taken from the spider Argiope trifasciata. Atomic force microscopy (AFM) and fluorescence spectroscopy were performed on manually drawn fibers. Circular dichroism spectroscopy and an initial low-resolution NMR structure both indicate that monomeric aciniform spidroin protein is predominantly α-helical in solution but changes to β-sheet in the fiber, as indicated by thioflavin T fluorescence co-localized with fibers. AFM characterization provides nano-scale surface chemistry and mechanical information for the silk fiber. Our ongoing work is correlating structure to mechanical properties to elucidate the mechanism by which spider silk fibers form.

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Structural Analysis of the Pf1 Subunit of the Sin3S/Rpd3S Complex and its Implications in Chromatin Targeting and Complex Assembly

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Multi-protein complexes containing histone deacetylase (HDAC) activities play key roles in regulating eukaryotic gene transcription by altering 'the histone code' and modulating chromatin structure dynamics. The evolutionarily-conserved, HDAC-associated, ~0.5 MDa Sin3S/Rpd3S complex is implicated in repressing transcription from cryptic start sites in the intragenic regions of transcriptionally-active genes and in limiting DNA damage due to genotoxic stress. Unlike the related ~2 MDa Sin3L/Rpd3L complex, which is targeted to promoter regions of genes through interactions with sequence-specific DNA-binding repressors, the smaller Sin3S/Rpd3S complex is targeted to intragenic regions through interactions with H3 K36(me_2/me_3)-modified histones. The Sin3S/Rpd3S complex comprises at least five subunits of which the MRG15 and Pf1 subunits are unique to this complex and play important roles in chromatin targeting and complex assembly. Pf1 harbors two PHD zinc fingers of unknown function and two Sin3 interaction domains - SID1 and SID2 - that interact with the PAH2 and PAH1 domains, respectively, of Sin3. Pf1 SID1 overlaps with a segment that we recently identified as being critical for interactions with MRG15 - the subunit that is thought to interact directly with H3 K36(me_2/me_3). We have used solution NMR spectroscopy to investigate the network of protein-protein interactions involving Pf1 and to characterize the structure and potential functions of its PHD domains. These studies provide unexpected insights into the assembly of this co-repressor complex besides affording new insights into Sin3 PAH-SID interactions and into the structure and function of PHD fingers. The results of these studies will be presented and their implications in chromatin/transcription biology discussed.

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Structure-Function Analysis of MRG15, a Chromatin-Targeting Protein Invovled in Cell Growth and Aging

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MRG15 is a member of the mortality factor family of proteins that have been implicated in processes involving cell growth and aging and is found in at least two distinct chromatin-modifying complexes, a 5-subunit histone deacetylase (HDAC)-complex containing the Sin3 corepressor and the 11-subunit NuA4 histone acetyltransferase complex. At the molecular level, MRG15 has been implicated in targeting the Sin3 corepressor complex to histones enriched in H3 K36(me_2/me_3) found in actively transcribed chromatin via a N-terminus chromodomain. The MRG domain at its C-terminus is thought to link MRG15 with Pf1 and thereby to the Sin3 and HDAC proteins in the Sin3 complex. MRG domains are common to all the mortality factor proteins and although the structure of the MRG domain is known, there is no information about the nature of the targets and how it interacts with them.

We have characterized the interaction of the MRG15 chromodomain with its cognate chromatin target and also that of the MRG15 MRG domain with Pf1. Our results indicate a surprisingly low affinity interaction between the chromodomain and its chromatin target, but a much stronger interaction between the MRG domain and Pf1; interestingly, other H3 methylated lysine peptides display comparable affinities to H3 K36(me_2/me_3). We have identified and characterized the MRG-interaction motif in Pf1 using a combination of mutagenesis, biochemical assays, informatics, and solution NMR; an analogous motif is also found in other MRG-domain interactors such as PALB2 and PAM14. The motif binds to MRG15 MRG with high affinity and competitively inhibits the oligomerization activity of MRG15 MRG.

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NMR Dissection of the Detailed Mechanism for Antibiotic Binding to Asite RNA

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A growing number of studies are suggesting the importance of non-coding RNAs that play a variety of roles by undergoing conformational changes in response to a specific cellular signal. It has been increasingly believed that this predisposition of RNA conformational changes in a specific manner is encoded by the flexibility that in turn is encoded in RNA sequences. Base flipping is a conformational transition that occurs ubiquitously across diverse RNA functional and structural contexts. A prominent example is Asite ribosomal RNA that contains two highly conserved internal loop adenines A1492 and A1493, which serve to decode the mRNA message by looping out and stabilizing a codon-anticodon mini-helix when it is formed between mRNA and its cognate aa-tRNA. Asite is also known to bind to many antibiotics where drug binds the internal loop, flips the two adenines out and the adenines are forced to bind the codon-anticodon minihelix irrespective of correctness of tRNA.

In this study, we attempt to dissect the process of Asite drug binding further using NMR spin relaxation techniques. Site-specific measurement of fast dynamics (using transverse and longitudinal relaxation measurements) and slow dynamics (using R1rho dispersion measurements) on free and bound Asite RNA provide insights into the transition states of binding. Our results highlight the significance of various neighboring bases that dynamically encode the process of base flipping that is vital for an effective antibiotic binding.

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Enzymatic Synthesis of Site-Specific Labeled NTPS used for *In Vitro* Transcription of RNAs to Facilitate Multi-Dimensional Nuclear Magnetic Resonance Spectroscopic Studies

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RNAs, more than ever before, are increasingly viewed as biomolecules at the forefront of the life and chemical sciences, given their diverse functional capabilities and ability to adopt intricate three-dimensional folds. Structural biologists faced with two critical challenges of chemical shift overlap and fast signal decay for large RNAs (>15 KDa) have resorted to stable-isotope labeling to circumvent the overcrowding problem. In this work, we have developed a facile and streamlined means of producing eight non-commercially available recombinant enzymes from the pentose phosphate pathway for synthesizing such labeled nucleotides. The enzyme preparations were purified to >90% homogeneity by a one-step Ni-NTA affinity chromatography resulting in specific activities comparable to or higher than those previously reported. These new site-specific labeled nucleotides made by these enzymes can be used to transcribe RNAs of any sequence and length. To demonstrate the usefulness of the site selectively labeled nucleotides, the highly conserved 36-nucleotide domain 5 (D5) RNA structure, which is the heart of the group II intron ribozyme machinery, was transcribed and various multidimensional NMR experiments tailored for these labels were run. The availability and reliability of these constructs would render production of fully and site-specific labeled nucleotides for transcribing RNA more accessible and straightforward, and facilitate high-resolution NMR spectroscopic and other biophysical studies.

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Mapping Col E1 RNA I - RNA II Kissing Complex and ROM Binding Interface using Paramagnetic Relaxation Enhancement NMR

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The RNA II transcript of ColE1 plasmid acts as the primer of plasmid replication, while RNA I transcript, an RNA antisense to the 5' end of RNA II, acts as a suppressor of replication. The RNA I and RNA II interaction involves the formation of a kissing complex that is stabilized by plasmid encoded RNA One Modulator (ROM) protein. High-resolution structures of both the ROM protein and the RNA kissing complex have been determined. Alanine scanning mutagenesis experiments of ROM have shown that the surface residues Asn10, Phe14, Glu18, Lys25, and Lys3 of helix 1 interact with RNA loop residues. To date, there is no high resolution structure of the complex of ROM with the kissing complex and the details of this interaction remain unknown. Towards our effort to determine this RNA-protein complex, an RNA construct capable of forming a C2 symmetric kissing complex, to reduce NMR spectral complexity, has been designed by introducing a U-U mismatch into the kissing helix. 2D NMR spectra of the kissing dimer formed by this construct reveal a structural fold similar to the wild type. The challenge of elucidating a high resolution structure of this complex, however, lies in availability of few, if any, NOE contacts between ROM and RNA. To overcome this problem, the RNA UU kissing dimer construct has been 5′-end labeled with 3-(2-Iodoacetamido)-proxyl group so distance restraints could be measured using the paramagnetic relaxation enhancement (PRE) effect of the spin label on the protons in the protein. Using T1/T2 relaxation measurements, structural restraints for the complex are being pursued to enable a high resolution description of the how ROM specifically binds the RNA kissing complex.