MicroRNAs (miRNAs) are small regulatory noncoding RNAs that are generated in the canonical RNA interference (RNAi) pathway. drosha, DiGeorge syndrome critical region 8 (DGCR8) and Dicer are key players in miRNA biogenesis. Argonaute (Ago) proteins bind to miRNAs and are guided by them to find messenger RNA targets and carry out post-transcriptional silencing of protein-coding genes. Recently, emerging evidence suggests that RNAi factors have a range of noncanonical functions that are beyond miRNA biogenesis. These functions pertain to various biological processes, such as development, transcriptional regulation, RNA processing and maintenance of genome integrity. Here, we review recent literature reporting miRNA-independent, noncanonical functions of Drosha, DGCR8, Dicer and Ago proteins and discuss the importance of these functions.

Keywords: Argonaute proteins; Dicer; Drosha and DGCR8

The human genome is pervasively transcribed [1,2] even though only a small proportion (~3%) of it codes for proteins [3]. The question of whether the nonprotein-coding component of our genome, besides ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs), is functional, has been receiving increasing attention. Recent research has provided insights into the functions of some noncoding transcripts. For example, certain noncoding RNAs (ncRNAs) are processed into small ncRNAs, which guide RNA-binding proteins to regulate gene expression in a process commonly known as RNA interference (RNAi). RNAi is best demonstrated by miRNAs, a group of small ncRNAs of ~22 nucleotides in length, that guide effectors to messenger RNAs (mRNAs) targets via complementary binding, leading to downregulation of target transcripts. miRNA-mediated gene regulation is regarded as a post-transcriptional gene silencing (PTGS) mechanism because it reduces protein levels by mRNA cleavage or translational inhibition [4].

The maturation of miRNA involves three central factors, namely Drosha, DiGeorge syndrome critical region 8 (DGCR8) and Dicer [5]. In addition, Argonaute (Ago) proteins directly associate with mature miRNAs to perform PTGS. Primary transcripts (pri-miRNAs) are generated from miRNA-encoding loci by RNA polymerase II (RNAPII) activity [6] and undergo nuclear processing by Microprocessor, a heterotrimeric complex that comprises one molecule of Drosha and two molecules of DGCR8. Pre-miRNAs are exported into the cytoplasm by Exportin 5 (XPO5), where they are processed by Dicer, with its partner protein TRBP into ~22-nucleotide-long miRNA duplexes. A miRNA duplex is subsequently loaded onto one of the Ago family proteins, together with several auxiliary proteins from the GW182 family, to form the RNA-induced silencing complex (RISC). The biological active (guide) strand of miRNA is retained, while the other (template) strand is discarded and degraded [7]. The miRNA guides the
RISC to specific target mRNAs by base-pairing with sequences in the transcripts. The key sequences within miRNAs for target recognition are called the ‘seed sequences’. The process is complete when the target mRNA is degraded or dissociated from the translation machinery resulting in no protein production. The canonical miRNA pathway is illustrated in Fig. 1.

A recent study re-evaluated the contribution of Drosha, XPO5 and Dicer in miRNA biogenesis. By using the colorectal cancer cell line HCT116, the authors concluded that Drosha and Dicer are crucial for miRNA biogenesis, with 96.5% and 96% of miRNA species decreased to less than 10% of their initial levels, when the proteins are knocked out respectively. Interestingly, upon deletion of XPO5, only 29% of miRNA levels decreased, indicating that XPO5 is dispensable for the maturation of a substantial proportion of miRNAs [8].

RNAi factors have been extensively studied due to the importance of miRNA-mediated PTGS, which is involved in many biological processes including development and pathogenesis [5]. Recently, it has become apparent that Drosha, DGCR8, Dicer and Ago proteins, in addition to their roles in miRNA pathway, have functions that are independent of miRNA biogenesis, and these functions are related to various biological processes, including development, transcriptional regulation, RNA processing and maintenance of genome integrity. Here, we discuss the emerging noncanonical functions of miRNA pathway enzymes in various biological processes.

Various roles of Drosha and DGCR8

Drosha and DGCR8 together form the Microprocessor complex and are crucial for the initial step of miRNA maturation: nuclear processing of pri-miRNAs. Drosha is a type III RNase, which has two tandem RNase III domains that recognise stem loop structures, and is the catalytic subunit of the Microprocessor complex [9]. DGCR8 is a cofactor of Drosha, which interacts with and stabilises Drosha through its C-terminal tail domain and its double-stranded RNA (dsRNA)-binding domains [10]. However, according to recent reports [11], the functions of Drosha and DGCR8 may not be limited to pri-miRNA processing (Fig. 2).

Drosha and DGCR8 in mRNA destabilisation

Even though pri-miRNAs are primary targets of Drosha, Drosha can also target other hairpin-containing transcripts. Genome-wide mapping of nascent

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**Fig. 1.** Canonical microRNA pathway. Schematic diagram representing the canonical microRNA (miRNA) pathway. The pathway starts with miRNA-coding loci being transcribed by RNA polymerase II (RNA Pol II) into primary miRNA transcripts (pri-miRNA), followed by recognition by the Microprocessor complex, constituted by Drosha and DiGeorge syndrome critical region B (DGCR8). After being cropped by the Microprocessor into miRNA precursors (pre-miRNA), the pre-miRNAs are exported into the cytoplasm by Exportin 5 (XPO5), catalysed by RAN-GTP hydrolysis. Once in the cytoplasm, the pre-miRNA is bound by Dicer, with its partner protein TRBP, and undergoes processing into miRNA duplex. The duplex is then loaded onto Argonaute (Ago) proteins and other proteins such as TNRC6A/B/C to form the RNA-induced silencing complex (RISC), where one of its strands is discarded and degraded. The RISC is then guided by the biological active strand to messenger RNA (mRNA) targets, which leads to gene silencing via mRNA degradation or translational inhibition.
Drosha substrates using formaldehyde cross-linking immunoprecipitation and sequencing (fCLIP-seq) revealed that Drosha also processes noncanonical substrates, suggesting that it may have functions other than miRNA maturation. Some of these hairpin structures, which are targets of Drosha, are found in exons of mRNAs. The levels of such host transcripts are upregulated upon depletion of Drosha [12]. Another striking example is that the Microprocessor complex regulates the levels of DGCR8 mRNA post-transcriptionally in the nucleus by cleaving the hairpin localised in the 5' UTR of the transcript. This mechanism is proposed to be important for the homeostatic regulation of miRNA biogenesis [13,14].

There are also reports of Drosha-mediated mRNA destabilisation in development. Loss of the Microprocessor complex leads to loss of stem cell properties and premature differentiation in murine forebrain neural progenitors. Drosha directly destabilises hairpin-containing Neurogenin 2 (Ngn2) mRNA and thus lowering the levels of Ngn2, a neurogenic transcription factor [15]. Another study has shown that the ablation of DGCR8 impairs corticogenesis in mice more pronouncedly than Dicer depletion, a phenomenon caused by dysregulation of the T-box brain protein 1 (Tbr1) transcript, which has predicted hairpin structures in its coding sequence. However, the authors did not observe a change in Ngn2 levels and proposed the existence of distinct Microprocessor complexes with different target affinities [16]. Drosha and DGCR8 are therefore important for maintaining the self-renewal property of murine neural progenitors, which is achieved by destabilisation of transcripts that encode differentiation factors. In addition to neurogenesis, Drosha is implicated in myeloid development wherein it directly destabilises Myosin Light Chain 9 (Myl9) mRNA and target of Drosha 1 (Todr1) mRNA, whose protein products are inhibitors of myelopoiesis and are essential for the development of dendritic cells [17].

Microprocessor-mediated regulation of retrotransposons in human cells has also been reported [18]. DGCR8 binds to RNAs derived from human long interspersed element 1 (LINE-1), Alu and SINE-VNTR-Alus retrotransposons. The Microprocessor complex cleaves the 5' UTR of LINE-1 mRNAs and regulates LINE-1 and Alu transpositions in vivo,
evidenced by increase in specific LINE-1 mRNAs and retrotransposition of LINE-1 and Alu elements upon expression of dominant negative forms of Drosha and DGCR8 [18].

**Drosha and DGCR8 in transcriptional regulation and RNA processing**

Both Drosha and DGCR8 are associated with promoter-proximal regions of human protein-coding genes. As evidenced by immunoprecipitation, Drosha interacts both with CBP80, an RNA-binding protein, and RNAPII via its N-terminal tail to positively regulate gene expression. Knocking down Drosha impairs RNAPII activity [19]. Using HIV-1 promoter as a model, Wagschal et al. showed that the Microprocessor complex helps initiate RNAPII pausing and premature transcription termination at the promoter both by recruiting termination factors Setx and Xrn2, as well as exosomal component Rrp6 and by processing of the transactivation response element (TAR). TAR is a stem-loop RNA, generated from the transcription of the long terminal repeat (LTR) of the HIV-1 promoter. The Microprocessor complex cleaves TAR, providing a substrate for Rrp6, which produces small RNAs derived from TAR. These small RNAs, by an unknown mechanism, repress the activity of the LTR [20]. Furthermore, Dhir et al. showed, by characterising the termination of the long ncRNA containing miR-122, that the transcription termination is not achieved by canonical cleavage and polyadenylation (CPA) but rather through Microprocessor-mediated cleavage of a hairpin in the transcript. Genome-wide analysis of HeLa cells demonstrated that the Microprocessor complex terminates transcription of most long ncRNAs that contain miRNA sequences, while knocking down Dicer does not affect transcription termination, indicating that the mechanism is miRNA-independent [21].

Additionally, Drosha is involved in regulating alternative splicing. Drosha binds to the hairpin-containing exon 5 of the *eukaryotic Translation Initiation Factor 4H* (*eIF4H*) mRNA and cleaves the transcript at the binding site. The primary function of Drosha binding to *eIF4H* mRNA, however, may not be destabilisation of the transcript since overexpression of Drosha does not reduce the levels of *eIF4H* transcript. Instead, Drosha enhances the splicing of exon 5 of the transcript, independent of its cleavage function [22]. The Microprocessor complex also targets an evolutionarily conserved mammalian hairpin embedded in the exon-intron junction of *DROSHA* mRNA for regulation of alternative splicing. In this case, the complex is recruited to the splice site between exon 7 and intron 7 of *DROSHA* transcript, creating a steric hindrance for U1 small nuclear ribonucleoprotein (U1 snRNP), a component of the spliceosome, and thus promotes skipping of the exon [23].

**Drosha and DGCR8 in maintenance of genome integrity**

In 2012, Francia et al. [24] reported that both Drosha and Dicer are important for efficient activation of the DNA damage response (DDR) in human, mouse and zebrafish models. The DDR is a signal transduction pathway for sensing and detecting DNA damage, and instigating cellular response to DNA damage in order to safeguard genome integrity [25]. Depletion of Drosha or Dicer by small-interfering RNAs (siRNAs) impairs formation of DDR foci, which contain auto-phosphorylated ATM, phosphorylated substrates of ATM and ATR (pS/TQ) and MDC1, in irradiated cells. Absence of RNAi factors also attenuates DNA-damage-induced cell cycle arrests at G1/S and G2/M checkpoints. A similar phenotype is observed in irradiated HeLa cells that were permeabilised and treated with RNase A, which digests RNA nonspecifically, suggesting that loss of RNAs can affect DDR. The authors showed that an RNA fraction of 20–35 nucleotides in length, which are proposed to be products of Drosha and/or Dicer, is responsible for regulating efficient DDR foci formation. These small RNAs, termed DNA damage response RNAs (DDRNAs), are derived from sites where DNA damage occurs. The addition of locus-specific synthetic RNAs triggers reformation of 53BP1, an important downstream repair factor, in RNase A-treated NIH 2/4 mouse cells, in which DNA damage is introduced to specific loci through the expression of a restriction enzyme [24]. It is important to note that, despite drawing a great deal of attention, RNA-dependent DDR remains controversial, as we will discuss below. A recent study expands on the role of Drosha in DDR by showing that depletion of Drosha affects DNA repair modulated by both homologous recombination and nonhomologous end joining (NHEJ), as measured by number of 53BP1, BRCA1 and Rad51 foci, and that Drosha is required relatively early after induction of double-strand breaks (DSBs). Furthermore, DNA : RNA hybrids, also called R-loops, are formed around DNA breaks in a Drosha-dependent manner and are important for DNA repair. However, molecular details of R-loop-dependent DDR remain largely elusive [26].

Drosha also exhibits antiviral functions in human cells infected by Sindbis virus (SINV), a positive-
stranded RNA virus, regardless of its association with DGCR8 and miRNA maturation. Drosha impedes the replication of SINV by directly inhibiting RNA-dependent RNA polymerase [27]. Another independent study also found that Drosha translocates to the cytoplasm in response to infection of various RNA viruses and cytoplasmic Drosha cleaves viral RNA, shown by in vitro experiments [28].

DGCR8 is also required for DDR. Specifically, DGCR8 promotes nucleotide excision repair (NER) in human cells through a mechanism that is independent of its RNA processing activity and association with Drosha. In response to UV radiation, Serine (S)153 of DGCR8 is phosphorylated by c-jun N-terminal kinases. Phosphorylated DGCR8 mediates cellular resistance and repair of UV-induced DNA damage via transcription-coupled NER [29].

Various roles of Dicer

Dicer is best known for its canonical function in the generation of mature miRNA duplexes from pre-miRNAs, endogenous small-interfering RNAs (endo-siRNAs) and long dsRNAs [30]. The cleavage of pre-miRNAs in mammals with a single DICER gene is fine-tuned by partner proteins, such as TRBP (HIV trans-activating response RNA-binding protein) and PACT (protein activator of PKR), which help to determine the cleavage site of some pre-miRNAs and thus the length of the resulting miRNAs [31]. Endo-siRNAs are derived from transposable elements (TE), convergent transcripts and self-complementary hairpin elements in worms, flies, mice and humans [32]. These two types of endogenous small RNAs are involved in gene silencing through establishment of repressive epigenetic marks by recruiting methyltransferases and acetyltransferases to the sites where they originate from [4,32].

Dicer is conventionally thought to be a cytoplasmic protein that serves as a processor for pre-miRNAs. However, an increasing body of evidence indicates that functional Dicer can localise to the nucleus [33–38]. A noncanonical nuclear localisation signal (NLS) was detected within the C-terminal dsRNA binding domain (dsRBD) of Dicer. The dsRBD of Dicer also associates with nuclear transport receptors Impβ, Imp7 and Imp8. However, in the context of full length Dicer, the dsRBD NLS may be masked either by other domains of the protein or associating molecules [33]. A study focusing on the murine Dicer protein has challenged the notion of nuclear Dicer and asserted that an endogenously HA-tagged Dicer is exclusively cytoplasmic protein in developing and mature mice [39].

Recently, Burger et al. addressed the question using super-resolution microscopy followed by 3D reconstitution and sensitive biochemical assays and demonstrated that a small fraction (~5%) of HA-tagged murine Dicer indeed localises in the nucleus. Upon DNA damage, HA-tagged murine Dicer is phosphorylated and localises in the nucleus [36]. As we will discuss below, the nuclear localisation of Dicer is important for many of its noncanonical functions (Fig. 3).

Dicer in biogenesis of tRNA-derived fragments

Apart from miRNAs and endo-siRNAs, Dicer has been implicated in the generation of a group of small RNAs that are derived from tRNAs, called tRNA-derived fragments (tRFs) in human cells, mice and zebrafish [40–42].

tRFs are classified into three groups according to the position of cleavage. 5’ tRFs and 3’ tRFs are derived from the 5’ and 3’ ends of mature tRNA transcripts, which are characterised by the addition of the trinucleotide sequence CCA at the 3’ terminus. 3’-U tRFs refer to the tRFs that originate from tRNA precursors that have poly-U residues at the 3’ terminus [43]. Depletion of Dicer in HeLa and HEK293 cells by RNAi affects the steady-state levels of an RNA fragment that corresponds to the 5’ end of tRNA^Gin. In vitro experiments also showed that recombinant Dicer can produce the aforementioned tRF [40].

Another study reported the expression of a miRNA-like tRF, called CU1276, in developing and mature B cells, and the tRF can impair expression of mRNAs in a sequence-specific manner. Biogenesis of CU1276 is Dicer-dependent and it associates with all four human Ago proteins. Expression of an exogenous CU1276 hairpin represses endogenous levels of single-stranded DNA binding protein RPA1 in 293T and lymphoma B cells by miRNA-like PTGS. CU1276 expression in Burkitt Lymphoma cells reduces cellular proliferation and increases sensitivity to etoposide-induced DNA damage through an RPA1-dependent mechanism [44].

Evidence for Dicer-dependent tRFs is not limited to human cells. In zebrafish, at least two tRFs that are derived from 5’ ends of tRNAs, 5’ tRF-Glu^CTC and 5’ tRF-Pro^CGG, can be cleaved by Dicer in vitro. RNA-Shapes, a software that predicts RNA secondary structure, estimated that 5% of tRNA^Glu and 50% of tRNA^Pro in zebrafish are likely to fold into long hairpin structures, indicating that they can be recognised by Dicer for processing [42]. In Arabidopsis thaliana, 5’
tRF accumulation is observed in ddm1 mutants, which lack the Decrease in DNA Methylation 1 (DDM1) chromatin remodelling protein. In dcr1/ddm1 double mutants, wherein Dicer-like1 (DCL1) protein is knocked out, 5’ tRF accumulation reduces, indicating that DCL1 is important for its biogenesis. These 5’ tRFs have been found to protect gametes from TE reactivation by targeting and repressing transcripts originating from TE in ddm1 background [45]. Analysis of small RNA sequencing in mouse embryonic stem cells revealed tRFs that map to 3’ end of the isoleucine tRNA gene transcript. These tRFs can potentially fold into a long hairpin, the canonical RNA structure recognised by Dicer [46]. Furthermore, a study found that Lupus autoantigen La, an RNA-binding protein that stabilises RNA polymerase III (RNAPIII), facilitate proper folding of some tRNAs, preventing them from entering the miRNA pathway, i.e. recognised by XPO5, which can export into the cytoplasm for Dicer processing and Ago loading. However, in the presence of La, a specific isoleucine pre-tRNA still can produce both a functional tRNA and a miRNA-like small RNA that can potentially lead to gene regulation [47].

However, a number of studies claim that Dicer is dispensable for tRF biogenesis [48–50]. Meta-analysis of sequencing data from mice, Drosophila and Schizosaccharomyces pombe revealed that mutation in Dicer does not significantly affect tRF levels [49]. Another study looking at A. thaliana mutants that lack Dicer-like proteins also supports the idea that Dicer may be dispensable for tRF biogenesis in plants [50]. It is tempting to speculate that Dicer-dependent tRFs processing has evolved in certain organisms as a mechanism with physiological relevance.

Dicer in transcriptional regulation and RNA processing

Does detection of Dicer in the nucleus point towards a functional role of nuclear Dicer? Immunoprecipitation of both Dicer and RNAPIII from nuclear extracts of HeLa cells shows association between Dicer and

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**Fig. 3.** Noncanonical functions of Dicer. Schematic diagram representing the noncanonical functions of Dicer (blue ovals) in biogenesis of transfer RNA-derived fragments (tRFs), transcriptional regulation and RNA processing, and maintenance of genome integrity. tRNA, transfer RNA; dsRNA, double-stranded RNA; RNAPII, RNA polymerase II; G9a, euchromatic histone-lysine N-methyltransferase 2; H3K9me2, dimethylation of histone H3 at lysine 9; ETNK1, ethanolamine kinase 1; diRNAs, damage-induced RNAs; UV, ultraviolet; MMSET, Multiple myeloma set domain; H4K20me2, dimethylation of histone H4 at lysine 20; XPA, Xeroderma Pigmentosum Group A-Complementing protein.
RNAPII, which is substantially affected in the absence of dsRNA. ChIP-sequencing (seq) data revealed that Dicer interacts with multiple human loci at the chromatin level. Knockdown of Dicer leads to enhanced transcription of sense and antisense transcription of four tested Dicer-associating loci and enrichment in repressive trimethylation of histone H3 at lysine 9 (H3K9me3) marks are detected over these loci, suggesting Dicer-dependent transcriptional gene silencing (TGS). Furthermore, knocking down Dicer leads to an accumulation of dsRNAs, which originate from Dicer-associating loci, in the nucleus in a miRNA-independent manner. Unprocessed dsRNA is eventually exported into the cytoplasm where it activates interferon response resulting in apoptotic cell death [34].

In Drosophila melanogaster, Dicer-2 (Dcr2) preferentially associates with transcriptionally active loci and depletion of Dcr2 changes the behaviour of RNAPII in the promoter-proximal region of several selected genes. Particularly, Dcr2 participates in transcriptional control during heat-shock response [51]. Cytoplasmic polyadenylation of Toll mRNA in D. melanogaster is carried out through a noncanonical mechanism. Recently, Dcr2 has been shown to be necessary for this process. Dcr2 interacts with Wispy, the cytoplasmic poly-A polymerase and promotes cytoplasmic polyadenylation of Toll mRNA and r2d2 mRNA [52].

Dicer regulates alternative cleavage and polyadenylation (APA), which leads to generation of mRNA isoforms based on the choice of cleavage and polyadenylation (CPA) sites. Dicer knockdown changes the APA profiles of a subset of transcripts in the cytoplasm (3%) and nucleus (6%). ChIP analyses revealed that Dicer associates with proximal CPA site of ETNK1, whose proximal APA isoform reduces when Dicer is depleted, and induces repressive epigenetic mark, dimethylation of histone H3 lysine 9 (H3K9me2) through the action of G9a histone methyltransferase. Heterochromatin induced by nuclear Dicer slows down RNAPII and promotes the proximal CPA site recognition [37].

**Dicer in maintenance of genome integrity**

Dicer is highly expressed in developing cerebellum in mice and deletion of Dicer leads to loss of cerebellar progenitor cells. Moreover, Dicer deficiency leads to accumulation of DNA damage in these cells, causing apoptosis. Similar observations were made in rapidly proliferating, Dicer-deficient medulloblastoma cells in which the cells are more sensitive to DNA damaging agents and exhibit increased cell death when compared to wild-type tumours [53]. This study indicates that Dicer is involved in DNA repair.

As discussed above, inactivation of Dicer and Drosha leads to inefficient DDR and the mechanism by which these two proteins regulate DDR involves Dicer/Drosha-dependent small RNA molecules, DDRNAs [24]. Coincidentally, another independent study also reported Dicer involvement in DSB repair in A. thaliana wherein DSB repair efficiency is reduced in dc12, del3 and del4 mutants. Deep sequencing uncovered accumulation of small damage-induced RNAs (diRNAs) that are derived from both sense and antisense strands of the sequence around the restriction sites where DSB were introduced. A similar phenomenon was observed in human U2OS cell line [54]. Another study observed an upregulation of Dicer upon DSB introduction. Knocking down Dicer increases SIRT7 binding and decreases the level of acetylation of histone H3 at lysine 18 (H3K19Ac) at DSB sites, which in turn repress the recruitment of NHEJ factors, 53BP1, Ku70 and Ku80, to DSBs while overexpression of Dicer enhances NHEJ. Remarkably, Dicer protein expression in patient colon cancer tissues is inversely proportional to chemosensitivity of the tissues and tumour progression [55]. These evidences establish a direct connection between Dicer and DDR. A more recent study showed that Dicer is phosphorylated at position S1016 in the platform-PIWI/Argonaute/Zwille-connector helix cassette upon induction of DSBs and recruited to DSB sites to facilitate DDR. Dicer deficiency leads to impaired recruitment of repair factors MDC1 and 53BP1 [35]. This is the first report on phosphorylation-induced nuclear localisation of Dicer in human cells.

Nuclear Dicer has also been implicated in transcription-independent, global-genomic NER, in which it mediates the recruitment of methyltransferase multiple myeloma set domain (MMSET) to the DNA damage site. MMSET induces demethylation of histone H4 at lysine 20 (H4K20me2), which helps to recruit XPA, a crucial factor for NER [56]. In a separate report by the same research group, Dicer facilitates chromatin decondensation in NER, allowing for repair factors to bind. ZRF-1, the H2A-ubiquitin-binding protein, recruits Dicer to the chromatin and this interaction is RNA-dependent but independent of its ribonuclease activity. Dicer and ZRF-1 mediates chromatin decondensation in a PARP-1-dependent manner [57].

Mammalian Dicer also takes part in antiviral immunity via a mechanism in which Dicer processes viral dsRNA precursors into siRNAs that suppresses viral activity. These viral-derived siRNAs were not detected until recently because the viruses have evolved.
strategies to counteract such antiviral immunity by suppressing RNAi [58–61].

**Various roles of Ago proteins**

Ago proteins bind to small RNAs and are characterised by N domain, PAZ (PIWI-Argonaute-Zwille), MID (middle) and PIWI (P-body-induced wimpy testes) domains. Ago proteins are mediators of miRNA function through forming the RISC complex with the small RNAs [62]. The miRNA duplex is first loaded onto an Ago protein, a process which requires the ATP-dependent Hsc70/Hsp90 chaperone machinery [63]. The duplex is then unwound through the action of the N domain, leaving the biological active strand in the complex, thus forming mature RISC [64]. Among all four human Ago proteins, Ago1-4, only Ago2 was thought to have slicer activity [65,66], which mediates endonucleolytic cleavage of mRNA [4]. Recently, Ago3 has been reported to also possess slicer ability but such activity is substrate-dependent [67]. Ago proteins have extensive properties that are not limited to miRNA-mediated RNAi in the cytoplasm (Fig. 4).

**Ago proteins in functions of tRNA-derived fragments**

Although controversial, some tRFs have been reported to be products of Dicer processing. Hence, many studies also looked at the association between tRFs and Ago proteins. Selected Dicer-dependent tRFs co-immunoprecipitate (IP) with all four FLAG-tagged Ago proteins while selected RNaseZ-dependent, Dicer-independent tRFs exhibit a preference for FLAG-tagged Ago3 and 4 over FLAG-tagged Ago1 and 2. On the other hand, overexpression of Ago proteins increases the abundances of some tRFs, suggesting that Ago proteins may stabilise the tRNAs or tRFs directly or indirectly. Interestingly, transfection of exogenous DNA oligonucleotides that are complementary to RNase Z-dependent, Dicer-independent tRFs triggers robust gene silencing [41]. Another study found that both 3’ and 5’ tRFs strongly associate with Ago1, 3 and 4 but not with Ago2 by meta-analysing photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) data from HEK293 cells. Read counts for 3’ and 5’ tFs in Ago 1, 3 and 4 PAR-CLIP data are comparable to miRNAs, which are considered as canonical binding substrates for Ago proteins but read counts of tRFs in Ago2 PAR-CLIP are almost zero. Similar patterns were observed in independent Ago1 and Ago2 PAR-CLIP data sets in HEK293 cells [49,68,69].

A recent study reported that endogenous 3’ tRFs can regulate gene expression via a Dicer-independent but Ago-dependent pathway. These 3’ tRFs can base-pair with target transcripts in association with GW182 proteins-containing RISC. Induction of 3’ tRF through overexpression of parental tRNA leads to downregulation of endogenous target genes, as evidenced by RNA-seq [70]. This indicates that tRFs may bypass Dicer processing and directly associate with Ago proteins to execute their functions.

**Ago proteins in transcriptional regulation and RNA processing**

The nuclear localisation of Ago proteins in various organisms is well documented. Ago proteins carry out TGS in various organisms by inducing heterochromatin through recruitment of methyltransferases and acetyltransferases to the chromatin [71–75].

Apart from heterochromatin formation, Ago proteins are also involved in RNAi-independent transcriptional regulation and RNA processing mechanisms. *Drosophila* Ago2 preferentially associates with euchromatin [51,76] and interacts with core components of the transcriptional machinery [51]. Deletion of Ago2 dysregulates the transcriptional response towards heat shock. Deep sequencing of Ago2-associated small RNAs uncovered strong enrichment of small RNAs derived from sense and antisense strands of promoter regions, regions of heat shock and other genetic loci [51]. Additionally, Ago2 associates with the chromatin via physically interaction with CTCF/CP190 chromatin insulator and is required for the activity of Fab-δ insulator, independent of its catalytic activity [76]. Nuclear Ago2 in *Drosophila* was subsequently shown to participate in pre-mRNA splicing and transcriptional repression regardless of its catalysis activity. The sites, where splicing is affected upon deletion of Ago2, do not correlate with the loci associated with nuclear Ago2 from ChiP data. Therefore, the effect of Ago2 on pre-mRNA splicing may be mediated by a cofactor of Ago2. Ago2 also downregulates the genes it associates with and these target genes are also bound by Polycomb group, transcriptional repressor proteins [77].

A study on the genome-wide regulation of Ago1 in plants revealed that Ago1 binds to active genes at the chromatin through interaction with small RNAs and SWI/SNF chromatin remodelling complexes. Deletion of Ago1 results in lower occupancy of RNAPII at Ago1-associated loci, suggesting that Ago1 promotes gene expression by directly or indirectly recruiting the transcriptional machinery. When subjected to
hormones and stresses, plant Ago1 binds to stimulus-responsive genes and induce their expression [78].

Human Ago1 associates with RNAPII and promoters of actively transcribed genes and regulates expression of genes that are implicated in oncogenic pathways, such as cell cycle progression, growth and survival [79]. Ago1 and Ago2 can regulate alternative splicing, by inducing H3K9me3 on variant regions of genes, which consequently leads to RNAPII slowdown and spliceosome recruitment [80]. Ago2 binds specifically to actively transcribed tRNA genes and 5S rRNA gene but not to other RNAPIII binding sites. Moreover, it regulates gene expression in a tRNA gene insulator region on chromosome 17 and RNAPII-associated genes flanking active tRNA genes, in a miRNA-independent manner [81].

**Ago proteins in maintenance of genome integrity**

DiRNAs, which are small RNA products generated from the vicinity of DSB sites, are associated with Ago2. A study that investigated the relationship between Ago2 and DSB repair found that Ago facilitates the recruitment of Rad51 to DSB sites. DiRNA binding and catalytic activity of Ago2 are required for the recruitment of the Rad51 recombinase to DSBs but not the interaction between Ago2 and Rad51 [82]. Another group has found that diRNAs, alongside Ago2, facilitate recruitment of methyltransferase MMSET and acetyltransferase Tip60, which induce di- and trimethylation of histone H4 at lysine 20 (H4K20me2 and 3) and acetylation of histone H4 at lysine 16 (H4K16Ac) respectively. Similar to previous findings, the diRNA binding and catalytic activity of Ago2 is dispensable for interaction between MMSET and Tip60 and the DSBs but crucial for their recruitment [83].

By using high throughput sequencing of RNA isolated by cross-linking immunoprecipitation (HITS-CLIP), a recent study has shown that Ago2 binds multiples sites of HIV-1 pre-mRNA. Knocking out Ago2 or depletion of Ago1 results in decrease in intracellular and extracellular viral capsid production while Dicer
depletion does not, indicating that the mechanism by which Ago proteins positively regulate viral activity is independent of miRNA function [84].

Conclusions and perspectives

Here, we summarise the recent findings on the noncanonical functions of Drosha, DGCR8, Dicer and Ago proteins in various biological processes. This is by no means an exhaustive list of the noncanonical functions of all the proteins involved in the miRNA pathway. For instance, the partner protein of Dicer, TRBP, also possesses functions in biological pathways that are not related to miRNA, such as stress response and neuronal development [85,86]. The diversity of the noncanonical functions reflects the importance of these enzymes beyond the realm of miRNAs. In the following section, we will further discuss these noncanonical functions and interesting questions that can be pursued in the future.

Despite the importance of these miRNA pathway enzymes and their diverse cellular roles, there are reports on viable cell lines that completely lack expression of these proteins [8,27,84,87]. In Dicer-deficient mouse embryonic stem (ES) cells, viability of the cells is not compromised but the cells fail to differentiate. Chromatin modifications and DNA methylation of centromeric repeats are affected upon Dicer knockout in mouse ES cells, leading to accumulation of RNAs transcribed from the centromeric repeats [88]. Ablation of Dicer in early B cell progenitors also lowers their survival rate and deprives them of their differentiating capacity [89]. Bogard et al. reported the first human Dicer-deficient 293T-based cell line called NoDice, wherein all three copies of dicer gene are deleted. These cells proliferate much more slowly than their parental cell line and lose expression of virtually all miRNAs [87], similar to Drosha- and Dicer-knockout HCT116 cell lines [8]. These evidences point to the fact that Dicer and Drosha may not be essential for cell survival, but they are incredibly important to robustness and at least the differentiating capacity of cells. However, these studies mostly focus on the impact on miRNAs upon deletion of these enzymes, very few have looked at the physiological consequence from the angle of their noncanonical functions. For instance, one may ask the question of whether the deletion of Dicer or Drosha affects DDR in a more pronounced way.

The relationship between Drosha and DGCR8 is complex, as mentioned above. The Microprocessor complex can destabilise DGCR8 mRNA and create a feedback loop that regulates its own levels [13,14]. Normally, Drosha and DGCR8 act as a complex to generate pre-miRNAs in the nucleus but sometimes the proteins can also act independently to carry out their noncanonical functions, for instance, in the case of participation of DGCR8 in transcription-coupled NER [29]. Future studies should look carefully at the individual roles of Drosha and DGCR8 to better comprehend the relationship between these two enzymes in the context of their noncanonical functions.

The reports on DDRNAs and diRNAs have similarities, in which they both suggest an unexpected, direct role for small RNAs in DDR [90]. More recently, a study from the research group that discovered DDRNAs has shown that DDRNAs interact with dihncRNAs through RNA–RNA pairing to facilitate DDR focus formation and association with 53BP1 [91]. However, another study, which focused on the role of Drosha in DDR, failed to detect such small RNAs in small RNA-seq data from the cells where sequence-specific DSBs were introduced. Authors suggested that previously reported DDRNAs may come from DNA damage, induced in repetitive sequences or may be degradation products of highly overexpressed loci [26]. Inactivation of Dicer or Drosha and RNase A treatment impairs DDR activation in various biological systems [24]. However, there is no direct evidence demonstrating that the precursors of these small RNAs contain structures that are recognisable and can be processed by either Dicer or Drosha. It is also unclear whether Dicer and Drosha work together or individually to mediate the processing of these precursors, yielding damage-induced small RNAs. More research needs to be done to elucidate the molecular mechanisms underlying RNA-dependent DDR.

Is Dicer responsible for tRF biogenesis? As current data suggest, some tRNAs can fold into structures that are canonical Dicer targets and upon Dicer knockdown, levels of selected tRFs are reduced [40]. However, there are reports that showed otherwise: knocking down Dicer has no effect on most tRF levels [49,50]. Just like DDRNAs, it is important to understand whether tRNAs, which can fold into various structures according to secondary structure prediction software, can be recognised and processed by Dicer in vivo. Overwhelming evidence shows the association between tRFs and Ago proteins but so far, no report has elucidated the role of Ago proteins in tRF biological functions.

Most studies of Ago proteins focused on Ago1 and 2. Only recently, a report has claimed that human Ago3 has slicer activity, which depends on the nature of the guide RNAs [67]. Very little is known about the noncanonical functional roles of Ago3 and 4, especially their functions in the nucleus.
The two main characteristics of miRNA pathway enzymes that can help distinguish their noncanonical functions from canonical ones are subcellular localisation and mechanism of action. Drosha can translocate to the cytoplasm in response to viral infection [28]. Dicer is phosphorylated upon DNA damage and recruited to DSB sites for efficient DDR activation [35]. Dicer is also recruited to the chromatin upon UV radiation for facilitation of NER [56,57]. Ago proteins are found in both subcellular compartments. In the cytoplasm, their main function is to destabilise mRNAs through miRNA-mediated complementary binding. Ago proteins have extensive roles in the nucleus, ranging from TGS to recruitment of DNA repair factors and transcriptional machinery. In many cases of their noncanonical functions, the catalytic or endonucleolytic activities of miRNA pathway enzymes are not required. They may act as scaffolding proteins for recruitment of other protein complexes or simply directly interacting with other proteins to alter their functions. 

Drosha, DGCR8, Dicer and Ago proteins were put on the spotlight owing to their roles in miRNA biogenesis and function. However, gradually it becomes apparent that their roles in the miRNA pathway are just the tip of the iceberg of their functional repertoire. It is therefore important to see them as more than miRNA pathway enzymes and more holistically in future research so that we will not fail to capture the full extent of their functions when studying a particular pathway or phenomenon.

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References

1. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F et al. (2012) Landscape of transcription in human cells. Nature 489, 101–108.

2. Hangauer MJ, Vaughn IW and McManus MT (2013) Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genet 9, e1003569.

3. Alexander RP, Fang G, Rozowsky J, Snyder M and Gerstein MB (2010) Annotating non-coding regions of the genome. Nat Rev Genet 11, 559–571.

4. Jonas S and Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 16, 421–433.

5. Ha M and Kim VN (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15, 509–524.

6. Lee Y, Kim M, Han J, Yeom K-H, Lee S, Baek SH and Kim VN (2004) MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23, 4051–4060.

7. Kobayashi H and Tomari Y (2016) RISC assembly: coordination between small RNAs and Argonaute proteins. Biochim Biophys Acta - Gene Regul Mech 1859, 71–81.

8. Kim Y-K, Kim B and Kim VN (2016) Re-evaluation of the roles of DROSHA, Exportin 5, and DICER in microRNA biogenesis. Proc Natl Acad Sci USA 113, E1881–E1889.

9. Kwon SC, Nguyen TA, Choi YG, Jo MH, Hohng S, Kim VN and Woo JS (2016) Structure of human DROSHA. Cell 164, 81–90.

10. Nguyen TA, Jo MH, Choi Y-G, Park J, Kwon SC, Hohng S, Kim VN and Woo J-S (2015) Functional anatomy of the human microprocessor. Cell 161, 1374–1387.

11. Lee D and Shin C (2017) Emerging roles of DROSHA beyond primary microRNA processing. RNA Biol 15, 186–193.

12. Kim B, Jeong K and Kim VN (2017) Genome-wide mapping of DROSHA cleavage sites on primary microRNAs and noncanonical substrates. Mol Cell 66, 258–269.e5.

13. Han J, Pedersen JS, Kwon SC, Belair CD, Kim YK, Yeom KH, Yang WY, Haussler D, Blelow R and Kim VN (2009) Posttranscriptional Crossregulation between Drosha and DGCR8. Cell 136, 75–84.

14. Triboulet R, Chang H-M, LaPierre RJ and Gregory RI (2009) Post-transcriptional control of DGCR8 expression by the microprocessor. RNA 15, 1005–1011.

15. Knuckles P, Vogt MA, Lugert S, Milo M, Chong MMW, Hautbergue GM, Wilson SA, Littman DR and Taylor V (2012) Drosha regulates neurogenesis by controlling Neurogenin 2 expression independent of microRNAs. Nat Neurosci 15, 962–969.

16. Marinaro F, Marzi MJ, Hoffmann N, Amin H, Perlinzoli R, Nicola F, Nicassio F and De Pietri Tonelli D (2017) MicroRNA-independent functions of DGCR8 are essential for neocortical development and TBR1 expression. EMBO Rep 18, 603–618.

17. Johanson TM, Keown AA, Cmero M, Yeo JHC, Kumar A, Lew AM, Zhan Y and Chong MMW (2015) Drosha controls dendritic cell development by cleaving messenger RNAs encoding inhibitors of myelopoiesis. Nat Immunol 16, 1134–1141.
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18 Heras SR, Macias S, Plass M, Fernandez N, Cano D, Eyras E, Garcia-Perez JL and Caceres JF (2013) The microprocessor controls the activity of mammalian retrotransposons. Nat Struct Mol Biol 20, 1173–1183.
19 Gromak N, Dienstbier M, Macias S, Plass M, Eyras E, Caceres JF and Proudfoot NJ (2013) Drosha regulates gene expression independently of RNA cleavage function. Cell Rep 5, 1499–1510.
20 Wagschal A, Rousset E, Basavaraajia P, Contreras X, Harwig A, Laurent-Chabalier S, Nakamura M, Chen X, Zhang K, Meziane O et al. (2012) Microprocessor, Setx, Xrn2, and Rrp6 co-operate to induce premature termination of transcription by RNAPII. Cell 150, 1147–1157.
21 Dhir A, Dhir S, Proudfoot NJ and Jopling CL (2015) Microprocessor mediates transcriptional termination of long noncoding RNA transcripts hosting microRNAs. Nat Struct Mol Biol 22, 319–327.
22 Havens MA, Reich AA and Hastings ML (2014) Drosha promotes splicing of a pre-microRNA-like alternative exon. PLoS Genet 10, e1004312.
23 Lee D, Nam J-W and Shin C (2017) DROSHA targets its own transcript to modulate alternative splicing. RNA 23, 1035–1047.
24 Francia S, Michelini F, Saxena A, Tang D, de Hoon AE, Anelli V, Mione M, Carninci P and d’Adda di Fagagna F (2012) Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 488, 231–235.
25 Ciccia A and Elledge SJ (2010) The DNA damage response: making it safe to play with knives. Mol Cell 40, 179–204.
26 Lu W-T, Hawley BR, Skalka GL, Baldock RA, Smith EM, Bader AS, Malewicz M, Watts FZ, Wilczynska A and Bushell M (2018) Drosha drives the formation of DNA:RNA hybrids around DNA break sites to facilitate DNA repair. Nat Commun 9, 532.
27 Aguado LC, Schmid S, May J, Sabin LR, Panis M, Blanco-Melo D, Shim JV, Sachs D, Cherry S, Simon AE et al. (2017) RNase III nuclease from diverse kingdoms serve as antiviral effectors. Nature 547, 114–117.
28 Shapiro JS, Schmid S, Aguado LC, Sabin LR, Yasunaga A, Shim JV, Sachs D, Cherry S and TenOever BR (2014) Drosha as an interferon-independent antiviral factor. Proc Natl Acad Sci USA 111, 7108–7113.
29 Calses PC, Dhillon KK, Tucker N, Chi Y, Huang J, Kawasumi M, Nghiem P, Wang Y, Clurman BE, Jacquemont C et al. (2017) DGCR8 mediates repair of UV-induced DNA damage independently of RNA processing. Cell Rep 19, 162–174.
30 Song M-S and Rossi JJ (2017) Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem J 474, 1603–1618.
31 Fukunaga R, Han BW, Hung JH, Xu J, Weng Z and Zamore PD (2012) Dicer partner proteins tune the length of mature miRNAs in flies and mammals. Cell 151, 533–546.
32 Okamura K and Lai EC (2008) Endogenous small interfering RNAs in animals. Nat Rev Mol Cell Biol 9, 673–678.
33 Doyle M, Badertscher L, Jaskiewicz L, Guttenger S, Jurado S, Huschegmschtid T, Kutay U and Filipowicz W (2013) The double-stranded RNA binding domain of human Dicer functions as a nuclear localization signal. RNA 19, 1238–1252.
34 White E, Schlackow M, Kamieniarz-Gdula K, Proudfoot NJ and Gullerova M (2014) Human nuclear Dicer restricts the deleterious accumulation of endogenous double-stranded RNA. Nat Struct Mol Biol 21, 552–559.
35 Burger K, Schlackow M, Potts M, Hester S, Mohammed S and Gullerova M (2017) Nuclear phosphorylated Dicer processes doublestranded RNA in response to DNA damage. J Cell Biol 216, 2373–2389.
36 Burger K and Gullerova M (2018) Nuclear re-localization of Dicer in primary mouse embryonic fibroblast nuclei following DNA damage. PLoS Genet 14, e1007151.
37 Neve J, Burger K, Li W, Hoque M, Patel R, Tian B, Gullerova M and Furger A (2016) Subcellular RNA profiling links splicing and nuclear DICER1 to alternative cleavage and polyadenylation. Genome Res 26, 24–35.
38 Gagnon KTT, Li L, Chu Y, Janowski BAA and Corey DRR (2014) RNAi factors are present and active in human cell nuclei. Cell Rep 6, 211–221.
39 Much C, Aachynnikava T, Pavlinic D, Buness A, Rappssilber J, Benes V, Alshire R and O’Carroll D (2016) Endogenous mouse dicer is an exclusively cytoplasmic protein. PLoS Genet 12, e1006095.
40 Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JWS, Green PJ, Barton GJ and Hutvagner G (2009) Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. RNA 15, 2147–2160.
41 Haussecker D, Huang Y, Lau A, Parameswaran P, Fire AZ and Kay MA (2010) Human tRNA-derived small RNAs in the global regulation of RNA silencing. RNA 16, 673–695.
42 Soares AR, Fernandes N, Reverendo M, Araujo HR, Oliveira JL, Moura GMR and Santos MAS (2015) Conserved and highly expressed tRNA derived fragments in zebrafish. BMC Mol Biol 16, 22.
43 Soares AR and Santos M (2017) Discovery and function of transfer RNA-derived fragments and their role in disease. Wiley Interdiscip Rev RNA 8, e1423.
44 Maute RL, Schneider C, Sumazin P, Holmes A, Califano A, Basso K and Dalla-Favera R (2013) tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proc Natl Acad Sci USA 110, 1404–1409.

45 Martinez G, Choudury SG and Slotkin RK (2017) TRNA-derived small RNAs target transposable element transcripts. Nucleic Acids Res 45, 5142–5152.

46 Babiarz JE, Ruby JG, Wang Y, Bartel DP and Belloch R (2008) Mouse ES cells express endogenous shRNAs, siRNAs, and other microprocessor-independent, dicer-dependent small RNAs. Genes Dev 22, 2773–2785.

47 Hasler D, Lehmann G, Murakawa Y, Klironomos F, Jakob L, Grässer FA, Rajewsky N, Landthaler M and Meister G (2016) The lupus autoantigen La prevents mis-channeling of tRNA fragments into the human microRNA pathway. Mol Cell 63, 110–124.

48 Li Z, Ender C, Meister G, Moore PS, Chang Y and John B (2012) Extensive terminal and asymmetric processing of small RNAs from rRNAs, snRNAs, snRNAs, and tRNAs. Nucleic Acids Res 40, 6787–6799.

49 Kumar P, Anaya J, Mudunuri SB and Dutta A (2014) Meta-analysis of tRNA derived RNA fragments reveals that they are evolutionarily conserved and associate with AGO proteins to recognize specific RNA targets. BMC Biol 12, 78.

50 Alves CS, Vicentini R, Duarte GT, Pinoti VF, Vincentz M and Nogueira FTS (2017) Genome-wide identification and characterization of tRNA-derived RNA fragments in land plants. Plant Mol Biol 93, 35–48.

51 Cernilogar FM, Onorati MC, Kothe GO, Burroughs AM, Parsi KM, Breiling A, Lo Sardo F, Saxena A, Miyoshi K, Siomi H et al. (2011) Chromatin-associated RNA interference components contribute to transcriptional regulation in Drosophila. Nature 480, 391–395.

52 Coll O, Guittat T, Villalba A, Papin C, Simonelig M and Gebauer F (2018) Dicer-2 promotes mRNA activation through cytoplasmic polyadenylation. RNA 24, 529–539.

53 Swahari V, Nakamura A, Baran-Gale J, Garcia I, Crowther AJ, Sons R, Gershon TR, Hammond S, Sethupathy P and Deshmukh M (2016) Essential function of dicer in resolving DNA damage in the rapidly dividing cells of the developing and malignant cerebellum. Cell Rep 14, 216–224.

54 Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, White CI, Danielsen JMR, Yang YG and Qi Y (2012) A role for small RNAs in DNA double-strand break repair. Cell 149, 101–112.

55 Chen X, Li WF, Wu X, Zhang HC, Chen L, Zhang PY, Liu LY, Ma D, Chen T, Zhou L et al. (2017) Dicer regulates non-homologous end joining and is associated with chemosensitivity in colon cancer patients. Carcinogenesis 38, 873–882.

56 Chitale S and Richly H (2017) DICER- and MMSET-catalyzed H4K20me2 recruits the nucleotide excision repair factor XPA to DNA damage sites. J Cell Biol, 217, 527–540.

57 Chitale S and Richly H (2017) DICER and ZRF1 contribute to chromatin decondensation during nucleotide excision repair. Nucleic Acids Res 5, e12175.

58 Maillard P V, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW and Voinnet O (2013) Antiviral RNA interference in mammalian cells. Science 342, 235–238.

59 Li Y, Lu J, Han Y, Fan X and Ding SW (2013) RNA interference functions as an antiviral immunity mechanism in mammals. Science 342, 231–234.

60 Li Y, Basavappa M, Lu J, Dong S, Cronkite DA, Prior JT, Reinecker HC, Hertzog P, Han Y, Li WX et al. (2016) Induction and suppression of antiviral RNA interference by influenza A virus in mammalian cells. Nat Microbiol 2, 16250.

61 Qiu Y, Xu Y, Zhang Y, Zhou H, Deng YQ, Li XF, Miao M, Zhang Q, Zhong B, Hu Y et al. (2017) Human virus-derived small RNAs can confer antiviral immunity in mammals. Immunity 46 (992–1004), e5.

62 Meister G (2013) Argonaute proteins: functional insights and emerging roles. Nat Rev Genet 14, 447–459.

63 Iwasaki S, Kobayashi M, Yoda M, Sakaguchi Y, Katsuma S, Suzuki T and Tomari Y (2010) Hsc70/ Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. Mol Cell 39, 292–299.

64 Kwak PB and Tomari Y (2012) The N domain of Argonaute drives duplex unwinding during RISC assembly. Nat Struct Mol Biol 19, 145–151.

65 Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G and Tuschi T (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell 15, 185–197.

66 Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L and Hannon GJ (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305, 1437–1441.

67 Park MS, Phan HD, Busch F, Hinckley SH, Brackbill JA, Wysocki VH and Nakanishi K (2017) Human Argonaute3 has slicer activity. Nucleic Acids Res 45, 11867–11877.

68 Hafner M, Landthaler M, Burger L, Khorshid M, Hauser J, Berninger P, Rothballer A, Ascano M, Jungkamp AC, Munschauer M et al. (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141, 129–141.

69 Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M et al. (2013) Circular RNAs are a large
class of animal RNAs with regulatory potency. Nature 495, 333–338.
70 Kuscu C, Kumar P, Kiran M, Su Z, Malik A and Dutta A (2018) tRNA fragments (tRFs) guide Ago to regulate gene expression post-transcriptionally in a Dicer independent manner. RNA 24, 1093–1105.
71 Martienssen RA, Zaratiegui M and Goto DB (2005) RNA interference and heterochromatin in the fission yeast Schizosaccharomyces pombe. Trends Genet 21, 450–456.
72 Grishok A, Sinskey JL and Sharp PA (2005) Transcriptional silencing of a transgene by RNAi in the soma of C. elegans. Genes Dev 19, 683–696.
73 Fagegaltier D, Bougé A-L, Berry B, Poisot E, Sismeiro O, Coppée J-Y, Théodore L, Voinnet O and Antoniewski C (2009) The endogenous siRNA pathway is involved in heterochromatin formation in Drosophila. Proc Natl Acad Sci USA 106, 21258–21263.
74 Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SIS and Moazed D (2004) RNAi-mediated targeting of heterochromatin by the RITS complex. Science 303, 672–676.
75 Zaratiegui M, Castel SE, Irvine DV, Kloc A, Ren J, Li F, De Castro E, Marín L, Chang AY, Goto D et al. (2011) RNAi promotes heterochromatic silencing through replication-coupled release of RNA Pol II. Nature 479, 135–138.
76 Moshkovitch N, Nisha P, Boyle PJ, Thompson BA, Dale RK and Lei EP (2011) RNAi-independent role for argonaute2 in CTCF/CP190 chromatin insulator function. Genes Dev 25, 1686–1701.
77 Taliaferro JM, Aspenl JL, Bradley T, Marwha D, Blanchette M and Rio DC (2013) Two new and distinct roles for Drosophila Argonaute-2 in the nucleus: alternative pre-mRNA splicing and transcriptional repression. Genes Dev 27, 378–389.
78 Liu C, Xin Y, Xu L, Cai Z, Xue Y, Liu YY, Xie D, Liu YY and Qi Y (2017) Arabidopsis ARGONAUTE 1 binds chromatin to promote gene transcription in response to hormones and stresses. Dev Cell 44, 348–361.e7.
79 Huang V, Zheng J, Qi Z, Wang J, Place RF, Yu J, Li H and Li LC (2013) Ago1 interacts with RNA polymerase II and binds to the promoters of actively transcribed genes in human cancer cells. PLoS Genet 9, e1003821.
80 Ameyar-Zazoua M, Rachez C, Souidi M, Robien P, Fritsch L, Young R, Morozova N, Fenuill R, Descostes N, Andraud JC et al. (2012) Argonaute proteins couple chromatin silencing to alternative splicing. Nat Struct Mol Biol 19, 998–1005.
81 Woolnough JL, Atwood BL and Giles KE (2015) Argonaute 2 binds directly to tRNA genes and promotes gene repression in cis. Mol Cell Biol 35, 2278–2294.
82 Gao M, Wei W, Li M-M, Wu Y-S, Ba Z, Jin K-X, Li M-M, Liao Y-Q, Adhikari S, Chong Z et al. (2014) Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. Cell Res 24, 532–541.
83 Wang Q and Goldstein M (2016) Small RNAs recruit chromatin-modifying enzymes MMSET and Tip60 to reconfigure damaged DNA upon double-strand break and facilitate repair. Cancer Res 76, 1904–1915.
84 Eckenfelder A, Ségéal E, Pinzon N, Ulveling D, Amadori C, Charpentier M, Nidelet S, Concordet JP, Zagury JF, Paillart JC et al. (2017) Argonaute proteins regulate HIV-1 multiply spliced RNA and viral production in a Dicer independent manner. Nucleic Acids Res 45, 4158–4173.
85 Chukwurah E and Patel RC (2018) Stress-induced TRBP phosphorylation enhances its interaction with PKR to regulate cellular survival. Sci Rep 8, 1020.
86 Antoniou A, Khudayberdiev S, Idziak A, Bicker S, Jacob R and Schratt G (2018) The dynamic recruitment of TRBP to neuronal membranes mediates dendritogenesis during development. EMBO Rep 19, e44853.
87 Bogerd HP, Whisnant AW, Kennedy EM, Flores O and Cullen BR (2014) Derivation and characterization of Dicer- and microRNA-deficient human cells. RNA 20, 923–937.
88 Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM and Rajewsky K (2005) Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 19, 489–501.
89 Korolov SB, Muljo SA, Giller GR, Krek A, Chakraborty T, Kanellopoulou C, Jensen K, Cobb BS, Merkenschlager M, Rajewsky N et al. (2008) Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. Cell 132, 860–874.
90 D’Adda di Fagagna F (2014) A direct role for small non-coding RNAs in DNA damage response. Trends Cell Biol 24, 171–178.
91 Micheli F, Pichayia S, Vitelli V, Sharma S, Gioia U, Pessina F, Cabrini M, Wang Y, Capozzo I, Iannelli F et al. (2017) Damage-induced IncRNAs control the DNA damage response through interaction with DDRRNAs at individual double-strand breaks. Nat Cell Biol 19, 1400–1411.