Activating silent Argonautes

Mary Anne Kidwell & Jennifer A Doudna

Multiple Argonaute proteins are implicated in gene silencing by RNA interference (RNAi), but only one is known to be an endonuclease that can cleave target mRNAs. Chimeric Argonaute proteins now reveal an unexpected mechanism by which mutations distal to the catalytic center can unmask intrinsic catalytic activity, results hinting at structurally mediated regulation.

© 2013 Nature America, Inc. All rights reserved.

During RNAi, RNAs of 20–30 nucleotides post-transcriptionally downregulate target mRNAs containing complementary sequences. The guide RNA molecules in this pathway serve to recruit key protein components including the enzyme Argonaute (Ago), which functions as the catalytic engine of the RNA-induced silencing complex (RISC). Within the RISC, Argonaute accepts small RNA duplexes and selects one strand as the guide to mediate mRNA silencing by Ago-catalyzed target-mRNA cleavage or translational silencing. Humans have four Argonaute proteins (Ago1–Ago4) associated with RNAi; however, only one is known to cleave target RNAs through its 'slicer' activity, whereas others silence mRNAs through translational repression followed by decay[1]. But what is the basis for this marked difference in Ago function, given the remarkable sequence and architectural similarity of these proteins? Two papers by Hauptmann *et al.*[2] and Schürmann *et al.*[3] in this issue of *Nature Structural & Molecular Biology* now reveal the roles of noncatalytic structural features within Argonaute that can influence enzymatic activity. This work elucidates the mechanism of the functional divergence of Ago family members and provides a basis for further analysis of their biological and biochemical behavior.

The Argonaute clade of proteins includes four structural domains: N-terminal (N), PAZ, MID and PIWI (**Fig. 1a**). These domains form a bilobed architecture, a structure that helps explain how a guide RNA is recognized through two conserved sets of interactions[4–6]. The 5′-phosphate and the first nucleotide of the guide strand are anchored by the MID domain, whereas the 3′-hydroxyl end is bound by the PAZ domain (**Fig. 1b**). The PIWI domain contains a catalytic tetrad of acidic residues that trigger the endonucleolytic cut in a target RNA[5] (**Fig. 1a**,**b**). The human Ago1 and Ago4 proteins lack the intact catalytic tetrad, and neither has RNA-cleaving activity. Surprisingly, however, both the catalytically active Ago2 and the catalytically inactive Ago3 possess these residues necessary for RNA cleavage, although only Ago2 is an active slicer enzyme. This leads to the following two questions: what renders Ago3 to be catalytically inactive, and what does this imply about the evolution of multiple Ago variants?

Hauptmann *et al.*[2] tackled these questions with chimeric proteins, first combining domains from human Ago2 and Ago3. The PIWI domain from Ago3, in the context

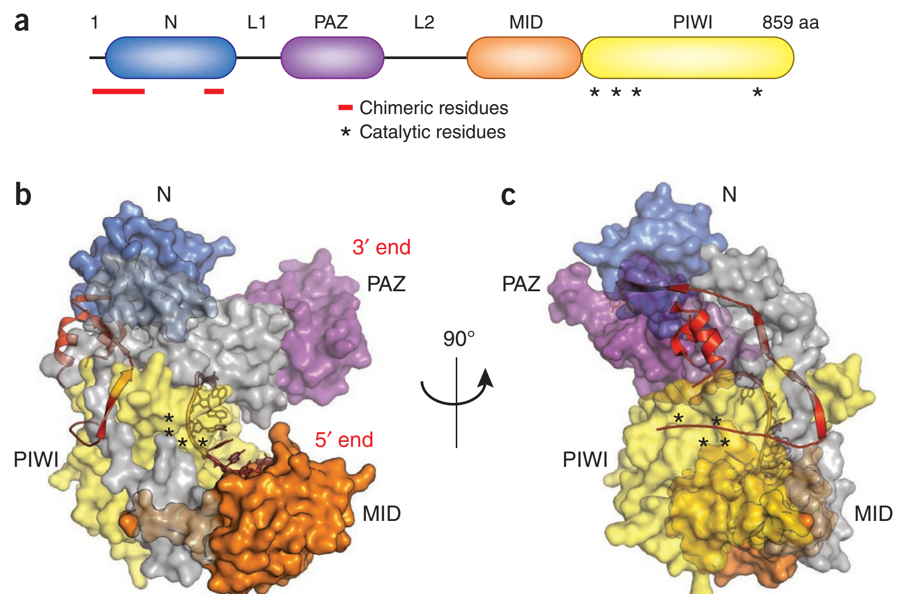

Figure 1 Model of the elements in the N domain that affect cleavage activity. (**a**) Domain architecture of Ago2 with the chimeric residues in the N domain indicated in red and the acidic catalytic residues with asterisks; aa, amino acids. (**b**) Model of the Ago2 crystal structure with the N-terminal regions affecting PIWI cleavage activity highlighted in red and the acidic catalytic residues with asterisks[4]. Nucleotides 1–8 of a guide RNA are drawn in, and the ends of the RNA are noted in red. The linker regions (L1 and L2) are drawn in gray. (**c**) Counterclockwise rotation of the Ago2 representation from **b** to match the orientation in Hauptmann *et al.*[2].

Mary Anne Kidwell and Jennifer A. Doudna are at the University of California, Berkeley, Berkeley, California, USA, and the Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, California, USA.
e-mail: doudna@berkeley.edu

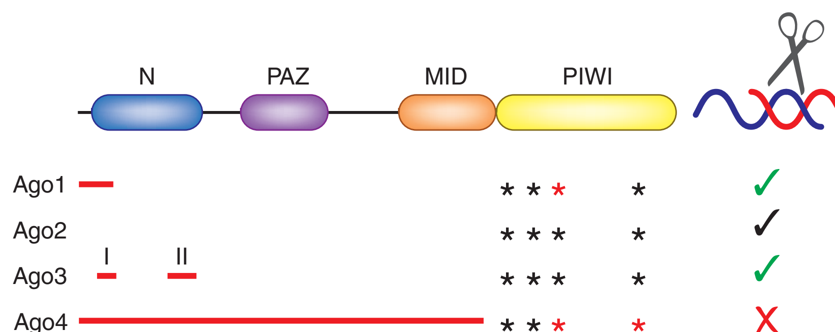

Figure 2 Minimal changes required to impart activity to Argonaute. Ago2 is the only Argonaute known to cleave target RNAs through its 'slicer' activity and needs no mutations (denoted by black check). To become an active slicer enzyme (denoted by green check) on perfect small-RNA duplexes, Ago1, similar to Ago2, required mutation of both the N and PIWI domains, whereas Ago3 required only mutation of the N domain. No chimeric protein containing Ago4 domains generated a competent enzyme (denoted by red x). Motifs I and II identified in Schürmann *et al.*[3] are indicated for Ago3.

of the remaining domains from Ago2, was found to be catalytically competent, thus confirming that the Ago3 PIWI domain does not lack intrinsic functionality. This led to the hypothesis that Ago3 prevents RNA cleavage with additional inhibitory elements. By analyzing the sequence conservation between the two Argonaute proteins, the authors designed and tested a series of chimeras, ultimately revealing two sequences in the N domain that inhibit the activity of the PIWI domain. One comprises the N terminus of the protein and forms little secondary structure, whereas the other is located toward the C-terminal end of the N domain and contains two α-helices (**Fig. 1c**). The authors speculated that the amino acids in the loops of these elements help Ago2 align the catalytic center to cleave a target RNA.

A complementary approach, called DNA family shuffling, was used by Schürmann *et al.*[3] to create a library of chimeric Argonaute proteins. This technique can generate up to tens of thousands of chimeric cDNAs at once and has been used for vaccine development and protein engineering[7]. In this modified version of PCR, highly similar gene sequences are fragmented with enzymes, heated and reannealed to allow the single-stranded DNA to bind homologous sequences. PCR is then used to extend the overlapping regions and create chimeric full-length genes. With DNA family shuffling, Schürmann *et al.*[3] isolated comparable parts of the N domain that overlap with the regions outlined above. One part (motif I) contains just five amino acids at the N-terminal end of the protein, and the other (motif II) contains a segment at the C-terminal end of the N domain (**Fig. 2**). Protein modeling based on the human Ago2 crystal structure showed that these two motifs reside adjacent to the PIWI domain. Similarly to Hauptmann *et al.*[2], Schürmann *et al.*[3] speculated that motif I in Ago2 helps position the catalytic residues to activate Argonaute, specifically a glutamate whose contributions to catalysis were not recognized until the recent crystal structures of Argonaute were published[5]. They predicted that the unique region in Ago3 prevents this reorientation, inhibiting the activity of the protein. They further proposed that motif II inhibits guide-target pairing with additional amino acids in the loop between the α-helices; this bulky loop blocks a channel in Argonaute needed to propagate base-pairing with target RNAs.

The same trend of inhibitory regions in the N domain is observed with human Ago1. Ago1 has a PIWI domain lacking the acidic residues, but once it is made catalytically active by the appropriate mutation, the N domain of Ago1 still prevents cleavage (**Fig. 2**). By replacing just the residues at the N terminus of the protein, Hauptmann *et al.*[2] created a fully competent slicer enzyme. These results, in combination with data from other species, indicate that the regulatory role of the N domain is well conserved among Argonautes. The crystal structure of a bacterial Argonaute from *Thermus thermophilus* revealed that the N domain prevents proper base-pairing with target RNA through steric clashes[8]. However, once this part of the N domain (corresponding to the first 106 amino acids of the protein) was deleted, the *T. thermophilus* Ago was rendered catalytically inactive, a result suggesting that this domain could stabilize the active complex. In *Drosophila melanogaster*, mutations to the N-terminal lobe also affected the activity of the PIWI domain[9]. Finally, another study using alanine scanning identified amino acids in the same region of Ago2 that could affect RISC assembly[10].

Although it is now clear that mutations distal to the catalytic center can dramatically change the activity of Argonaute, it remains unclear why there are four distinct Argonaute proteins in humans. In considering the evolution of the Ago proteins, it is notable that Ago4 is the most divergent of the four proteins and is not expressed in most human cell lines[11]. Using their library of chimeric proteins, Schürmann *et al.*[3] noted that no chimera containing any part of Ago4 was competent for slicing, reinforcing the idea that Ago4 is evolutionarily and functionally distinct from the other human Ago proteins (**Fig. 2**). The library created by Schürmann *et al.*[3], containing >35,000 chimeras, could help unravel the mystery of gene duplications for Argonaute. This library could probe Argonaute structural features' complex effects not only on RNA cleavage but also on gene silencing. It may be that the different natural Argonautes can fine-tune the rate and extent of mRNA suppression, to provide exquisite control over biological outcomes.

Beyond Argonaute's intrinsic behavior, the interactions between Argonaute and other proteins also contribute to gene-silencing efficiency. Various proteins assist Argonaute in translational repression of mRNAs, including glycine-tryptophan proteins that directly bind the PIWI domain[12]. The studies from Hauptmann *et al.*[2] and Schürmann *et al.*[3] raise the interesting possibility that such interactions could activate Ago3 or inactivate Ago2 by rearranging Argonaute's N-terminal extensions. Dramatic conformational rearrangements mediated by the chaperone machinery are already known to help create a mature RISC[13,14]. Corroborating the results discussed here, a related recently published crystal structure of Ago1 confirms the regulatory role of the N domain[15]. Future studies should reveal the properties of different Argonaute complexes and how they contribute to small-RNA biology.

Corrected after print 12 July 2013

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Wilson, R.C. & Doudna, J.A. *Annu. Rev. Biophys.* **42**, 217–239 (2013).
2. Hauptmann, J. *et al. Nat. Struct. Mol. Biol.* **20**, 814–817 (2013).
3. Schürmann, N. *Nat. Struct. Mol. Biol.* **20**, 818–826 (2013).
4. Schirle, N.T. & MacRae, I.J. *Science* **336**, 1037–1040 (2012).
5. Nakanishi, K., Weinberg, D.E., Bartel, D.P. & Patel, D.J. *Nature* **486**, 368–374 (2012).
6. Elkayam, E. *et al. Cell* **150**, 100–110 (2012).

© 2013 Nature America, Inc. All rights reserved.
npg

7. Crameri, A., Raillard, S.A., Bermudez, E. & Stemmer, W.P. *Nature* **391**, 288–291 (1998).
8. Wang, Y. *et al. Nature* **461**, 754–761 (2009).
9. Hur, J.K., Zinchenko, M.K., Djuranovic, S. & Green, R. *J. Biol. Chem.* **288**, 7829–7840 (2013).
10. Kwak, P.B. & Tomari, Y. *Nat. Struct. Mol. Biol.* **19**, 145–151 (2012).
11. Petri, S. *et al. RNA* **17**, 737–749 (2011).
12. Till, S. *et al. Nat. Struct. Mol. Biol.* **14**, 897–903 (2007).
13. Iwasaki, S. *et al. Mol. Cell* **39**, 292–299 (2010).
14. Iki, T. *et al. Mol. Cell* **39**, 282–291 (2010).
15. Faehnle, C.R., Elkayam, E., Haase, A.D., Hannon, G.J. & Joshua-Tor, L. *Cell Rep.* published online, http://dx.doi.org/doi:10.1016/j.celrep.2013.05.033 (6 June 2013).

Antibodies expose multiple weaknesses in the glycan shield of HIV

Max Crispin & Thomas A Bowden

A shield of glycans coats the viral-envelope proteins of HIV. Recent work shows how broadly neutralizing antibodies can recognize this shield despite structural variation in these 'self' carbohydrate structures.

© 2013 Nature America, Inc. All rights reserved.

npg

Although the HIV proteins responsible for attachment and fusion spike (gp120 and gp41) are encoded by the viral genome, they contain a dense array of glycans attached by infected cells during viral biogenesis (**Fig. 1**). These 'self' glycan structures have been shown to protect the virus from antibody neutralization[1]. However, there is a growing body of evidence suggesting that antibodies capable of neutralizing a broad range of viral isolates can do so by binding these 'self' glycans. One critical factor that makes HIV glycans an attractive vaccine target is that their structural variation is considerably lower than that of the viral protein sequence[2,3]. In this issue, Kong *et al.*[4] and Pancera *et al.*[5] demonstrate that there are multiple modes of viral neutralization involving glycan recognition and show how such broadly neutralizing antibodies (bnAbs) can tolerate structural diversity within both highly conserved and more variable regions of the glycan shield. Together, these studies reveal sites of vulnerability in the viral envelope.

Although assembled by the cell, the processing of gp120 glycans differs from that typically seen on host cell glycoproteins. Glycans constitute approximately half the mass of gp120. Such dense packing of glycans structurally precludes full enzymatic processing from oligomannose to hybrid-type and then complex-type glycosylation, leading to the emergence of oligomannose-type glycosylation, also called the 'mannose patch' phenomenon.

Max Crispin is at the Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford, UK. Thomas A. Bowden is at the Division of Structural Biology, University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, UK.
e-mail: max.crispin@bioch.ox.ac.uk

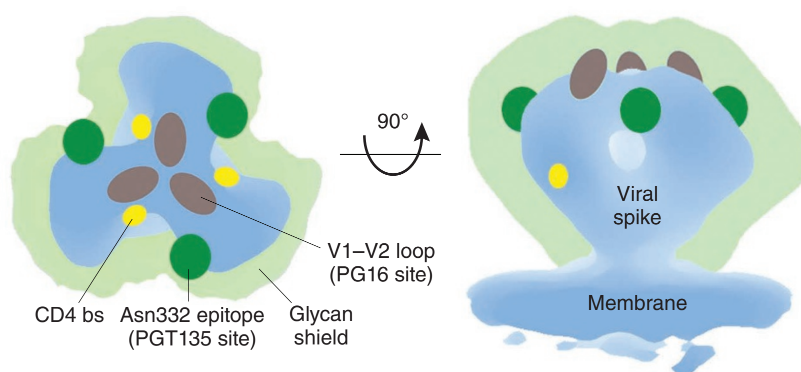

Figure 1 Organization of the viral spike of HIV. The protein surface (blue) is based on cryo-EM analysis[20]. The glycan shield is shown in light green, the CD4-binding site (bs) in yellow, the glycan epitope at Asn332 in dark green and the V1–V2 loop in brown.

Virus neutralization through antibody recognition of this mannose patch was originally demonstrated for 2G12, an antibody that binds the termini of oligomannose-type glycans and achieves avidity by unusual domain exchange of the heavy chains[6–8]. Many glycan-binding bnAbs have since been isolated[9], and a few (PGT128, PG9 and PGT121) have been crystallized in complex with their respective glycan-bearing antigens[10–12] to reveal how glycan recognition may be achieved by a single Fab without domain exchange.

Now Kong *et al.*[4] reveal the epitope of PGT135 by crystallographic analysis of the Fab in complex with gp120, CD4 and Fab 17b (ref. 7). The PGT135 epitope is dominated by two glycans (at Asn332 and Asn392) that flank a central protein-protein interface. The structure of the PGT135 epitope, together with previous analysis of the epitopes of 2G12 and PGT128, reveals that the Asn332 oligomannose-type glycan is structurally conserved and can be recognized by a large range of clonally unrelated antibodies exhibiting diverse binding modes (**Fig. 2**)[8,10]. Consequently, there may be multiple immunological solutions to the recognition of this epitope, and these may potentially be inducible by vaccination.

A major challenge for the generation of bnAbs to the glycan shield is how to maintain neutralization breadth and potency despite microheterogeneity at individual glycan sites together with the shifting or mutation of glycosylation sites across viral isolates[13]. Within the mannose patch, microheterogeneity is more restricted than that observed on the variable loops, which can exhibit a larger range of structures, depending on cell type and the viral biogenesis pathway adopted. Kong *et al.*[4] show that the binding mode of PGT135 can tolerate a limited variation in the composition of the oligomannose-type glycans, particularly at the second Asn392 site.

Pancera *et al.*[5] reveal the architecture of PG16 in complex with a V1-V2-loop scaffold expressed to contain hybrid-type glycans. PG16 recognizes a mixed glycan/protein epitope, but there are important differences in its recognition mode compared to those of bnAbs that recognize glycans of the

Erratum: Activating silent Argonautes

Mary Anne Kidwell & Jennifer A Doudna

***Nat. Struct. Mol. Biol.* 20, 769–771 (2013); published online 3 July 2013; corrected after print 12 July 2013**

In the version of this article initially published, in Figure 2 the asterisks representing mutations to the PIWI domain that turn Argonaute into an active enzyme should have been colored red. The error has been corrected in the HTML and PDF versions of the article.

© 2013 Nature America, Inc. All rights reserved.
npg