Structure, Vol. 12, Feburary, 2004, ©2004 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.str.2004.01.020

Gauging Length: Recognition of Small Interfering RNAs

The first crystal structures of small interfering RNA (siRNA)-protein complexes revealed an elegant caliper-like principle of length selection of siRNAs.

Initially identified as a conserved defense mechanism triggered by dsRNAs in a broad range of eukaryotic organisms against molecular parasites such as viruses, RNA silencing has now emerged as a universal interfering mechanism of endogenous gene expression that operates at multiple levels including mRNA degradation, translational suppression, and chromatin remodeling (Hannon, 2002; Zamore, 2002). Induced RNA interference has also becoming a widely used tool to knock down specific gene expressions and to probe gene functions (Tuschl, 2003).

The major intermediates of any RNA silencing pathway appear to be siRNAs of 21–26 nucleotides (nt) in length with 5′-phosphates and 3′-hydroxyls that form duplexes with 2 nt 3′-overhangs. They are generated from dsRNAs or RNA hairpin-containing microRNA precursors by ribonuclease III-like enzymes such as Dicer. siRNAs are incorporated into RNA-induced silencing complexes (RISCs) or other effector complexes for targeting homologous sequences for gene silencing at multiple levels. They may also act as primers for RNA-dependent RNA polymerases during the amplification of silencing signals.

Recent literature has seen the first example of siRNA recognition from the crystal structures of an siRNA (21 nt, 19 base pair [bp]) in complex with the RNA silencing suppressor p19 of two plant viruses of the tombusvirus genus, tomato bushy stunt virus (TBSV) at 1.85 Å resolution (Ye et al., 2003), and carnation Italian ringspot virus (CIRV) at 2.5 Å resolution (Vargason et al., 2003), respectively. Tombusviruses possess ssRNA genomes and replicate through dsRNA intermediates that are strong inducers of RNA silencing. As a counter defense, tombusvirus p19 proteins interact with siRNAs, preferably with duplex regions of 18–22 bp in lengths, and sequester them from being incorporated into silencing effector complexes.

In both structures, each homodimeric p19 forms a central saddle-like 8-stranded β sheet that embraces the entire 19 bps along one face of the siRNA duplex (Figure 1), in which the asymmetric siRNA interacts with the symmetric dimeric p19 in a mixture of two opposite and almost superimposable orientations. The siRNA adopts basically an A-form conformation with its helical axis bending by 40° toward the protein. It contacts the protein with the central minor groove and two adjacent partial major grooves of its duplex region. The use of an extended β sheet to serve as the main surface for interaction with dsRNAs presents a new mode of protein-dsRNA interaction, although partial structural homology of p19 to dsRNA-interacting ribosomal protein L1 was observed. Most of the p19-siRNA interactions are limited to the sugar-phosphate backbone of the siRNA, characteristic of sequence independent recognition.

Most elegantly, the structures revealed that p19 recognizes siRNAs almost exclusively by length selection of their duplex regions, in a manner similar to accurate length measurements by caliper-like devices (Vargason et al., 2003; Ye et al., 2003). Two symmetry-related "reading head" helices of the p19 dimer bracket both ends of the siRNA duplex. A pair of critical tryptophan residues, W42 and W39, project from the helix to form stacking interactions with the 5′- and the 3′-bases, respectively, to extend the terminal base pairs at either end by an additional step (Figure 1). Surprisingly, W39 is not well conserved among tombusvirus p19s, suggesting variable recognition of the 3′-end of the RNA duplex. Blunt ended dsRNAs have higher affinity to p19 than their siRNA counterparts with 3′-overhangs, perhaps due to the additional energy required to peel away the 3′-overhangs before stacking could occur. Consistent with the importance of end-base stacking in the recognition of siRNAs by p19, structure-based mutational analyses showed that substitutions of either one or both of the tryptophan residues to arginines resulted in attenuation of the lethal necrosis phenotype of the wild-type virus and that removal of the side chains as in W39G mutation completely abolished p19 suppressor activity (Vargason et al., 2003).

Additional structural insights were obtained in each of the two p19-siRNA complexes. The TBSV p19-siRNA complex revealed an unusual hydroxyl-group hydrogen-bonding network between the 2′-hydroxyls of the central minor groove of the siRNA and a bed of serine and threonine side chains at the surface of the central β sheet of p19, explaining the specificity of p19 for dsRNA and the lack of affinity for dsDNA (Ye et al., 2003). The siRNA used in complex with CIRV p19 has 5′-phosphates, which were revealed to form hydrogen bonds with the imino nitrogens of stacked W42, indicating a preference of p19 for 5′-phosphorylated siRNAs. Consistent with this observation, removal of 5′-phosphates from a 21 nt siRNA was shown to reduce its affinity to p19 from a dissociation constant of $\sim 10^{-10}$ to $\sim 10^{-9}$ M (Vargason et al., 2003).

How does p19 accommodate siRNAs of a range of lengths? Although the structures do not provide a definitive answer to this question, it appears that the "reading head" helices of p19 exhibit certain degree of structural plasticity, as suggested by the partial disordering in the linker between the helices and the main β sheet. It is possible then that the precise positioning of the "reading head" helices in the structures is determined by the end-base stacking interactions. Therefore, although the exact structural basis of this adaptability remains to be seen, perhaps from structures of p19 alone and in complex with siRNAs of different lengths, it is clear that

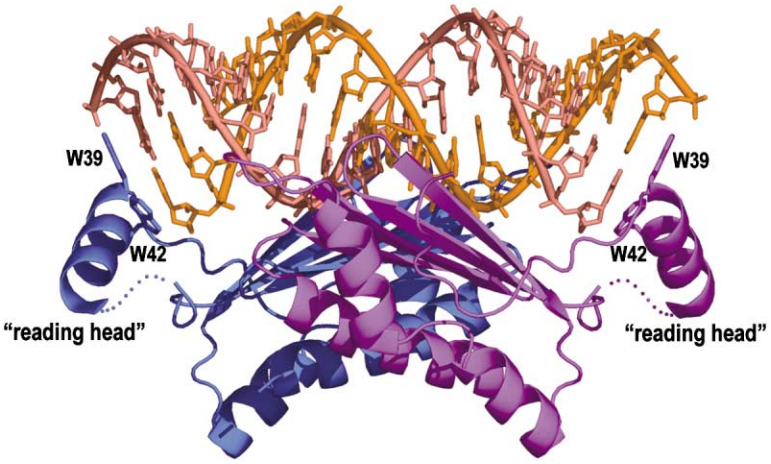

Figure 1. Structure of p19-siRNA Complex
Adapted from Ye et al., 2003.

p19 is capable of accurate yet adaptable interactions with siRNAs.

Do the current structures have implications to siRNA recognition in RNA silencing pathways? Specific recognition of siRNAs may be required during both the generation of siRNAs and the incorporation of siRNAs into RISCs or other effector complexes. Since siRNAs have the characteristics of specific lengths, 5′-phosphates and 2 nt 3′-overhangs, it is predictable that siRNA recognition would utilize one, several or all of these features. In an optimal case, perhaps all distinguishing features of siRNAs are recognized simultaneously by a combination of proteins or domains. Recent structures of PAZ domains from RISC components revealed that they might recognize 3′-overhangs and 5′-phosphates of siRNAs and/or ssRNAs (Lingel et al., 2003; Song et al., 2003; Yan et al., 2003). How about length recognition? Is it far fetched to envision the existence of a similar mode of length selection in RNA silencing pathways? In any case, nature has a way of surprising the mind and many excitements are bound to arrive with more structural and biochemical studies in this ever-accelerating field.

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Selected Reading

Hannon, G.J. (2002). Nature *418*, 244–251.

Lingel, A., Simon, B., Izaurralde, E., and Sattler, M. (2003). Nature *426*, 465–469.

Song, J.J., Liu, J., Tolia, N.H., Schneiderman, J., Smith, S.K., Martienssen, R.A., Hannon, G.J., and Joshua-Tor, L. (2003). Nat. Struct. Biol. *10*, 1026–1032.

Tuschl, T. (2003). Nature *421*, 220–221.

Vargason, J.M., Szittya, G., Burgyan, J., and Tanaka Hall, T.M. (2003). Cell *115*, 799–811.

Yan, K.S., Yan, S., Farooq, A., Han, A., Zeng, L., and Zhou, M.M. (2003). Nature *426*, 468–474.

Ye, K., Malinina, L., and Patel, D.J. (2003). Nature *426*, 874–878.

Zamore, P.D. (2002). Science *296*, 1265–1269.

Structure, Vol. 12, January, 2004, DOI 10.1016/j.str.2004.01.018

The 447-52D Antibody: Hitting HIV-1 Where Its Armor Is Thickest

The problem of recognizing "non-self" is immensely more difficult when the "non-self" in question is hypervariable. In this issue of *Structure*, Stanfield et al. (2004) reveal the atomic-level basis for broad recognition of a hypervariable loop of HIV-1.

Antigenic variation is a dominant mechanism of viral escape from the immune system (Sharp, 2002) and, as the annual aches and fevers from influenza virus show, a highly successful one. The human immunodeficiency virus type-1 (HIV-1) is a "master of disguise," able to alter itself at an evolutionary rate estimated to be a million times faster than that of its human host. This variation allows HIV-1 to stay several steps ahead of the immune system and is an essential component of the virus's ability to establish a persistent ultimately fatal infection.

If variation is armor, then the site where HIV-1's armor is thickest is arguably the hypervariable V3 loop. This loop, on the exterior gp120 envelope glycoprotein, is a site of extraordinary sequence variation in the HIV-1