger killing of chronic lymphocytic leukemia cells. *Cancer Res.* **72**, 1763–1772 doi:10.1158/0008-5472.CAN-11-3671
- 97 Gibbings, D., Mostowy, S., Jay, F., Schwab, Y., Cossart, P. and Voinnet, O. (2012) Selective autophagy degrades DICER and AGO2 and regulates miRNA activity. *Nat. Cell Biol.* **14**, 1314–1321 doi:10.1038/ncb2611
- 98 Melo, S.A., Sugimoto, H., O'Connell, J.T., Kato, N., Villanueva, A., Vidal, A. et al. (2014) Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell* **26**, 707–721 doi:10.1016/j.ccell.2014.09.005
- 99 Squadrino, M.L., Baer, C., Burdet, F., Maderna, C., Gilfillan, G.D., Lyle, R. et al. (2014) Endogenous RNAs modulate MicroRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep.* **8**, 1432–1446 doi:10.1016/j.celrep.2014.07.035
- 100 Rybak-Wolf, A., Jens, M., Murakawa, Y., Herzog, M., Landthaler, M. and Rajewsky, N. (2014) A variety of Dicer substrates in human and *C. elegans*. *Cell* **159**, 1153–1167 doi:10.1016/j.cell.2014.10.040
- 101 Ding, S.-W. (2010) RNA-based antiviral immunity. *Nat. Rev. Immunol.* **10**, 632–644 doi:10.1038/nri2824
- 102 Kalantari, R., Chiang, C.-M. and Corey, D.R. (2016) Regulation of mammalian transcription and splicing by nuclear RNAi. *Nucleic Acids Res.* **44**, 524–537 doi:10.1093/nar/gkv1305

- 103 Liang, X.-H. and Crooke, S.T. (2011) Depletion of key protein components of the RISC pathway impairs pre-ribosomal RNA processing. *Nucleic Acids Res.* **39**, 4875–4889 doi:10.1093/nar/gkr076
- 104 Burger, K., Muhl, B., Rohrmoser, M., Coords, B., Heidemann, M., Kellner, M. et al. (2013) Cyclin-dependent kinase 9 links RNA polymerase II transcription to processing of ribosomal RNA. *J. Biol. Chem.* **288**, 21173–21183 doi:10.1074/jbc.M113.483719
- 105 Wu, H., Xu, H., Miraglia, L.J. and Crooke, S.T. (2000) Human RNase III is a 160-kDa protein involved in preribosomal RNA processing. *J. Biol. Chem.* **275**, 36957–36965 doi:10.1074/jbc.M005494200
- 106 Lafontaine, D.L.J. (2015) Noncoding RNAs in eukaryotic ribosome biogenesis and function. *Nat. Struct. Mol. Biol.* **22**, 11–19 doi:10.1038/nsmb.2939
- 107 Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I.S. and Martienssen, R.A. (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297**, 1833–1837 doi:10.1126/science.1074973
- 108 Hall, I.M., Shankaranarayana, G.D., Noma, K.-i., Ayoub, N., Cohen, A. and Grewal, S.I.S. (2002) Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232–2237 doi:10.1126/science.1076466
- 109 Creamer, K.M. and Partridge, J.F. (2011) RITS-connecting transcription, RNA interference, and heterochromatin assembly in fission yeast. *Wiley Interdiscip. Rev. RNA* **2**, 632–646 doi:10.1002/wrna.80
- 110 Zilberman, D., Cao, X. and Jacobsen, S.E. (2003) *ARGONAUTE4* control of locus-specific siRNA accumulation and DNA and histone methylation. *Science* **299**, 716–719 doi:10.1126/science.1079695
- 111 Vaistij, F.E., Jones, L. and Baulcombe, D.C. (2002) Spreading of RNA targeting and DNA methylation in RNA silencing requires transcription of the target gene and a putative RNA-dependent RNA polymerase. *Plant Cell.* **14**, 857–867 doi:10.1105/tpc.010480
- 112 Pal-Bhadra, M., Bhadra, U. and Birchler, J.A. (2002) RNAi related mechanisms affect both transcriptional and posttranscriptional transgene silencing in *Drosophila*. *Mol. Cell* **9**, 315–327 doi:10.1016/S1097-2765(02)00440-9
- 113 Morris, K.V., Chan, S.W.-L., Jacobsen, S.E. and Looney, D.J. (2004) Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* **305**, 1289–1292 doi:10.1126/science.1101372
- 114 Fukagawa, T., Nogami, M., Yoshikawa, M., Ikeno, M., Okazaki, T., Takami, Y. et al. (2004) Dicer is essential for formation of the heterochromatin structure in vertebrate cells. *Nat. Cell Biol.* **6**, 784–791 doi:10.1038/ncb1155
- 115 Kim, K.M., Abdelmohsen, K., Mustapic, M., Kapogiannis, D. and Gorospe, M. (2017) RNA in extracellular vesicles. *Wiley Interdiscip. Rev. RNA* e1413–e1n/a doi:10.1002/wrna.1413
- 116 Hergenreider, E., Heydt, S., Treguer, K., Boettger, T., Horrevoets, A.J.G., Zeiher, A.M. et al. (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat. Cell Biol.* **14**, 249–256 doi:10.1038/ncb2441
- 117 Montecalvo, A., Larregina, A.T., Shufesky, W.J., Stolz, D.B., Sullivan, M.L.G., Karlsson, J.M. et al. (2012) Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* **119**, 756–766 doi:10.1182/blood-2011-02-338004
- 118 Willms, E., Johansson, H.J., Mäger, I., Lee, Y., Blomberg, K.E.M., Sadiq, M. et al. (2016) Cells release subpopulations of exosomes with distinct molecular and biological properties. *Sci. Rep.* **6**, 22519 doi:10.1038/srep22519
- 119 Crescitelli, R., Lässer, C., Szabo, T.G., Kittel, A., Eldh, M., Dianzani, I. et al. (2013) Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. *J. Extracell. Vesicles* **2** doi:10.3402/jev.v2i0.20677
- 120 Gould, S.J. and Raposo, G. (2013) As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J. Extracell. Vesicles* **2** doi:10.3402/jev.v2i0.20389
- 121 Iftikhar, H. and Carney, G.E. (2016) Evidence and potential in vivo functions for biofluid miRNAs: from expression profiling to functional testing: potential roles of extracellular miRNAs as indicators of physiological change and as agents of intercellular information exchange. *BioEssays* **38**, 367–378 doi:10.1002/bies.201500130
- 122 Bronisz, A., Godlewski, J. and Chiocca, E.A. (2016) Extracellular vesicles and MicroRNAs: their role in tumorigenicity and therapy for brain tumors. *Cell. Mol. Neurobiol.* **36**, 361–376 doi:10.1007/s10571-015-0293-4
- 123 Cheng, L., Sharples, R.A., Scicluna, B.J. and Hill, A.F. (2014) Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. *J. Extracell. Vesicles* **3**, 23743 doi:10.3402/jev.v3.23743
- 124 Hagiwara, S., Kantharidis, P. and Cooper, M.E. (2014) MicroRNA as biomarkers and regulator of cardiovascular development and disease. *Curr. Pharm. Des.* **20**, 2347–2370 doi:10.2174/13816128113199990495
- 125 Bryniarski, K., Ptak, W., Martin, E., Nazimek, K., Szczepanik, M., Sanak, M. et al. (2015) Free extracellular miRNA functionally targets cells by transfecting exosomes from their companion cells. *PLoS ONE* **10**, e0122991 doi:10.1371/journal.pone.0122991
- 126 Xu, J., Chen, Q., Zen, K., Zhang, C. and Zhang, Q. (2013) Synaptosomes secrete and uptake functionally active microRNAs via exocytosis and endocytosis pathways. *J. Neurochem.* **124**, 15–25 doi:10.1111/jnc.12057
- 127 Patton, J.G., Franklin, J.L., Weaver, A.M., Vickers, K., Zhang, B., Coffey, R.J. et al. (2015) Biogenesis, delivery, and function of extracellular RNA. *J. Extracell. Vesicles* **4**, 27494 doi:10.3402/jev.v4.27494
- 128 Morel, L., Regan, M., Higashimori, H., Ng, S.K., Esau, C., Vidensky, S. et al. (2013) Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1. *J. Biol. Chem.* **288**, 7105–7116 doi:10.1074/jbc.M112.410944
- 129 Narayanan, A., Iordanskiy, S., Das, R., Van Duyn, R., Santos, S., Jaworski, E. et al. (2013) Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA. *J. Biol. Chem.* **288**, 20014–20033 doi:10.1074/jbc.M112.438895
- 130 Nakagawa, A., Shi, Y., Kage-Nakadai, E., Mitani, S. and Xue, D. (2010) Caspase-dependent conversion of Dicer ribonuclease into a death-promoting deoxyribonuclease. *Science* **328**, 327–334 doi:10.1126/science.1182374
- 131 Li, Y., Lu, J., Han, Y., Fan, X. and Ding, S.-W. (2013) RNA interference functions as an antiviral immunity mechanism in mammals. *Science* **342**, 231–234 doi:10.1126/science.1241911
- 132 Maillard, P.V., Ciaudo, C., Marchais, A., Li, Y., Jay, F., Ding, S.W. et al. (2013) Antiviral RNA interference in mammalian cells. *Science* **342**, 235–238 doi:10.1126/science.1241930
- 133 Li, Y., Basavappa, M., Lu, J., Dong, S., Cronkite, D.A., Prior, J.T. et al. (2016) Induction and suppression of antiviral RNA interference by influenza A virus in mammalian cells. *Nat. Microbiol.* **2**, 16250 doi:10.1038/nmicrobiol.2016.250
- 134 Kennedy, E.M., Whisnant, A.W., Kornepati, A.V. R., Marshall, J.B., Bogerd, H.P. and Cullen, B.R. (2015) Production of functional small interfering RNAs by an amino-terminal deletion mutant of human Dicer. *Proc. Natl Acad. Sci. U.S.A.* **112**, E6945–E6954 doi:10.1073/pnas.1513421112

- 135 Kitajewski, J., Schneider, R.J., Safer, B., Munemitsu, S.M., Samuel, C.E., Thimmappaya, B. et al. (1986) Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2 α kinase. *Cell* **45**, 195–200 doi:10.1016/0092-8674(86)90383-1
- 136 Bhat, R.A. and Thimmappaya, B. (1984) Adenovirus mutants with DNA sequence perturbations in the intragenic promoter of VAI RNA gene allow the enhanced transcription of VAI RNA gene in HeLa cells. *Nucleic Acids Res.* **12**, 7377–7388 doi:10.1093/nar/12.19.7377
- 137 Thimmappaya, B., Weinberger, C., Schneider, R.J. and Shenk, T. (1982) Adenovirus VAI RNA is required for efficient translation of viral mRNAs at late times after infection. *Cell* **31**, 543–551 doi:10.1016/0092-8674(82)90310-5
- 138 Subramanian, S., Bhat, R.A., Rundell, M.K. and Thimmappaya, B. (1986) Suppression of the translation defect phenotype specific for a virus-associated RNA-deficient adenovirus mutant in monkey cells by simian virus 40. *J. Virol.* **60**, 363–368 PMID:PMC288901
- 139 Lu, S. and Cullen, B.R. (2004) Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. *J. Virol.* **78**, 12868–12876 doi:10.1128/JVI.78.23.12868-12876.2004
- 140 Andersson, M.G., Haasnoot, P.C.J., Xu, N., Berenjian, S., Berkhout, B. and Akusjarvi, G. (2005) Suppression of RNA interference by adenovirus virus-associated RNA. *J. Virol.* **79**, 9556–9565 doi:10.1128/JVI.79.15.9556-9565.2005
- 141 Kamel, W., Segerman, B., Öberg, D., Punga, T. and Akusjärvi, G. (2013) The adenovirus VA RNA-derived miRNAs are not essential for lytic virus growth in tissue culture cells. *Nucleic Acids Res.* **41**, 4802–4812 doi:10.1093/nar/gkt172
- 142 Bennasser, Y., Chable-Bessia, C., Triboulet, R., Gibbings, D., Gwizdek, C., Dargemont, C. et al. (2011) Competition for XPO5 binding between Dicer mRNA, pre-miRNA and viral RNA regulates human Dicer levels. *Nat. Struct. Mol. Biol.* **18**, 323–327 doi:10.1038/nsmb.1987
- 143 Xu, N., Segerman, B., Zhou, X. and Akusjarvi, G. (2007) Adenovirus virus-associated RNAI-derived small RNAs are efficiently incorporated into the RNA-induced silencing complex and associate with polyribosomes. *J. Virol.* **81**, 10540–10549 doi:10.1128/JVI.00885-07
- 144 Grinberg, M., Gilad, S., Meiri, E., Levy, A., Isakov, O., Ronen, R. et al. (2012) Vaccinia virus infection suppresses the cell microRNA machinery. *Arch. Virol.* **157**, 1719–1727 doi:10.1007/s00705-012-1366-z
- 145 Schnettler, E., Sterken, M.G., Leung, J.Y., Metz, S.W., Geertsema, C., Goldbach, R.W. et al. (2012) Noncoding flavivirus RNA displays RNA interference suppressor activity in insect and mammalian cells. *J. Virol.* **86**, 13486–13500 doi:10.1128/JVI.01104-12
- 146 Pijlman, G.P. (2014) Flavivirus RNAi suppression: decoding non-coding RNA. *Curr. Opin Virol.* **7**, 55–60 doi:10.1016/j.coviro.2014.04.002
- 147 Kakumani, P.K., Ponia, S.S., S, R.K., Sood, V., Chinnappan, M., Banerjee, A.C. et al. (2013) Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. *J. Virol.* **87**, 8870–8883 doi:10.1128/JVI.02774-12
- 148 Moon, S.L., Dodd, B.J.T., Brackney, D.E., Wilusz, C.J., Ebel, G.D. and Wilusz, J. (2015) Flavivirus sRNA suppresses antiviral RNA interference in cultured cells and mosquitoes and directly interacts with the RNAi machinery. *Virology* **485**, 322–329 doi:10.1016/j.viro.2015.08.009
- 149 Iizasa, H., Wulff, B.-E., Alla, N.R., Maragkakis, M., Megraw, M., Hatzigeorgiou, A. et al. (2010) Editing of Epstein-Barr virus-encoded BART6 microRNAs controls their dicer targeting and consequently affects viral latency. *J. Biol. Chem.* **285**, 33358–33370 doi:10.1074/jbc.M110.138362
- 150 Li, W.-X., Li, H., Lu, R., Li, F., Dus, M., Atkinson, P. et al. (2004) Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. *Proc. Natl Acad. Sci. U.S.A.* **101**, 1350–1355 doi:10.1073/pnas.0308308100
- 151 Soldan, S.S., Plassmeyer, M.L., Matukonis, M.K. and Gonzalez-Scarano, F. (2005) La Crosse virus nonstructural protein NSs counteracts the effects of short interfering RNA. *J. Virol.* **79**, 234–244 doi:10.1128/JVI.79.1.234-244.2005
- 152 Sullivan, C.S. and Ganem, D. (2005) A virus-encoded inhibitor that blocks RNA interference in mammalian cells. *J. Virol.* **79**, 7371–7379 doi:10.1128/JVI.79.12.7371-7379.2005
- 153 Fabozzi, G., Nabel, C.S., Dolan, M.A. and Sullivan, N.J. (2011) Ebola virus proteins suppress the effects of small interfering RNA by direct interaction with the mammalian RNA interference pathway. *J. Virol.* **85**, 2512–2523 doi:10.1128/JVI.01160-10
- 154 Haasnoot, J., de Vries, W., Geutjes, E.-J., Prins, M., de Haan, P. and Berkhout, B. (2007) The Ebola virus VP35 protein is a suppressor of RNA silencing. *PLoS Pathog.* **3**, e86 doi:10.1371/journal.ppat.0030086
- 155 Brodersen, P. and Voinnet, O. (2006) The diversity of RNA silencing pathways in plants. *Trends Genet.* **22**, 268–280 doi:10.1016/j.tig.2006.03.003
- 156 Margis, R., Fusaro, A.F., Smith, N.A., Curtin, S.J., Watson, J.M., Finnegan, E.J. et al. (2006) The evolution and diversification of Dicers in plants. *FEBS Lett.* **580**, 2442–2450 doi:10.1016/j.febslet.2006.03.072
- 157 Wang, X.-B., Jovel, J., Udornporn, P., Wang, Y., Wu, Q., Li, W.X. et al. (2011) The 21-nucleotide, but not 22-nucleotide, viral secondary small interfering RNAs direct potent antiviral defense by two cooperative Argonautes in *Arabidopsis thaliana*. *Plant Cell.* **23**, 1625–1638 doi:10.1105/tpc.110.082305
- 158 Andika, I.B., Maruyama, K., Sun, L., Kondo, H., Tamada, T. and Suzuki, N. (2015) Differential contributions of plant Dicer-like proteins to antiviral defences against potato virus X in leaves and roots. *Plant J.* **81**, 781–793 doi:10.1111/tpj.12770
- 159 Czech, B., Malone, C.D., Zhou, R., Stark, A., Schlingeheyde, C., Dus, M. et al. (2008) An endogenous small interfering RNA pathway in *Drosophila*. *Nature* **453**, 798–802 doi:10.1038/nature07007
- 160 Wang, Z., Wu, D., Liu, Y., Xia, X., Gong, W., Qiu, Y. et al. (2015) *Drosophila*Dicer-2 has an RNA interference-independent function that modulates Toll immune signaling. *Sci. Adv.* **1**, e1500228 doi:10.1126/sciadv.1500228
- 161 Sabin, L.R., Zheng, Q., Thekkat, P., Yang, J., Hannon, G.J., Gregory, B.D. et al. (2013) Dicer-2 processes diverse viral RNA species. *PLoS ONE* **8**, e55458 doi:10.1371/journal.pone.0055458
- 162 Bogerd, H.P., Skalsky, R.L., Kennedy, E.M., Furuse, Y., Whisnant, A.W., Flores, O. et al. (2014) Replication of many human viruses is refractory to inhibition by endogenous cellular MicroRNAs. *J. Virol.* **88**, 8065–8076 doi:10.1128/JVI.00985-14
- 163 Hussain, M. and Asgari, S. (2014) MicroRNA-like viral small RNA from dengue virus 2 autoregulates its replication in mosquito cells. *Proc. Natl Acad. Sci. U.S.A.* **111**, 2746–2751 doi:10.1073/pnas.1320123111
- 164 Hussain, M., Torres, S., Schnettler, E., Funk, A., Grundhoff, A., Pijlman, G.P. et al. (2012) West Nile virus encodes a microRNA-like small RNA in the 3' untranslated region which up-regulates GATA4 mRNA and facilitates virus replication in mosquito cells. *Nucleic Acids Res.* **40**, 2210–2223 doi:10.1093/nar/gkr848
- 165 Arur, S., Ohmachi, M., Nayak, S., Hayes, M., Miranda, A., Hay, A. et al. (2009) Multiple ERK substrates execute single biological processes in *Caenorhabditis elegans* germ-line development. *Proc. Natl Acad. Sci. U.S.A.* **106**, 4776–4781 doi:10.1073/pnas.0812285106
- 166 Drake, M., Furuta, T., Suen, K.M., Gonzalez, G., Liu, B., Kalia, A. et al. (2014) A requirement for ERK-Dependent Dicer phosphorylation in coordinating oocyte-to-embryo transition in *C. elegans*. *Dev. Cell* **31**, 614–628 doi:10.1016/j.devcel.2014.11.004

- 167 Chiosea, S., Jelezcova, E., Chandran, U., Luo, J., Mantha, G., Sobol, R.W. et al. (2007) Overexpression of Dicer in precursor lesions of lung adenocarcinoma. *Cancer Res.* **67**, 2345–2350 doi:10.1158/0008-5472.CAN-06-3533
- 168 Karube, Y., Tanaka, H., Osada, H., Tomida, S., Tatematsu, Y., Yanagisawa, K. et al. (2005) Reduced expression of Dicer associated with poor prognosis in lung cancer patients. *Cancer Sci.* **96**, 111–115 doi:10.1111/j.1349-7006.2005.00015.x
- 169 Merritt, W.M., Lin, Y.G., Han, L.Y., Kamat, A.A., Spannuth, W.A., Schmandt, R. et al. (2008) Dicer, Drosha, and outcomes in patients with ovarian cancer. *N. Eng. J. Med.* **359**, 2641–2650 doi:10.1056/NEJMoa0803785
- 170 Pampalakis, G., Diamandis, E.P., Katsaros, D. and Sotiropoulou, G. (2010) Down-regulation of dicer expression in ovarian cancer tissues. *Clin. Biochem.* **43**, 324–327 doi:10.1016/j.clinbiochem.2009.09.014
- 171 Sand, M., Gambichler, T., Skrygan, M., Sand, D., Scola, N., Altmeyer, P. et al. (2010) Expression levels of the microRNA processing enzymes Drosha and dicer in epithelial skin cancer. *Cancer Investig.* **28**, 649–653 doi:10.3109/07357901003630918
- 172 Dedes, K.J., Natrajan, R., Lambros, M.B., Geyer, F.C., Lopez-Garcia, M.A., Savage, K. et al. (2011) Down-regulation of the miRNA master regulators Drosha and Dicer is associated with specific subgroups of breast cancer. *Eur. J. Cancer* **47**, 138–150 doi:10.1016/j.ejca.2010.08.007
- 173 Chiosea, S., Jelezcova, E., Chandran, U., Acquafondata, M., McHale, T., Sobol, R.W. et al. (2006) Up-regulation of dicer, a component of the MicroRNA machinery, in prostate adenocarcinoma. *Am. J. Pathol.* **169**, 1812–1820 doi:10.2353/ajpath.2006.060480
- 174 Kaul, D. and Sikand, K. (2004) Defective RNA-mediated c-myc gene silencing pathway in Burkitt's lymphoma. *Biochem. Biophys. Res. Commun.* **313**, 552–554 doi:10.1016/j.bbrc.2003.12.002
- 175 Klein, S., Lee, H., Ghahremani, S., Kempert, P., Ischander, M., Teitell, M.A. et al. (2014) Expanding the phenotype of mutations in DICER1: mosaic missense mutations in the RNase IIIb domain of *DICER1* cause GLOW syndrome. *J. Med. Gen.* **51**, 294–302 doi:10.1136/jmedgenet-2013-101943
- 176 Kim, Y.-K., Kim, B. and Kim, V.N. (2016) Re-evaluation of the roles of *DROSHA*, *Exportin 5*, and *DICER* in microRNA biogenesis. *Proc. Natl Acad. Sci. U. S.A.* **113**, E1881–E1889 doi:10.1073/pnas.1602532113
- 177 Fukunaga, R., Colpan, C., Han, B.W. and Zamore, P.D. (2014) Inorganic phosphate blocks binding of pre-miRNA to Dicer-2 via its PAZ domain. *EMBO J.* **33**, 371–384 doi:10.1002/embj.201387176
- 178 Hellwig, S. and Bass, B.L. (2008) A starvation-induced noncoding RNA modulates expression of Dicer-regulated genes. *Proc. Natl Acad. Sci. U.S.A.* **105**, 12897–12902 doi:10.1073/pnas.0805118105