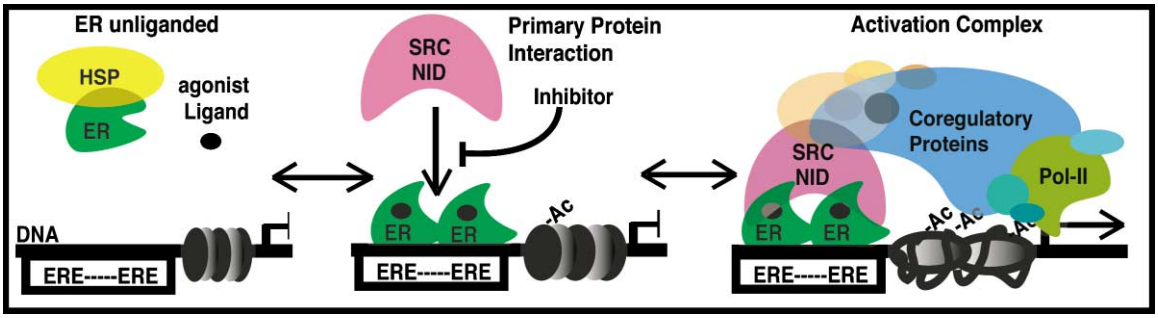

Figure 1. Ligand-Dependent Nuclear Receptor Regulatory Transcription Complex Assembly
This is Figure 1 from Geistlinger et al. [11].

agonist [11]. They show that two NR receptors, ERα and ERβ, bind to the same agonist ligand, which then exposes distinctive structural areas of that NR•agonist complex to the SRC2 NID (i.e., NR box). Using a NR box proteomimetic they had developed earlier [8], Guy and coworkers demonstrate selective recruitment of either ERα or ERβ with the same agonist hormone. These proteomimetic NR boxes were shown to selectively inhibit the binding of SRC2 to the ERα•agonist complex over the ERβ•agonist complex, preferentially forming the ERα•agonist•proteomimetic. Interestingly, Guy and coworkers found that the proteomimetic was unique to the agonist ligand used with the ER receptor. That is, three different agonists were used, estradiol, diethyl stilbesterol, or genistein, and each ERα•agonist•proteomimetic complex had unique binding selectivity compared to using the same agonist for the ERβ•agonist•proteomimetic complex.

Furthermore, in contrast to earlier work that utilized linear peptides [9, 10], Guy and coworkers found that the SRC binding pockets of the ERs have a strong selectivity for phenylalanine when utilizing a constrained scaffold. It is believed that the entropic cost of the necessary α-helix formation, which is high in linear peptides, is overcome in the constrained peptide system described by Guy. Thus, a more realistic method for screening small molecules that prevent binding of SRC to a NR•agonist complex is to utilize a constrained, induced fit, α-helix to appropriately present these hydrophobic residues. The success of these small molecule proteomimetics (Guy NR boxes) is a significant achievement, as it will allow one to observe the consequences of selectively recruiting ERβ to regulate transcription. In addition, the Guy proteomimetic approach can be generalized where one can envision screening small molecule "NR box" libraries in search of tools that will eventually uncouple the function of all NRs. This will allow the selective regulation of individual NRs and, therefore, reveal the connection between individual transcription regulation of these NRs and their associated diseases.

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Chemistry & Biology, Vol. 11, February, 2004, DOI 10.1016/j.chembiol.2004.02.006

How to Silence Silencing

Two recent reports [1, 2] describe the stunning crystal structures of complexes between a viral protein that suppresses RNA silencing and a 21 nucleotide small interfering (si)RNA.

RNA silencing is part of an innate defense strategy against viruses in plants [3]. Both suppressor proteins come from plant viruses belonging to the Tombusvirus

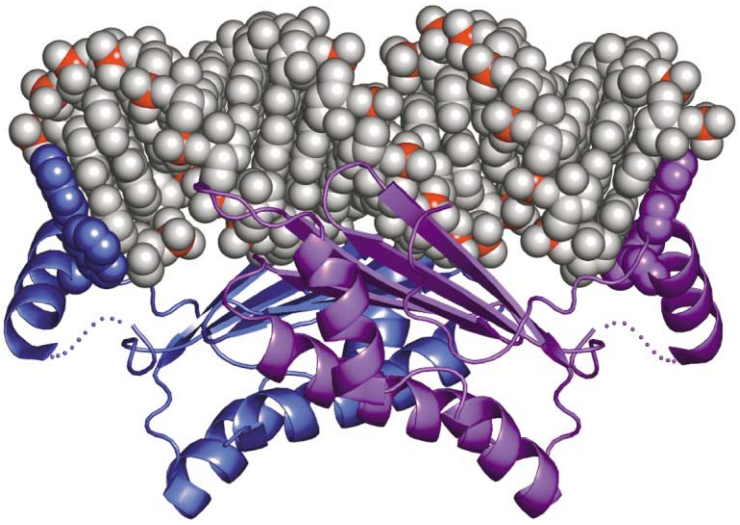

Figure 1. The Structure of the p19-siRNA Complex

The p19 dimer cradles the siRNA duplex with the concave surface of a β sheet and caps the duplex ends with two projecting α helices. Individual monomers of the p19 dimer are colored blue and magenta. The RNA and the two tryptophans from each monomer of the p19 dimer bracket the terminal base pairs at either end of the siRNA duplex and are shown in space-filling representation. Phosphate atoms of RNA are colored red. I am grateful to K. Ye and D. Patel (Sloan Kettering Institute) for allowing me to reproduce this drawing.

genus: one from Tomato bushy stunt virus (TBSV) [2] (which is the type member of the genus and a virus with historical importance in crystallography [4]) and the second from the carnation strain of TBSV called the Carnation Italian ringspot virus (CIRV) [1]. Those icosahedral viruses have positive sense single-stranded RNA genomes with five open reading frames, the fifth one being a 19 kDa protein (p19), the function of which has been unknown for a long time. Recently, it was shown that the 19 kDa protein, like other so-called viral pathogenecity determinants of many viruses, suppresses posttranscriptional gene silencing [5]. Simultaneously, it was noticed that RNA molecules 21–23 nucleotides in length accumulated in cells with gene silencing activity [6]. Similarly, in animals undergoing RNA interference, 21–23 nt RNAs were isolated and found to be associated with a sequence-specific nuclease activity, the RNA-induced silencing complex (RISC) [6–8]. Later, the protein responsible for the processing of double-stranded RNAs into 21–23 nt RNAs was assigned to the RNase III-related protein, Dicer, in *Drosophila* [9]. In 2002, Silhavy and coworkers [10] showed that p19 binds 21 nt synthetic double-stranded RNAs with 2-nt long 3′ overhanging ends in vitro. They concluded that the silencing suppression mediated by p19 is due to a depletion of the 21–23 nt dsRNAs generated by the posttranscriptional gene silencing processes. The two crystal structures [1, 2] highlighted below reveal how the p19 protein recognizes and binds 21 nt dsRNAs.

Each complex contains a 19 bp RNA duplex with two uridines dangling at the 3′ ends. Such RNAs are the products (siRNAs) that result when the ribonuclease DICER processes double-stranded RNAs. One complex was solved at 2.5 Å [1], and the other was solved at 1.85 Å [2]. The two proteins are very similar (with 12 amino acid changes among the 172 residues). The p19 proteins bind as a dimer to the 21 nt siRNA. The monomer is composed of a four-stranded β sheet stacked on three α helices (α+β sandwich), and the dimerization leads to an eight-stranded β sheet forming a concave surface that binds to the shallow minor groove in the center part of the double-stranded RNA. The fold of the protein is unusual; it is very different from the αββββα fold of several double-stranded binding proteins [11] or from the OB fold recently found rather close to the RNA binding motif present in the Argonaute2 Paz, also part of the RNAi machinery [12]. Vargason and coworkers [1] found a similarity in topology with the ribosomal protein L1 (if one assumes a circular permutation) [13]. The diversity in viral suppressors of silencing has already been noticed and attributed to evolutionary convergence [3]. Circularly permuted proteins, not a rare occurrence in nature, are thought to arise mainly through gene duplication or exon shuffling [14].

Dimerization of the protein places tryptophans W39 and W42, separated by one α-helical turn, symmetrically so that the terminal base pairs of the double-stranded 19 bp RNA helix can stack below them on either side (see Figure 1). Only the second tryptophan, W42, stacked on the 5′ end base, is conserved. In addition, W42 is framed by two conserved residues that form important contacts: R43 interacts with the 2 nt 3′ end overhang, and E41 forms a conserved salt bridge with R75 in the β sheet. Overall, there are three salt bridges between conserved residues, and all of these occur at the interface between the N- and C-terminal domains of p19 [1]. W39 is semiconserved and can be replaced with arginine, not a surprising observation, but it can also be replaced by serine and leucine.

The RNA duplex is regular, with no distortion in one crystal structure but with a 40° bend in the other one. Similarly, although in one case the 2 nt 3′ dangling residues were observed in the electron density with precise contacts with the protein, this was not the case in the other crystal structure. It is unclear, however, whether these differences are really meaningful. Indeed, the space groups of the crystals are different ($P6_122$ [1] and R32 [2]) and in one (R32), the asymmetric unit is a monomer of p19 with one RNA strand, whereas in the other crystal ($P6_122$), the dimeric complex forms the asymmetric unit. However, in both cases the RNA can bind in two ways about the RNA pseudo-dyad axis

(which is coincident in R32 with a crystallographic 2-fold and with a pseudo-2-fold in $P6_122$). The end result is that, in the two crystals, the double-stranded RNA is present twice at half occupancy. Interestingly, in the hexagonal crystal, the RNA sequence is almost a palindrome (with the central base pair being a C...C opposition), whereas it is not in the rhombohedral crystal. The different crystal packings, the symmetry considerations, and the presence of a C…C opposition in the center of one siRNA may limit the structural comparisons between the two siRNAs.

Thus, the protein architecture accommodates the entire length of a 19 bp RNA duplex by capping the polymer and sandwiching it between two tryptophan residues independent of the RNA sequence. Indeed, as is the case for many dsRNA binding proteins [11], the lack of sequence specificity is a component of the molecular recognition modes. Most of the recognition contacts between the protein and the RNA occur via the phosphate groups and the RNA specific O2′ hydroxyl group, with almost no specific contacts with the bases [15]. The interactions with the ribose hydroxyl groups are especially worth noticing. First, the floor of the β sheet is rich in serine and threonine [2]. Second, several water molecules are integral to the interface. For example, the hydroxyl of conserved serine 124 of the β4 strand (which hydrogen bonds to its symmetrically oriented counterpart in the dimer) contacts the 2′ hydroxyl group via a water molecule and also directly contacts the 2′ hydroxyl of the 11th RNA residue, in both complexes, and to atom N3 of the base in the two independent complexes of the hexagonal crystal. Conserved threonine 122 and glycine 118 also have similar water-mediated contacts in the two crystals. Several of those types of water-mediated contacts are frequently observed in crystal structures of uncomplexed RNA molecules too, especially the configuration in which a water molecule bridges the hydroxyl O2′ and the N3 (or O2) atom of the attached base [16]. In uncomplexed RNA crystals, such a bridging water molecule has been observed interacting either with another water molecule or with the hydroxyl of a serine or a threonine; alternatively, the water molecule is replaced altogether by an amino acid hydroxyl group. In the case of the P19-siRNA structures, the water has altogether been replaced by an amino acid hydroxyl group. Although, because of the usual crystallographic limitations, the full set of hydration water is far from being observed, one can realize that the RNA is not fully dehydrated in the bound state and that it is only partially dehydrated during the recognition process. Similar conclusions were reached for the binding of antibiotics to RNA molecules [17].

Both papers constitute marvelous examples of structural biology at its best. Furthermore, these two structures have numerous implications for our further understanding of the mechanisms of silencing and its suppression. They open also the way to the use of p19 protein for suppressing silencing in heterologous systems.

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