Double-stranded (ds) RNA is created through the pairing of complementary sequences. A ubiquitous motif in biological systems, dsRNA exhibits a limited conformational plasticity, but exerts a multitude of biological effects. dsRNA can possess a transient, dynamic nature, as seen in antisense RNA binding to target sites. Intramolecular base-pairing provides local double-helical structures that can function as architectural elements or as protein recognition sites. In the latter case, protein binding to dsRNA enables processes such as RNA transport and localization. dsRNA invokes potent cellular responses, as a single molecule of dsRNA can trigger interferon production in vertebrate cells. The cellular response underscores the important aspect of dsRNA as an indicator of pathogenic conditions. In fact, dsRNA is the chromosomal material of many viruses, and cell membrane receptors specifically recognize dsRNA as an initial event in the response pathway.1–5
The enzymatic cleavage of dsRNA is a conserved reaction of fundamental importance in cellular and viral gene expression and regulation, host defense, and genome surveillance. A conserved enzymatic mechanism has been characterized that catalyzes the coordinate cleavage of both RNA strands at selected target sites. Specifically, dsRNA processing is accomplished by members of a ribonuclease family, including the prototypical ribonuclease III (RNase III) of bacterial cells. Ongoing studies on RNase III, and in particular the enzyme from Escherichia coli, have revealed a global role for dsRNA processing in bacterial gene expression and regulation. In particular, analysis of the essential involvement of dsRNA processing in many eukaryotic family members has extended the cited above may serve as alternative sources. Processing and gene regulation. However, the reviews from many laboratories that are studying dsRNA is unable to include the important contributions summarized. Owing to page limitations this review physicochemical properties of dsRNA also will be characterized.10 The subsequent characterization of eukaryotic family members has extended the essential involvement of dsRNA processing in many additional pathways. In particular, analysis of the components of RNA interference (RNAi) and related pathways identified two RNase III family members, Dicer and Drosha, as essential functional partners in RNAi. Understanding how dsRNA is processed by RNase III family members, and characterizing the many consequences of this reaction remain as key objectives.

This review addresses current knowledge of dsRNA processing by RNase III family enzymes. Emphasis will be placed on the mechanisms of catalysis and target site selection. The salient physicochemical properties of dsRNA also will be summarized. Owing to page limitations this review is unable to include the important contributions from many laboratories that are studying dsRNA processing and gene regulation. However, the reviews cited above may serve as alternative sources.

**PHYSICAL PROPERTIES OF dsRNA**

The RNase III mechanism of dsRNA recognition, phosphodiester hydrolysis, and product release necessarily reflects the unique attributes of dsRNA. In contrast to the DNA double helix, dsRNA is structurally conservative, existing essentially as an A-form helix with an 11-fold pitch. The preferential adoption of the A-helix reflects the uniform presence of ribose 2′-hydroxyl groups that stabilize the C3′-endo sugar conformation. The sugar pucker in turn provides a shallow, solvent-exposed minor groove and a narrow, deep major groove. The 2′-hydroxyl groups that line each side of the minor groove create a polar, extensively hydrated surface, and many dsRNA–protein interactions, including those seen with RNase III family members, rely on the tracks of 2′-hydroxyl groups to provide binding energy and to distinguish dsRNA from DNA, or DNA–RNA hybrids. Alterations in solvent conditions yield the A′-helix that exhibits a 12 base-pair (bp) pitch and a widened major groove. The A′-helix has been implicated in specific protein-dependent RNA transport processes involving protein–major groove interactions.

A prominent feature of dsRNA is its thermodynamic stability that is conferred in part by metal cation interactions. Two modes of binding have been described, in which a metal (e.g., Mg2+) either can be sequestered within the major groove or occupy sites that span the major groove entrance. These interactions effectively reduce interstrand repulsion by providing significant charge screening, and also contribute to the ability of dsRNA to resist condensation caused by double helix denaturation, as seen with DNA. The metal ion interactions allow close packing of helices to provide stable structures, as seen in ribozymes and riboswitches. Another prominent feature of dsRNA is its relative rigidity, as evidenced by a persistence length greater than that for DNA. While dsRNA is not responsive to sequences than can permanently kink dsDNA, specific secondary structural elements such as bulges, loops, and mismatches can provide flexible joints. As discussed below, these elements can function as key substrate reactivity determinants.

**THE RIBONUCLEASE III FAMILY**

Catalysis of dsRNA cleavage is achieved by a unique fold, termed the RNase III domain. The domain has the capacity to dimerize, providing a stable homodimeric structure with a catalytic site in each subunit. The RNase III domain (150 aa) exhibits a characteristic consensus sequence of 10 aa, containing conserved residues that bind divalent metal ions important for catalytic activity, or stabilize the dimeric structure. RNase III enzymes exhibit additional domains (Figure 1) that function in concert with the RNase III domain. The additional domains contribute to substrate binding, broaden substrate range, participate in cleavage site selection, confer regulation, and enable protein–protein interactions (see below). RNase III enzymes are highly conserved in the Bacteria and Eukarya, but are only sporadically observed in the Archaea. From an evolutionary perspective, an RNase III enzyme may first have appeared in an early bacterium, then entered the eukaryotic lineage via an endosymbiont. Subsequent elaboration of the structure by acquisition of additional domains created the diversity of eukaryotic family members.
The first reported structure of the RNase III domain involved the successful crystallization of the enzyme from *Aquifex aeolicus* (Aa) that had the dsRBD deleted. The Aa-RNase III domain exhibits (1) a novel, essentially all-α-helical fold; (2) a dimeric structure with an extensive subunit interface; and (3) a symmetrically positioned divalent metal ion (Mn$^{2+}$ or Mg$^{2+}$) in each subunit, coordinated to conserved carboxylic acid side chains important for catalytic activity. The subunit interface is stabilized by symmetric, hydrophobic ‘ball-and-socket’ motifs, with the dimensions of the shallow valley, defined by the subunit interface, appropriate for accommodation of a dsRNA. Subsequent studies described structures of full-length Aa-RNase III bound to 11 or 12 bp dsRNAs, where in several cases the catalytic site was inactivated by point mutation. Together with a 2.0 Å structure of full-length enzyme from *Thermotoga maritima*, crystallized in the absence of dsRNA or divalent metal ion (unpublished; PDB entry 1O0W), these structures revealed the positional flexibility of the dsRBD, and suggested how RNase III can engage dsRNA in a noncatalytic manner. In this regard, a 2.1 Å structure of full-length RNase III of *Mycobacterium tuberculosis* (PDB entry 2A11) revealed only the RNase III domain, as the flexibility of the linker conferred significant positional variability to the dsRBDs. Subsequent studies attempted to define the structure of a precatalytic complex. To this end, the D44N mutation was incorporated into Aa-RNase III, with the expectation that it would suppress phosphodiester cleavage, thus allowing formation of an enzyme-substrate assemblage that approximates the precatalytic complex. However, the mutation did not fully inactivate the enzyme, and dsRNA cleavage occurred during crystallization. This reaction was apparently followed by re-binding of product, providing a structure with features of a post-catalytic complex (PDB entry 2EZ6) (see below).

In summary, the homodimeric structure of RNase III, and the relative positions of the catalytic sites with respect to the dsRNA binding surface form the structural basis for the characteristic action of RNase III and its family members: the scissile phosphodiesters of the bound dsDNA are on the same face of the double helix and on opposite sides of the minor groove; and each can be simultaneously accommodated in a catalytic site. As such, hydrolysis of both linkages would create product ends exhibiting 2-nucleotidyl(3′,3′) overhangs. Additional details on the catalytic mechanism are discussed below.

**RNase III Catalytic Mechanism**

RNase III activates water as a nucleophile to hydrolyze target site phosphodiester, creating 3′-hydroxyl,
5′-phosphomonoester product termini. An oxygen isotope incorporation study of Ec-RNase III action on a model substrate revealed the essential irreversibility of the hydrolytic step.34 RNase III requires a divalent metal ion for catalysis, and it was shown for Ec-RNase III that in addition to Mg$^{2+}$, the transition metal ions Mn$^{2+}$, Ni$^{2+}$, and Co$^{2+}$ also support catalysis, with differing efficiencies.34 Given the intracellular abundance of Mg$^{2+}$, and its ability to support optimal catalytic activity, this metal most likely is the biologically relevant species. A direct involvement of the metal ion in water activation is indicated by the dependence of the rate of the hydrolytic step on the metal ion pK.34 Since RNase III can bind dsRNA in the absence of Mg$^{2+}$, metal occupancy of the catalytic sites is not a prerequisite for substrate binding.35

A kinetic study of Ec-RNase III measured the rate of cleavage of a model substrate containing a single scissile bond as a function of Mg$^{2+}$ concentration. Single-turnover conditions were applied, such that the rate reflected the hydrolytic step. The concentration dependence of the rate indicated that two Mg$^{2+}$ ions participate in phosphodiester hydrolysis.36 This is consistent with crystal structures of wild-type Aa-RNase III bound to dsRNA cleavage products, exhibiting two Mg$^{2+}$ ions in each catalytic site that are coordinated to a set of highly conserved carboxylic acid side chains (PDB entries 2NUF, 2NUG).37 The adjacency of the two metal ions and their interaction with the scissile phosphodiester linkage fit the well-studied two-metal-ion catalytic mechanism, wherein one metal (MgA) binds and activates the water nucleophile, and the second metal (MgB) facilitates departure of the 3′-oxygen atom (Figure 2a). Both metal ions are jointly coordinated to the side chain of a highly conserved, functionally essential glutamic acid (E110 in Aa-RNase III, and E117 in Ec-RNase III).37 Additional carboxylic acid side chains and associated water molecules contribute to the proper positioning of MgA and MgB (Figure 2a). Since there is no evidence for the involvement of additional functional groups in the catalytic site, catalysis appears to be largely driven by the two metals. Ca$^{2+}$ ion does not support catalysis, perhaps reflecting its larger radius and differing ligand coordination properties.38 However, since Ca$^{2+}$ can stabilize RNase III-substrate complexes it is possible that Ca$^{2+}$ binding to the catalytic sites reduces local negative charge density, thereby promoting stable binding of substrate. While phosphodiester hydrolysis depends upon two closely positioned metal ions, there is structural evidence for a third metal binding site, near the A and B sites, whose occupancy either by a third metal ion—or transiently by one of the two catalytic metals—may contribute to the reaction pathway.37 Since product release is rate-limiting in the steady-state,34 and may also be subject to positive control by an additional process (see below), the third metal site may be important for this step.

The structures of Aa-RNase III-substrate complexes and the RNA-free structure of T. maritima RNase III (Figure 3), along with biochemical studies, led to a proposed catalytic pathway for dsRNA cleavage.37,39 (Figure 4). Initial engagement of substrate by a dsRBD is followed by engagement by the dimeric RNase III domain and the second dsRBD, forming the precatalytic complex. Phosphodiester cleavage is followed by disengagement of the products from the RNase III domain, then their release from each dsRBD. The catalytic pathway does not appear to involve an unwinding or other major distortion of the double helix, as none of the reported crystal structures reveal any unusual RNA conformations. Thus, there may only be a modest distortion of the double helix by one of the two catalytic metals.
The positioning of the db ~11 bp from the target site serves to establish a minimum substrate length (~11 bp) necessary for efficient reaction. The db does not exhibit a conserved sequence, either among substrates of a given RNase III, or across species. However, bp substitutions in the db reduce Ec-RNase III substrate reactivity, while having minimal effects on Aa-RNase III substrate reactivity. A short, nonconserved region in the RNase III domain, termed RBM4, interacts with the db. Thus, an emerging picture is an idiosyncratic yet energetically important db–RNase III interaction. Structural studies of RNase III-substrate complexes with anticipated precatalytic features can be expected to provide further insight on this interaction.

Structural and biochemical studies on the RNase III–pb interaction have shown how bp sequence controls reactivity and participates in target site selection. Side chains in the N-terminal portion of the dsRBD N-terminal α helix (termed RBM1) form an array of hydrogen bonds with functional groups within the pb, including ribose 2’-hydroxyl groups on both strands. One interaction of interest occurs in the minor groove, and involves a hydrogen bond between the carboxamide side chain of a highly conserved glutamine (Q157 in Aa-RNase III) and the O2 pyrimidine atom of the AU bp at pb position 2 (Figure 5b). The carboxamide group also forms a second hydrogen bond with an adjacent ribose 2’-OH group. These are functionally important interactions, as mutation of the glutamine to an alanine strongly reduces RNase III activity for substrate. Interestingly, pb position 2 also is the site of inhibition by bp substitution. Thus, while the AU and UA bp confer equivalent reactivity, a CG or GC bp blocks binding of Ec-RNase III. The purine 2-amino group is responsible for inhibition, as incorporation of an amino group on the adenine C2 atom of the AU pair (forming a 2,6-diaminopurine•U bp) also confers inhibition, and that removal of the 2-amino group from G (forming an inosine-cytosine pair) restores processing activity. On the basis of crystallographic data, the 2-amino group may prevent dsRBD binding by a steric clash with the glutamine side chain, prohibiting formation of the hydrogen bonds. The apparent invariance of the glutamine in the RBM1 site of bacterial RNase III sequences, and the preference for a UA or AU bp at pb position 2 underscores the important contribution of this interaction to binding of enzyme in the correct register. The absence of a conserved glutamine in the corresponding position of the dsRBDs of eukaryotic RNase III enzymes (AWN, unpublished) is consistent with different mechanisms for target site selection (see below).
FIGURE 4 | Proposed catalytic pathway for ribonuclease III (RNase III). The diagram is a modification of a proposed scheme. Initial recognition of dsRNA (black) by a dsRBD (pink) is followed by engagement of the RNase III domain (light blue) and the second dsRBD to form a precatalytic complex. Phosphodiester hydrolysis (red arrows) provides a post-catalytic complex with products still bound by the RNase III domain. Release of the products from the RNase III domain is followed by release from the dsRBDs. The rate-limiting step may be release of products from the RNase III domain (see text). (Reprinted with permission from Ref 7. Copyright 2011 Springer Science+Business Media).

FIGURE 5 | Reactivity determinants of ribonuclease III (RNase III) substrates. (a) Diagrammatic structure of an idealized dsRNA substrate of Ec-RNase III. The length of the helix from the end of each db is 22 bp (two A-helical turns). The boxed bp indicate the distal box (db), middle box (mb) and proximal box (pb). The twofold symmetry of the substrate is indicated by the black dot centered between the two cleavage sites (black arrows), and blue color of the lower portion of the substrate. Note that the symmetry does not include specific bp sequence. N-N′ indicate a standard bp of unspecified sequence. The sites of interaction of RBM1, RBM2, RBM3, and RBM4 with the db, mb, and pb are indicated by dashed lines. (b) Sequence-dependent interaction of the conserved glutamine side chain of the dsRBD α1 helix (RBM1) with the O2 uracil atom. Taken from the Aa-RNase III(D44N)-dsRNA crystal structure (PDB entry 2EZ6). The dotted lines indicate probable hydrogen bonds of the Q157 carboxamide group with the O2 atom and the adjacent 2′-hydroxyl group.

The inhibitory action of GC/CG bp substitution at pb position 2 may have a role in minimizing ‘off-target’ effects of RNase III. Antideterminants were originally described as tRNA sequence or structural features that block inappropriate recognition by noncognate aminoacyl-tRNA synthetases or other proteins. For dsRNA structures whose functions would be destroyed by RNase III action, the presence of antideterminant bp at key positions would prevent enzyme recognition, and without disruption of the regular double-helical structure. A qualitatively different type of bp-dependent inhibition is seen at pb position 4, where a GC or CG substitution prevents substrate cleavage, but does not inhibit binding of Ec-RNase III. This type of substitution may afford dsRNA structures that function as RNase III binding sites. In this regard, an in vitro selection (SELEX) study identified an RNA that can bind RNase III but is resistant to cleavage, due in part to a GC/CG bp adjacent to the cleavage site.

In summary, RNase III recognition of substrate involves at least one sequence-specific, energetically important interaction of the dsRBD with the pb, and an idiosyncratic interaction of the RNase III domain.
with the db. Interestingly, a truncated form of Ec-
RNase III that lacks the dsRBD also cleaves substrate at the canonical site, under conditions of reduced salt concentration and using Mn$^{2+}$ in place of Mg$^{2+}$.26 Thus, the dsRBD and RNase III domain engage substrate in a complementary register. This coincident specificity may provide an important reinforcement of target site selectivity, with the dsRBD performing a key role in initial discrimination of substrate. The mechanism by which the RNase III domain identifies the target site is not known, and the db interaction may be insufficient to achieve this. The natural RNase III variant, Mini-III lacks the dsRBD, yet site-specifically cleaves the immediate precursor to the mature 23S rRNA.25 While optimal activity of Mini-III requires ribosomal protein L3,46 the latter protein is not required for specificity. Finally, the proximal boxes of cellular substrates exhibit significant degeneracy. The generally constrained lengths ($\sim<20$ bp) of the double-helical structures of many cellular substrates may compensate for the degeneracy to provide a single target site. Regarding the apparent paradox of RNase III action (see above), the degeneracy of recognition sites also would allow RNase III to degrade much longer dsRNAs that are created, for example, by association of genomic transcripts.47,48 Thus, the long-

Regulation of RNase III

As with metabolic pathways in general, RNA processing and decay reactions and the associated enzymes are subject to regulation. This not only controls ribonucleotide flux, but as (or more) importantly, controls gene expression. Regulation of ribonuclease levels and activity can confer global control of the production of the processed products. Several lines of evidence demonstrate that dsRNA processing is regulated at multiple levels through control of RNase III. It has been estimated that there are several hundred copies of RNase III in the E. coli cell, the levels of which increase in proportion to growth rate.6 The limited amount of RNase III indicates a stringent regulation of expression. While there is no apparent transcriptional control, regulation of Ec-RNase III expression is imposed at the post-transcriptional level by the action of the enzyme on its own mRNA. Specifically, cleavage of an irregular hairpin in the mRNA 5′-UTR creates a new, unstructured 5′-end with a phosphomonoester group. This structure is recognized in turn by RNase E, leading to rapid decay of the mRNA.51 The negative autoregulation not only limits production of enzyme but also allows for upregulation of enzyme level with increased growth rate. Here, the increased number of substrates—in particular the abundantly produced rRNA precursors that are processed by RNase III—engage a larger proportion of the available enzyme, thereby reducing the level of autoregulation.

RNase III catalytic activity is subject to positive regulation. The T7 bacteriophage expresses a protein kinase that phosphorylates RNase III and enhances catalytic activity ~fourfold, as measured in vitro.52 Phosphorylation occurs on a serine in the RNase III domain,53 and biochemical analyses reveal that the covalent modification facilitates product release, which is the rate-limiting step in the catalytic pathway (S. Gone, M. Prieto and A.W.N., manuscript in preparation). The T7-induced catalytic enhancement of RNase III may serve to meet the demands placed on limited amounts of enzyme to process the abundant phage transcripts, most of which contain at least one RNase III target site, and whose maturation is required for optimal production of phage protein.54

RNase III is negatively regulated by the macrodomain protein, YmdB. Cohen and coworkers showed that ectopic overexpression of YmdB reduces RNase III activity, and that RNase III activity also is downregulated when cells undergo cold shock, which increases YmdB levels.55 YmdB-dependent regulation of RNase III appears to be a post-transcriptional feature of the global shift in physiological state in response to lower temperatures and perhaps other stress conditions. The mechanism of YmdB-dependent inhibition of RNase III is unclear, but it is thought to be dependent upon the direct interaction of the two proteins.55 YmdB also binds ADP-ribose, and hydrolyzes O-acetyl ADP-ribose.56 The latter compound is a product of sirtuin-catalyzed protein deacetylation, which uses NAD as
cosubstrate. Whether these activities are related to YmdB regulation of RNase III is unclear, but brings up the possibility of functional crosstalk between dsRNA-dependent gene regulation, the protein acetylome, and cellular redox state.

RNase III activity in vivo also is responsive to osmotic stress. The E. coli proU operon encodes an uptake system for the osmoprotectants proline betaine and glycine betaine. The proU operon transcript is relatively stable (half-life ~6.5 second) under conditions of high osmolarity, but under conditions of reduced osmolarity undergoes rapid degradation (half-life <4 second) through an initiating cleavage by RNase III. The rapid degradation ensures efficient inhibition of proU expression and further uptake of osmoprotectants. Other substrates are anticipated whose reactivity towards RNase III is responsive to changes in growth conditions. In summary, RNase III levels and catalytic activity are regulated at multiple levels, including mRNA stability, covalent modification, protein binding, and changes in ionic conditions, and reflect transitions between different physiological states. Additional regulatory mechanisms are predicted for other family members.

**Yeast RNase III**

RNase III family members within the fungi include Rnt1p of *Saccharomyces cerevisiae* and Pac1p of *Schizosaccharomyces pombe*. Rnt1p and Pac1p cleave hairpin structures in pre-rRNAs, pre-mRNAs, and transcripts containing noncoding RNAs such as snoRNAs, as part of the respective maturation pathways. The enzymes also are involved in RNA quality control. The Rnt1p polypeptide possesses a single RNase III domain, a C-terminal dsRBD, and an additional N-terminal domain that functions in nuclear localization and interaction with additional factors. (Figure 1). Many Rnt1p substrates contain a consensus [A/u]GNN tetraloop that functions as a recognition determinant. Rnt1p recognizes the tetraloop in a shape-dependent (rather than sequence-dependent) manner, and cleaves the stem ~14–16 bp from the structure. Tetraloop recognition involves the dsRBD $\alpha_1$ helix, which possesses an extended structure relative to the $\alpha_1$ helix of other RNases III. The elongated helix provides an expanded hydrophobic core, conferring a conformational plasticity to the dsRBD that is important for forming a functionally competent dsRBD-hairpin complex. The conformational changes that occur in the dsRBD and tetraoop are consistent with an induced-fit mechanism that can distinguish substrates from other hairpin structures.

Additional features of the Rnt1p-substrate interaction contribute to processing reactivity. Specific bp sequence elements can modulate substrate reactivity, and a network of hydrogen bonds provides an energetically important contribution to Rnt1p binding. A phylogenetic-based substrate alignment analysis revealed a statistically significant exclusion of the UA bp from the position adjacent to the tetraloop. Substitution of the UA bp at that position inhibited substrate cleavage but did not perturb enzyme binding. The mechanism of inhibition is unclear but is dependent on the purine 6-amino group immediately 3’ to the fourth nucleotide of the tetraloop. The inhibition does not appear to disrupt the tetraloop–dsRBD interaction, but instead perturbs a downstream event, perhaps engagement of the RNase III domain. The UA bp may afford an antideterminant that is used in other stem-loop structures that are not Rnt1p substrates.

The maturation of a number of snoRNAs, including U18 and snR38, involves Rnt1p-dependent excision from the respective host introns. The structures of the intronic precursors deviate from the canonical [A/u]GNN stem-loop substrates, and their processing requires participation of the nucleolar protein Nop1p, which physically interacts with Rnt1p. Rnt1p also interacts with Gar1p, a protein involved in pseudouridylation reactions. The C-terminal portion of Rnt1p, adjacent to the dsRBD, interacts with Gar1p. As these interactions imply an association of rRNA processing and RNA modification reactions, Rnt1p may be regarded as a core nucleolytic component of one or more dynamic nuclear/nucleolar protein complexes. The full characterization of these complexes remains an important goal.

**Drosha and Microprocessor Function**

Over 1000 micro(mi) RNAs are estimated to be encoded in mammalian genomes, and their expression and utilization is regulated by diverse inputs in a cell- and development-specific manner. Mammalian miRNA maturation involves, *inter alia*, the sequential action of two RNase III family enzymes, Drosha and Dicer. Drosha is localized in the nuclear compartment and acts on primary transcripts synthesized by RNA polymerase II, and that typically contain several miRNAs. Site-specific cleavage within irregular, extended hairpin structures (pri-miRNAs) creates the pre-miRNAs that then are delivered by Exportin5 to the cytoplasm for final maturation by Dicer. Drosha functions within a complex termed the microprocessor that contains a protein, DGCR8, that is required...
for Drosha action. There is scant structural information currently available on Drosha, which in part reflects a general challenge in studying large proteins that function in larger complexes. Thus, most current information derives from biochemical studies, including sequence and structural analyses of the pri-miRNAs (see below). The Drosha polypeptide (Figure 1) possesses tandem RNase III domains and a C-terminal dsRBD. The RNase III domains form an intramolecular pseudodimer with two catalytic sites. A solution structure determination of the Drosha dsRBD reveals an α1–α1 loop element with a dynamic, extended structure. The inability of the Drosha dsRBD to form a stable complex on its own with dsRNA may reflect the atypical mobility and negative charge of the loop, and raises questions on its function in pri-miRNA processing. The Drosha N-terminal region also contains proline-rich and arginine/serine-rich domains that are implicated in protein–protein and protein–nucleic acid interactions.

The DGCR8 polypeptide exhibits tandem dsRBDs and an N-terminal WW domain. The structure of DGCR8 is not known, but a crystallographic study of the C-terminal region reveals the specific, stable positioning of the two dsRBDs which are packed on the C-terminal helix, and a linker connecting the dsRBDs that exhibits an ordered structure (Figure 6a). Molecular dynamic simulations indicate a correlated motion of the two dsRBDs. This can be contrasted with the flexible linkers and independent action of the RNase III dsRBDs. DGCR8 also undergoes dimerization in a heme-dependent manner, and is important in providing optimal activity. The dimerization region is a component of the heme-binding domain, and involves the WW motif. The role of heme-dependent dimerization of DGCR8 in microprocessor function awaits further analysis.

Structural features of pri-miRNAs important for reactivity have been identified through extensive genomic sequence analyses, RNA structure probing, and biochemical assays. Pri-miRNAs exhibit nonuniform structures, and contain double-helical segments punctuated at each full helical turn by bulges, loops, or mismatches (Figure 6b). DGCR8 recognizes the ss/ds junction at the base of the extended stem-loop hairpins and allows engagement of Drosha. It was originally proposed that Drosha cleavage sites are identified by their distance (one helical turn) from the ss/ds junction. The recognition model was further elaborated by incorporating noncanonical secondary structural elements near the target site as reactivity determinants. Structure-probing protocols, coupled with computational modeling showed that the elements confer structural plasticity to pri-miRNAs. The deformability may be a prerequisite for engagement by the DGCR8 dsRBDs, whose fixed orientations require a conformational change in the pri-miRNA in order to achieve a stable complex and allow engagement of Drosha. Moreover, the conformational plasticity also would identify substrates by an induced-fit pathway. Structural studies involving new approaches (e.g., see analysis of Dicer below) are expected to shed light on other Drosha domains and the structural basis of microprocessor function. Recent studies have uncovered a cytoplasmic version of the microprocessor that acts on a viral-encoded pri-miRNA, ultimately providing the mature species (termed ‘virtron’) in a Dicer-dependent manner. The cytoplasmic microprocessor involves Drosha, which is shuttled from the nucleus in a virus-infection-dependent manner, but is not strictly dependent upon DGCR8. Whether Drosha associates with other factors, forming the cytoplasmic microprocessor, remains to be determined. In a similar light, studies are revealing a broader functional role of DGCR8 in RNA processing pathways that may involve nucleases other than Drosha.

Dicer
Eukaryotic genomes encode single or multiple forms of Dicer polypeptides. While a key function of Dicer is the conversion of pre-miRNAs to miRNAs, other roles include host defense and genome surveillance mediated by the processing of alternative, dsRNA-based substrates. Dicer polypeptides share a core structure consisting of tandem RNase III domains and a PAZ domain (Figure 1). One or two dsRBDs may be present in the C-terminal region, and the polypeptide also may carry an N-terminal helicase-like domain (Figure 1). The questions addressed here are how Dicer (1) provides precisely-sized products; (2) can act in a processive manner; and (3), achieves flexibility in processing regular dsRNAs as well as hairpin substrates that include pre-miRNAs.

Biochemical analysis of human Dicer first indicated that the tandem RNase III domains form an intramolecular pseudodimer with two catalytic sites. A crystallographic analysis of the ‘minimal’ Dicer of Giardia intestinalis (gDicer), containing the PAZ and tandem RNase III domains, confirmed the pseudodimeric RNase III domain, and revealed how product length derives from the physical spacing and functional cooperation of the PAZ and RNase III domains. The PAZ domain specifically recognizes the 2-nt, 3′-overhangs of a processed dsRNA terminus and is positioned ~65 Å from the RNase III catalytic
sites (Figure 7a). Engagement of substrate by the two domains allows production of the characteristic ~25 bp product of gDicer action\(^86\) (Figure 7b). Although a comparable crystallographic analysis of hDicer has been sought, the enzyme has been largely refractory to such an approach. Instead, domain-specific labeling, combined with single-molecule electron microscopy provided essential insight on the relative positions and functions of the additional domains, and afforded a structural model for hDicer action.\(^87\) The PAZ domain of the L-shaped protein is positioned at the top of the ‘L’, with the tandem RNase III domains occupying the bend (Figure 8a). The physical separation of the two domains establishes the ~21 bp dsRNA products. Here, PAZ domain recognition of the 2-nt, 3’-overhang and the 5’-phosphomonoester group provides the “3’-counting”\(^86\) and “5’-counting”\(^88\) mechanisms, respectively, for target site selection. The shorter size of the hDicer products compared to the ~25 bp products of gDicer reflects a translational rotation of the RNase III domain, relative to the PAZ domain.\(^87\) The positioning of the hDicer helicase domain is positioned adjacent to the RNase III domain, at the opposite end of the L from the PAZ domain.\(^87\) The hDicer helicase domain is positioned adjacent to the RNase III domain, at the opposite end of the L from the PAZ domain. The position of the helicase domain and its ability to engage dsRNA, confers processivity, wherein engagement of dsRNA allows successive rounds of cleavage prior to release of substrate\(^87\) (Figure 8b). The positioning of the helicase domain explains its ability, under specific conditions, to inhibit substrate processing, most likely by the occluding the catalytic sites. This facet of helicase domain function may ensure stringent control of the ribonucleolytic action of Dicer. Additional insight on helicase domain function has emerged from a high-throughput sequencing study of the products of Dicer action on small hairpin(sh) RNAs \textit{in vivo}. It was determined that placement of the cleavage site 2 nt from the hairpin loop confers maximal accuracy of processing. In addition, an insertion mutation of the helicase domain caused a reduction in processing accuracy for these substrates.\(^89\) A ‘loop-counting’ mechanism was proposed for target site selection, in which the helicase domain establishes an additional interaction with the RNA loop (either as a terminal or internal loop), thereby conferring an additional level of cleavage precision not afforded by the PAZ domain alone.\(^89\)

Biochemical and structural studies of hDicer have been challenged by the size of the polypeptide and the attendant issues of solubility and activity. As an alternative approach, individual domains or domain combinations were purified in recombinant form, and assays of substrate binding and cleavage performed that included single domain omission.\(^90\) It was shown that the helicase domain directly interacts with pre-miRNA hairpins, thus rationalizing how Dicer can process substrates structurally distinct from dsRNA, using an alternative substrate recognition
FIGURE 7 | Structural features of a ‘minimal’ Dicer. (a) Two views of the 3.3 Å crystal structure of Dicer from Giardia intestinalis.86 (PDB entry 2ffl). The PAZ domain (gold) is linked to the RNase IIIa domain (yellow) by the connector helix, or ‘ruler’ (red) (see also Figure 1), which determines the dsRNA product length. The RNase IIIb domain is shown in green, and the two catalytic sites (within the rectangle) are identified by the two metal (Er3+) ions (purple) in each site (see also Figure 2). (b) Two views of a modeled complex of gDicer bound to dsRNA.86 Blue and red coloration indicate basic and acidic regions, respectively. dsRNA is shown in yellow and gray strands, and the white arrows indicate the sites of cleavage. The yellow star indicates the site in the PAZ domain that binds the dsRNA 3’-overhang. (Reprinted with permission from Ref 86. Copyright 2006 AAAS)

pathway.90 The functional role of the dsRBD has been enigmatic, as it is not essential for Dicer action in standard assays. However, it was shown that this domain, in isolated form, binds dsRNA,91 and enables substrate processing by Dicer in the absence of the PAZ domain.90 These findings suggest that the dsRBD may establish a pathway for recognition of alternative substrates, including those not recognized by the PAZ domain.90 The occurrence of Dicer family members with multiple dsRBDs underscores the relevance of alternative recognition pathways connected with substrate diversification. On the basis of these findings it is anticipated that additional substrates will be identified that do not conform to the pre-miRNA or regular dsRNA structures. Dicer interacts with other proteins, including TRBP, PACT, and Ago2.92,93 While the structural details of these interactions are just beginning to be elucidated, their influence on Dicer activity is evident. For example, TRBP alters the manner in which hDicer processes pre-miRNAs, allowing formation of alternative miRNAs (‘isomiRs’) that are one nt longer than the canonical form, and possess different target specificities.94,95

RNase III Enzymes in Trypanosome Kinetoplasts

RNase III family enzymes participate in kinetoplast RNA editing, wherein multiple uridine nucleotides are inserted or deleted at multiple sites in the mRNA precursors, ultimately creating the mature, translationally competent species. The selection of editing sites; the determination whether U insertion or deletion occurs at a given site; and how many U residues are inserted or deleted at each site are determined by trans-acting, noncoding guide(g) RNAs.96 A segment of each gRNA is complementary to clusters of editing sites, allowing formation of an intermolecular duplex that directs endonucleolytic cleavage of the pre-mRNA at the ss/ds junction. The gRNA sequence also determines whether U residues are removed from the cleaved pre-mRNA via exonucleolytic action, or instead are inserted by a uridylyltransferase. A final ligation reaction establishes the mature sequence at the editing site.

Three distinct, multisubunit RNA editing complexes have been characterized that have differing editing site specificities, and that contain an RNase III family polypeptide termed KREN1, KREN2, or KREN3.97–100 These polypeptides are required for the endonucleolytic cleavage step, and they may specifically function in the form of heterodimers that involve partner proteins KREPB4 or KREPB5.101 The latter proteins also have RNase III domains, but sequence analysis and mutational studies reveal that the catalytic sites are nonfunctional.101 If such a heterodimeric complex exists, it would therefore have only a single functional catalytic site in the KREN1, KREN2, or KREN3 subunit, and therefore would cleave only one strand in a single binding event (here, the pre-mRNA at the editing site) while preserving the other strand (the gRNA).
this regard, the ability of RNase III heterodimers to ‘nick’ dsRNA has been shown elsewhere.\(^{39,40}\) Alternatively, the single-strand cleavage pattern may reflect local RNA structure at or near the target site (see also above). Further analyses are needed to determine whether the KREN1-3 polypeptides associate with KREPB4 and KREPB5 to form heterodimeric structures. The KREN1-3 polypeptides exhibit a distinctive modular structure that includes a C2H2 Zinc finger (ZnF) motif (Figure 1). A sequence alignment analysis\(^{101}\) showed that the five RNase III family polypeptides contain a PUF domain, rather than a dsRBD, as had been previously suggested. Since PUF domains of other proteins can recognize RNA in a sequence-dependent manner,\(^{102}\) it is suggested. Since PUF domains of other proteins can recognize RNA in a sequence-dependent manner,\(^{102}\) it is anticipated that the domain functions in cleavage site selection. The presence of the ZnF and PUF domains reflects the unique requirements for the highly specialized and complex process of RNA editing.

The maturation of the gRNAs from polycistronic precursors also involves an additional RNase III family enzyme, mRPN1.\(^{103}\) A knockdown of mRPN1 levels causes a drop in gRNA levels and concomitant accumulation of the precursor forms.\(^{103}\) mRPN1 possesses a homodimeric structure, and mutational analysis identified a glutamic acid essential for activity, and which corresponds to a catalytic site glutamic acid in bacterial RNase III.\(^{103}\) The polypeptide contains a C2H2 ZnF domain and a dsRBD (Figure 1) (although the identity of the latter domain remains to be confirmed—see above). mRPN1 associates with several proteins, one of which (TbRGG2) may mediate gRNA access to the RNA editing complexes.\(^{103}\) The structures of the gRNA precursors and the associated mRPN1 processing sites have not yet been described. However, the maturation of polycistronic gRNA precursors by mRPN1 is reminiscent of the action of E. coli RNase III on the five processing signals within the ~7000 nt polycistronic mRNA precursor of bacteriophage T7.\(^{27,54}\)

**Noncatalytic Action of RNase III-like Proteins**

Point mutations that selectively disable the catalytic site have been used to show that RNase III can regulate gene expression as a dsRNA-binding protein.\(^{104}\) Structural studies reveal how RNase III can engage naturally occurring RNase III substrates, as isolated by in vitro selection, can be bound by RNase III but are resistant to cleavage.\(^{43}\) The question is whether in fact RNase III family members have noncatalytic cellular roles. An example is provided in the chloroplasts of land plants. Here, a polypeptide (RNC1) that plays a role in supporting the splicing of group II introns, possesses tandem RNase III domains that are catalytically silent due to mutation of active site residues\(^{105}\) (Figure 1). The tandem RNase III domains are anticipated to self-associate, forming a dsRNA-binding fold that stabilizes structures important for intron splicing.\(^{106}\) The RNC1 polypeptide associates with at least one additional protein, WTF1, as part of a larger complex that supports splicing.\(^{107}\)

**BOX 1 OTHER WAYS OF PROCESSING dsRNA, AND A QUESTION ABOUT ARCHAEAL dsRNA**

The processing of dsRNA is not exclusively performed by RNase III family members. Specific members of the RNase A family of vertebrate-secreted proteins are capable of degrading dsRNA, using an alternative catalytic mechanism. These enzymes, in dimeric form, can bind dsRNA and stabilize localized ssRNA structures created through natural ‘breathing’ of the dsRNA. Each strand is cleaved via a phosphotransferase...
The functions of these enzymes are diverse, and include host defense, response to stress, and angiogenesis.109,110 The Lassa fever virus expresses a 3′→5′ exonuclease that degrades dsRNA (as well as the RNA strand of RNA-DNA hybrids).111,112 The degradation of the RNA strand apparently occurs without major disruption of the double-helical structure.112 Thus, at least one function of this protein is to suppress the host cell innate immune mechanism that recognizes dsRNA.111–113

RNase III orthologs appear to be only accidental to the Archaea. Thus, a question is how dsRNA is recognized and processed in this major branch of life. The Bulge-Helix-Bulge (BHB) splicing endoribonuclease functions in place of RNase III to process the pre-rRNAs, but it is not a dsRNA-specific nuclease.114 If dsRNA processing is a feature of Archaeal RNA metabolism (which is reasonable to assume), either an alternative, dsRNA-specific nuclease exists (e.g., see above), or dsRNA processing is accomplished by the cooperative action of several enzymes, such as a specific exo- or endoribonuclease working in conjunction with, for example, an RNA helicase. In any event, the pathways remain to be understood.

CONCLUSION

The broadening understanding of the role of dsRNA processing in gene expression and regulation continues to spur studies on the structures and mechanisms of RNase III family members, and their functional and physical interactions with other factors. How specificity is achieved in dsRNA cleavage continues to draw attention, especially in the maturation of miRNAs and siRNAs, for which accuracy is essential for correct function, but for which there is evidence for modulation of cleavage site selection as a means to diversify target sites. The demonstration that RNase III enzymes are regulated at multiple levels, as especially shown by bacterial family members, indicates the importance for such regulation in cellular processes. RNase III enzymes function in complexes that exhibit dynamic features and are subject to regulation. Studies are now increasingly focused on determining the components and structural features of the complexes. The regulated activity of these complexes in turn can confer global regulation of dsRNA-dependent processes, of which much remains to be understood. Definition of these networks is needed for a full understanding of the involvement of dsRNA processing on cell physiology in response to diverse inputs.

The question remains as to whether RNase III family members can act in a noncatalytic manner. There is now an example of this in chloroplasts, involving a noncatalytic RNase III polypeptide, and it is anticipated that cellular substrates will be uncovered that bind RNase III in this manner. Specific substrates of RNase III family members are being used as functional modules in synthetic gene networks,115,116 and research on dsRNA processing can be envisioned to extend to the nanotechnology arena.117 RNase III can function in dense nanopatches of dsRNA, and can provide a permanent topographic ‘imprint’ of dsRNA recognition by either a protein or inhibition by an intercalating agent.118 Such approaches can provide the basis for detecting dsRNA and related molecules as disease biomarkers at the single-cell level.

ACKNOWLEDGMENTS

The author thanks Ms. Samridhdhi Paudyal, Dr. Mercedes Prieto and Dr. Wenzhao Meng for help with the figures, and Dr. Rhonda Nicholson for comments on the manuscript. Research in the author’s laboratory is supported in part by grants from the NIH.

REFERENCES

1. Haines DS, Strauss KI, Gillespie DH. Cellular response to double-stranded RNA. J Cell Biochem 1991, 46:9–20.

2. Nicholson AW. Structure, reactivity and biology of double-stranded RNA. Prog Nucleic Acid Res Mol Biol 1996, 52:1–65.

3. Wang Q, Carmichael GG. Effects of length and location on the cellular response to double-stranded RNA. Microbiol Mol Biol Rev 2004, 68:432–452.

4. Gantier MP, Williams BR. The response of mammalian cells to double-stranded RNA. Cytokine Growth Factor Rev 2007, 18:363–371.
5. deFaria IJ, Olmo RP, Silva EG, Marques JT. dsRNA sensing during viral infection: lessons from plants, worms, insects, and mammals. *J. Interferon Cytokine Res* 2013, 33:239–253.

6. Court D. RNA processing and degradation by RNase III. In: Belasco J, Brawerman G, eds. *Control of Messenger RNA Stability*. New York: Academic Press, Inc; 1993, 71–116.

7. Nicholson AW. Ribonuclease III and the role of double-stranded RNA processing in bacterial systems. In: Nicholson AW, ed. *Ribonucleases*. *Nucleic Acids and Molecular Biology* 26. Berlin-Heidelberg: Springer-Verlag; 2011, 269–297. doi: 10.1007/978-3-642-21078-5_11.

8. Lamontagne B, LaRose S, Boulanger J, Elela SA. The RNase III family: a conserved structure and expanding functions in eukaryotic dsRNA metabolism. *Curr Issues Mol Biol* 2001, 3:71–78.

9. Nicholson AW. The ribonuclease III superfamily: forms and functions in RNA maturation, decay, and gene silencing. In: Hannon GJ, ed. *RNAi: A Guide to Gene Silencing*. Cold Spring Harbor, NY: Cold Spring Harbor Press; 2003, 149–174.

10. MacRae IJ, Doudna JA. Ribonuclease revisited: structural insights into ribonuclease III family enzymes. *Curr Opin Struct Biol* 2007, 17:1380145.

11. Saenger W. *Principles of Nucleic Acid Structure*. New York, Berlin: Springer-Verlag; 1983.

12. Tanaka Y, Fujii S, Hiroaki H, Sakata T, Tanaka T, Usugi S, Tomita K, Kyogoku Y. A-form RNA double helix in the single crystal structure of r(UGACCUUCGGCUC). *Nucleic Acids Res* 1999, 27:949–955.

13. Bullock SL, Ringel I, Ish-Horowicz D, Lukavsky PJ. A-form RNA helices are required for cytoplasmic mRNA transport in *Drosophila*. *Nat Struct Mol Biol* 2010, 17:703–710. doi: 10.1038/nsmb.1813.

14. Robinson H, Gao Y-G, Sanishvili R, Joachimiak A, Wang AH-J. Hexahydrated magnesium ions bind in the deep major groove and at the outer mouth of A-form nucleic acid duplexes. *Nucleic Acids Res* 2000, 28:1760–1766.

15. Pabit SA, Qiu X, Lamb JA, Li L, Meisburger SP, Pollack L. Both helix topology and counterion distribution contribute to the more effective charge screening in dsRNA compared with dsDNA. *Nucleic Acids Res* 2009, 37:3887–3896. doi: 10.1093/nar/gkp257.

16. Li L, Pabit SA, Meisburger SP, Pollack L. Double-stranded RNA resists condensation. *Phys Rev Lett* 2011, 106:108101. doi: 10.1103/PhysRevLett.106.108101.

17. Abels JA, Moreno-Herrero F, van der Heijden T, Dekker C, Dekker NH. Single-molecule measurements of the persistence length of double-stranded RNA. *Biophys J* 2005, 88:2737–2744. doi: 10.1529/biophysj.104.052811.

18. Faustino I, Pérez A, Orozco M. Toward a consensus view of duplex RNA flexibility. *Biophys J* 2010, 99:1876–1885. doi: 10:1016/j.bpj.2010.06.061.

19. Wang Y-H, Howard MT, Griffith JD. Phased adenine tracts in double-stranded RNA do not induce sequence-directed bending. *Biochemistry* 1991, 30:5443–5449.

20. Gast F-U, Hagerman PJ. Electrophoretic and hydrodynamic properties of duplex ribonucleic acid molecules transcribed in vitro: evidence that A-tracks do not generate curvature in RNA. *Biochemistry* 1991, 30:4268–4277.

21. Zacharias M, Hagerman PJ. The influence of symmetric internal loops on the flexibility of RNA. *J Mol Biol* 1996, 257:276–289.

22. Zacharias M, Hagerman PJ. Bulge-induced bends in RNA: quantification by transient electric birefringence. *J Mol Biol* 1995, 247:486–500.

23. Ji X. The mechanism of RNase III action: how dicer dices. *Curr Top Microbiol Immunol* 2008, 320:99–116.

24. Masliah G, Barraud P, Allain FHT. RNA recognition by double-stranded RNA binding domains: a matter of shape and sequence. *Cell Mol Life Sci* 2013, 70:1875–1895. doi: 10.1007/s00018-012-1119-x.

25. Redko Y, Bechhofer DH, Condon C. Mini-III, an unusual member of the RNase III family of enzymes, catalyses 23S ribosomal RNA maturation in *B. subtilis*. *Mol Microbiol* 2008, 68:1096–1106. doi: 11.111/j.1365-2958.2008.06207.x.

26. Sun W, Jun E-J, Nicholson AW. Intrinsic double-stranded-RNA processing activity of *Escherichia coli* ribonuclease III lacking the dsRNA-binding domain. *Biochemistry* 2001, 40:14976–14984.

27. Dunn JJ. RNase III cleavage of single-stranded RNA. Effect of ionic strength on the fidelity of cleavage. *J Biol Chem* 1976, 251:3807–3814.

28. Blaszczyk J, Tropea JE, Bubunenko M, Routzahn KM, Waugh DS, Court DL, Ji X. Crystallographic and modeling studies of RNase III suggest a mechanism for double-stranded RNA cleavage. *Structure* 2001, 9:1225–1236.

29. Blaszczyk J, Gan J, Tropea JE, Court DL, Waugh DS, Ji X. Noncatalytic assembly of ribonuclease III with double-stranded RNA. *Structure* 2004, 12:457–466. doi: 10.1016/j.str.2004.02.004.

30. Gan J, Tropea JE, Austin BP, Court DL, Waugh DS, Ji X. Intermediate states of ribonuclease III in complex with double-stranded RNA. *Structure* 2005, 13:1435–1442. doi: 10.1016/j.str.2005.06.014.

31. Ji X. Structural basis for non-catalytic and catalytic activities of ribonuclease III. *Acta Crystallogr D Biol Crystallogr* 2006, D62:933–940. doi: 10.1107/S090744490601153X.
32. Akey DL, Berger JM. Structure of the nuclease domain of ribonuclease III from M. tuberculosis at 2.1Å. *Protein Sci* 2005, 14:2744–2750. doi: 10.1110/ps.051665905.

33. Gan J, Tropea JE, Austin BP, Court DL, Waugh DS, Ji X. Structural insight into the mechanism of double-stranded RNA processing by ribonuclease III. *Cell* 2006, 1245:355–366. doi: 10.1016/j.cell.2005.11.034.

34. Campbell FE Jr, Cassano AG, Anderson VE, Harris ME. Pre-steady-state and stopped-flow fluorescence analysis of *Escherichia coli* ribonuclease III: insights into mechanism and conformational changes associated with binding and catalysis. *J Mol Biol* 2002, 317:21–40.

35. Li H, Nicholson AW. Defining the enzyme binding domain of a ribonuclease III processing signal. Ethylation interference and hydroxyl radical footprinting using catalytically inactive RNase III mutants. *EMBO J* 1996, 15:1421–1433.

36. Sun W, Pertzev A, Nicholson AW. Catalytic mechanism of *Escherichia coli* ribonuclease III: kinetic and inhibitor evidence for the involvement of two magnesium ions in RNA phosphodiester hydrolysis. *Nucleic Acids Res* 2005, 33:807–815.

37. Gan J, Shaw G, Tropea JE, Waugh DS, Court DL, Ji X. A stepwise model for double-stranded RNA processing by ribonuclease III. *Mol Microbiol* 2008, 67:143–154. doi: 10.1111/j.1365-2958.2007.06032.x.

38. Mordasini T, Curioni A, Andreoni W. Why do divalent metal ions either promote or inhibit enzymatic reactions? *J Biol Chem* 2003, 278:4381–4384. doi: 10.1074/jbc.C200664200.

39. Meng W, Nicholson AW. Heterodimer-based analysis of subunit and domain contributions to double-stranded RNA processing by *Escherichia coli* RNase III in vitro. *Biochem J* 2008, 410:39–48. doi: 10.1042/BJ20071047.

40. Conrad C, Schmitt JG, Evguenieva-Hackenberg E, Klug G. One functional subunit is sufficient for catalytic activity and substrate specificity for *Escherichia coli* endoribonuclease III artificial heterodimers. *FEBS Lett* 2002, 518:93–96.

41. Zhang K, Nicholson AW. Regulation of ribonuclease III processing by double-helical sequence antideterminants. *Proc Natl Acad Sci U S A* 1997, 94:13437–13441.

42. Pertzev A, Nicholson AW. Characterization of RNA sequence determinants and antideterminants of processing reactivity for a minimal substrate of *Escherichia coli* ribonuclease III. *Nucleic Acids Res* 2006, 34:3708–3721.

43. Shi Z, Nicholson RH, Jaggi R, Nicholson AW. Characterization of *Aquifex aeolicus* ribonuclease III and the reactivity epitopes of its pre-ribosomal RNA substrates. *Nucleic Acids Res* 2011, 39:2756–2768.

44. Rudinger J, Hillenbrandt R, Sprinzl M, Giegé R. Antideterminants present in minihelix(Sec) hinder its recognition by prokaryotic elongation factor Tu. *EMBO J* 1996, 15:650–657.

45. Calin-Jageman I, Nicholson AW. RNA structure-dependent uncoupling of substrate recognition and cleavage by *Escherichia coli* ribonuclease III. *Nucleic Acids Res* 2003, 31:2381–2392.

46. Redko Y, Condon C. Ribosomal protein L3 bound to 23S precursor rRNA stimulates its maturation by Min-III ribonuclease. *Mol Microbiol* 2009, 71:1145–1154. doi: 10.1111/j.1365-2958.2008.06591.x.

47. Lasa I, Toledo-Aranza A, Dobin A, Lasa I, Villanueva M, de los Mozos IR, Vergara-Irigaray M, Segura V, Fagegalitier D, Penadés JR, et al. Genomewide antisense transcription drives mRNA processing in bacteria. *Proc Natl Acad Sci USA* 2011, 108:20172–20177. doi: 10.1073/pnas.1113521108.

48. Lioliou E, Sharma CM, Caldeira L, Helfer A-C, Fechter P, Vandenes F, Vogel J, Romby P. Global regulatory functions of the *Staphylococcus aureus* endoribonuclease III in gene expression. *PLoS Genet* 2012, 8:e1002782. doi: 10.1371/journal.pgen.1002782.

49. Panayotatos N, Truong K. Cleavage within an RNase III site can control mRNA stability and protein synthesis in vivo. *Nucleic Acids Res* 1985, 13:2227–2240.

50. Calin-Jageman I, Nicholson AW. Mutational analysis of an RNA internal loop as a reactivity epitope for *Escherichia coli* ribonuclease III substrates. *Biochemistry* 2003, 42:5025–5034.

51. Matsunaga J, Simons EL, Simons RW. RNase III autoregulation: structure and function of mnoO, the posttranscriptional “operator”. *RNA* 1996, 2:1228–1240.

52. Mayer JE, Schweiger M. RNase III is positively regulated by T7 protein kinase. *J Biol Chem* 1983, 258:5340–5343.

53. Gone S, Nicholson AW. Bacteriophage T7 protein kinase: site of inhibitory autophosphorylation, and use of dephosphorylated enzyme for efficient modification of protein in vitro. *Protein Expr Purif* 2012, 85:218–223.

54. Dunn JJ, Studier FW. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J Mol Biol* 1983, 166:477–535.

55. Kim K, Manasherob R, Cohen SN. YmdB: a stress-responsive ribonuclease-binding regulator of E. coli RNase III activity. *Genes Dev* 2008, 22:3497–3508. doi: 10.1101/gad.1729508.

56. Chen D, Vollmar M, Ross MN, Phillips C, Kraehngebuehl R, Slade D, Mehrotra PV, von Delft F, Crosthwaite SK, Gileadi O, et al. Identification of macrodomain proteins as novel O-acetyl-ADP-ribose decacytelases. *J Biol Chem* 2011, 286:13261–13271. doi: 10.1074/jbc.M110.206771.
57. Kavalchuk K, Srinivasan M, Schnetz K. RNase III initiates rapid degradation of proU mRNA upon hypoosmotic stress in Escherichia coli. RNA Biol 2012, 9:1–12. doi: 10.4161/rna.9.1.18228.

58. Lamontagne B, Tremblay A, Abou ES. The N-terminal domain that distinguishes yeast from bacterial RNase III contains a dimerization signal required for efficient double-stranded RNA cleavage. Mol Cell Biol 2000, 20:1104–1115.

59. Lebars I, Lamontagne B, Yoshizawa S, Abou Elela S, Fourmy D. Solution structure of conserved AGNN tetraloops: insights into Rnt1p processing. EMBO J 2001, 20:7250–7258.

60. Wu H, Yang PK, Butcher SE, Kang S, Chanfreau G, Feigon J. A novel family of RNA tetraloop structure forms the recognition site for accharomyces cerevisiae RNase III. EMBO J 2001, 20:7240–7249.

61. Hartman E, Wang Z, Zhang Q, Roy K, Chanfreau G, Feigon J. Intrinsinc dynamics of an extended hydrophobic core in the S. cerevisiae RNase III dsRBD contributes to recognition of specific RNA binding sites. J Mol Biol 2013, 425:546–562. doi: 10.1016/j.jmb.2012.11.025.

62. Wang Z, Hartman E, Roy K, Chanfreau G, Feigon J. Structure of a yeast RNase III dsRBD complex with a noncanonical RNA substrate provides new insights into binding specificity of dsRBDS. Structure 2011, 19:999–1010. doi: 10.1016/j.str.2011.03.022.

63. Lamontagne B, Ghazal G, Lebars I, Yoshizawa S, Fourmy D, Abou ES. Sequence dependence of substrate recognition and cleavage by yeast RNase III. J Mol Biol 2003, 327:985–1000. doi: 10.1016/S0022-0283(03)00231-6.

64. Lavoie M, Abou ES. Yeast ribonuclease III uses a network of multiple hydrogen bonds for RNA binding and cleavage. Biochemistry 2008, 47:8514–8526. doi: 10.1021/bi080238u.

65. Sam M, Henras AK, Chanfreau G. A conserved major groove antideterminant for Saccharomyces cerevisiae RNase III recognition. Biochemistry 2005, 44:4181–4187. doi: 10.1021/bi047483u.

66. Giorgi C, Fatica A, Nagel R, Bozzi oni I. Release of U18 snoRNA from its host intron requires interaction of Nop1p with the Rnt1p endonuclease. EMBO J 2001, 20:6856–6865.

67. Lamontagne B, Catala M, Yam Y, Larose S, God L, Abou ES. A physical interaction between Gar1p and Rnt1p is required for the nuclear import of H/ACA small nucleolar RNA-associated proteins. Mol Cell Biol 2002, 22:4792–4802.

68. Han J, Lee Y, Yeom K-H, Nam J-W, Heo I, Rhee J-K, Sohn SY, Cho Y, Zhang B-T, Kim VN. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006, 125:887–901. doi: 10.1016/j.cell.2006.03.043.

69. Han J, Lee Y, Yeom K-H, Kim Y-K, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 2004, 18:3016–3027. doi: 10.1101/gad.1262504.

70. Macias S, Cordiner RA, Cáceres JE. Cellular functions of the microprocessor. Biochem Soc Trans 2013, 41:838–843.

71. Mueller GA, Miller MT, DeRose EF, Ghosh M, London RE, Tanaka-Hall TM. Solution structure of the Drosha double-stranded RNA-binding domain. Silence 2010, 1:2. doi: 10.1186/1758-907X-1-2.

72. Westenberg C, Quarles KA, Showalter SA. Dynamic origins of differential RNA binding function in two dsRBDS from the miRNA “Microprocessor” complex. Biochemistry 2010, 49:10728–10736. doi: 10.1021/bi1015716.

73. Fortin KR, Nicholson RH, Nicholson AW. Mouse ribonuclelease III. cDNA structure, expression analysis, and chromosomal location. BMC Genomics 2002, 3:26.

74. Yeom K-H, Lee Y, Han J, Suh MR, Kim VN. Characterization of DGCR8/Pasha, the essential cofactor for Drosha in primary microRNA processing. Nucleic Acids Res 2006, 34:4622–4629. doi: 10.1093/nar/gkl458.

75. Quarles KA, Sahu D, Havens MA, Forsyth ER, Westenberg C, Hastings ML, Showalter SA. Ensemble analysis of primary microRNA structure reveals an extensive capacity to deform near the Drosha cleavage site. Biochemistry 2013, 52:795–807. doi: 10.1021/bi301452a.

76. Sohn SY, Bae WJ, Kim JJ, Yeom K-H, Kim VN, Cho Y. Crystal structure of human DGCR8 core. Nat Struct Mol Biol 2007, 14:847–853. doi: 10.1038/nsmb1294.

77. Warf MB, Johnson WE, Bass BL. Improved annotation of C. elegans microRNAs by deep sequencing reveals structures associated with processing by Drosha and Dicer. RNA 2011, 17:563–577.

78. Westenberg C, Noid WG, Showalter SA. MD simulations of the dsRBP DGCR8 reveal correlated motions that may aid pri-miRNA binding. Biophys J 2010, 99:248–256. doi: 10.1016/j.bpj.2010.04.010.

79. Faller M, Matsuanga M, Yin S, Loo JA, Guo F. Heme is involved in microRNA processing. Nat Struct Mol Biol 2007, 14:23–29. doi: 10.1038/nsmb1182.

80. Senturia R, Faller M, Yin SM, Loo JA, Cascio D, Sawaya MR, Hwang D, Clubb RT, Guo F. Structure of the dimerization domain of DiGeorge Critical Region 8. Protein Sci 2010, 19:1354–1365. doi: 10.1002/pro.414.

81. Shapiro JS, Varble A, Pham AM, Tenenov BR. Noncanonical cytoplasmic processing of viral microRNAs. RNA 2010, 16:2068–2074.

82. Shapiro JS, Langlois RA, Pham AM, Tenenov BR. Evidence for a cytoplasmic microprocessor of pri-miRNAs. RNA 2012, 18:1338–1346.
83. Shapiro JS. Processing of virus-derived cytoplasmic primary-microRNAs. Wiley Interdiscip Rev RNA 2013, 4:463–471.

84. Macias S, Plass M, Stajuda A, Michlewski G, Eyras E, Cáceres JF. DGCR8 HITS-CLIP reveals novel functions for the microprocessor. Nat Struct Mol Biol 2012, 19:760–766.

85. Zhang H, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W. Single processing center models for human dicer and bacterial RNase III. Cell 2004, 118:57–68.

86. MacRae IJ, Zhou K, Li F, Repic A, Brooks AN, Cande WZ, Adams PD, Doudna JA. Structural basis for double-stranded RNA processing by Dicer. Science 2006, 311:195–198. doi: 10.1126/science.1121638.

87. Lau PW, Guiley KZ, De N, Potter CS, Carragher B, MacRae IJ. The molecular architecture of human dicer. Nat Struct Mol Biol 2012, 19:436–441. doi: 10.1038/nsmb.2268.

88. Park JE, Heo I, Tian Y, Simanshu DK, Chang H, Jee D, Patel DJ, Kim VN. Dicer recognizes the 5′ end of RNA for efficient and accurate processing. Nature 2011, 475:201–205.

89. Gu S, Jin L, Zhang Y, Huang Y, Zhang F, Valdmanis PN, Kay MA. The loop position of shRNAs and premiRNAs is critical for the accuracy of Dicer processing in vivo. Cell 2012, 151:900–911.

90. Ma E, Zhou K, Kidwell MA, Doudna JA. Coordinated activities of human dicer domains in regulatory RNA processing. J Mol Biol 2012, 422:466–476. doi: 10.1016/j.jmb.2012.06.009.

91. Wostenberg C, Lary JW, Sahu D, Acevedo R, Quarles KA, Cole JL, Showalter SA. The role of human dicer-dsRBBD in processing small regulatory RNAs. PLoS One 2012, 7:e51829. doi: 10.1371/journal.pone.0051829.

92. Lau PW, Potter CS, Carragher B, MacRae IJ. Structure of the human dicer-TRBP complex by electron microscopy. Structure 2009, 17:1326–1332. doi: 10.1016/j.str.2009.08.013.

93. Noland CL, Ma E, Doudna JA. siRNA repositioning for guide strand selection by human dicer complexes. Mol Cell 2011, 43:110–121.

94. Lee HY, Doudna JA. TRBP alters human pre-precursor microRNA processing in vitro. RNA 2012, 18:2012–2019. doi: 10.1261/rna.035301.112.

95. Fukunaga R, Han BW, Hung JH, Xu J, Weng Z, Zamore PD. Dicer partner proteins tune the length of mature miRNAs in flies and mammals. Cell 2012, 151:533–546. doi: 10.1016/j.cell.2012.09.027.

96. Simpson L, Sbicigo S, Aphashizhev R. Uridine insertion/deletion RNA editing in trypanosome mitochondria: a complex business. RNA 2003, 9:265–276.

97. Carnes J, Trotter JR, Ernst NL, Steinberg A, Stuart K. An essential RNase III insertion editing endonuclease in Trypanosoma brucei. Proc Natl Acad Sci USA 2005, 102:16614–16619. doi: 10.1073/pnas.0506133102.

98. Carnes J, Trotter JR, Peltan A, Fleck M, Stuart K. RNA editing in Trypanosoma brucei requires three different editosomes. Mol Cell Biol 2008, 28:122–130. doi: 10.1128/MCB.01374-07.

99. Hernandez A, Panigrahi A, Cifuentes-Rojas C, Sacharidou A, Stuart K, Cruz-Reyes J. Determinants for association and guide RNA-directed endonuclease cleavage by purified RNA editing complexes from Trypanosoma brucei. J Mol Biol 2008, 381:35–48. doi: 10.1016/j.jmb.2008.05.003.

100. Carnes J, Soares CZ, Wickham C, Stuart K. Endonuclease associations with three distinct editosomes in Trypanosoma brucei. J Biol Chem 2011, 286:19320–19330. doi: 10.1074/jbc.M111.228965.

101. Carnes J, Schnaufer A, Mcdermott SM, Domingo G, Proff R, Steinberg AG, Kurtz I, Stuart K. Mutational analysis of Trypanosoma brucei editosome endonucleases KREPB4 and KREPB5 reveal domains critical for function. RNA 2012, 18:1897–1909. doi: 10.1261/rna.035048.112.

102. Filipowska A, Rackham O. Modular recognition of nucleic acids by PUF, TALE and PPR proteins. Mol Biosyst. 2012, 8:699–708. doi: 10.1039/c2mb05392f.

103. Madina BK, Kuppan G, Vashisht AA, Liang Y-H, Downey KM, Wohlschlegel JA, Ji X, Sze S-H, Sacchettini JC, Read JK, et al. Guide RNA biogenesis involves a novel RNAIII family endoribonuclease in Trypanosoma brucei. RNA 2011, 17:1821–1830. doi: 10.1261/rna.2815911.

104. Dasgupta S, Fernandez L, Kameyama I, Inada T, Nakamura Y, Pappas A, Court DL. Genetic uncoupling of the dsRNA-binding and RNA cleavage activities of the Escherichia coli endoribonuclease RNAIII—the effect of dsRNA binding on gene expression. Mol Microbiol 1998, 28:629–640.

105. Watkins KP, Kroeger TS, Cooke AM, Williams-Carrier RE, Friso G, Belcher SE, van Wijk KJ, Barkan A. A ribonuclease III domain protein functions in group II intron splicing in maize chloroplasts. Plant Cell 2007, 19:2606–2623. doi: 10.1105/tpc.107.053736.

106. Kroeger TYS, Watkins KP, Friso G, van Wijk KJ, Barkan A. A plant-specific RNA-binding domain revealed through analysis of chloroplast group II intron splicing. Proc Natl Acad Sci USA 2009, 106:4537–4542. doi: 10.1073/pnas.0812503106.

107. Olinares PD, Ponnala L, van Wijk KJ. Megadalton complexes in the chloroplast stroma of Arabidopsis thaliana characterized by size exclusion chromatography, mass spectrometry, and hierarchical clustering. Mol Cell Proteomics 2010, 9:1594–1615. doi: 10.1261/mcp.M000038-MCP201.

108. Libonati M, Sorrentino M. Degradation of double-stranded RNA by mammalian pancreatic-type ribonucleases. Methods Enzymol 2001, 341:234–248.
109. D’Alessio G. The superfamily of vertebrate-secreted ribonucleases. In: Nicholson AW, ed. Ribonucleases. Nucleic Acids and Molecular Biology 26. Berlin-Heidelberg: Springer-Verlag; 2011, 1–34. doi: 10.1007/978-3-642-21078-5_1.

110. Rosenberg HF. Vertebrate secretory (RNase A) ribonucleases and host defense. In: Nicholson AW, ed. Ribonucleases. Nucleic Acids and Molecular Biology 26. Berlin-Heidelberg: Springer-Verlag; 2011, 36–53. doi: 10.1007/978-3-642-21078-5_2.

111. Hastie KM, Kimberlin CR, Zandonatti MA, MacRae IJ, Saphire EO. Structure of the Lassa virus nucleoprotein reveals a dsRNA-specific 3′ to 5′ exonuclease activity essential for immune suppression. Proc Natl Acad Sci U S A 2011, 108:2396–2401. doi: 10.1073/pnas.1016404108.

112. Hastie KM, King LB, Zandonatti MA, Saphire EO. Structural basis for the dsRNA specificity of the Lassa virus NP exonuclease. PLoS One 2012, 7:e44211. doi: 10.1371/journal.pone.0044211.

113. Hastie KM, Bale S, Kimberlin CR, Saphire EO. Hiding the evidence: two strategies for innate immune evasion by hemorrhagic fever viruses. Curr Opin Virol 2012, 2:151–156.

114. Tang TH, Rozhdestvensky TS, d’Orval BC, Bortolin ML, Huber H, Charpentier B, Branlant C, Bachellerie JP, Brosius J, Hüttenhofer A. RNomics in Archaea reveals a further link between splicing of archaeal introns and rRNA processing. Nucleic Acids Res 2002, 30:921–930.

115. Babiskin AH, Smolke CD. Engineering ligand-responsive RNA controllers in yeast through the assembly of RNase III tuning modules. Nucleic Acids Res 2011, 39:5299–5311. doi: 10.1093/nar/gkr090.

116. Babiskin AH, Smolke CD. Synthetic RNA modules for fine-tuning gene expression levels in yeast by modulating RNase III activity. Nucleic Acids Res 2011, 39:8651–8664. doi: 10.1093/nar/gkr445.

117. Castronovo M, Stopar A, Coral L, Redhu SK, Vidonis M, Kumar V, Del Ben F, Grassi M, Nicholson AW. Effects of nanoscale confinement on the functionality of nucleic acids: implications for nanomedicine. Curr Med Chem 2013, 20: 3539–3557.

118. Redhu SK, Castronovo M, Nicholson AW. Digital imprinting of RNA recognition and processing on a self-assembled nucleic acid matrix. Sci Rep 2013, 3:2530. doi: 10.1038/srep02530.