otherwise unstable and unattainable state by cryoradiolytic reduction of an oxymyoglobin equivalent (Compound III) to generate and trap the so-called peroxymyoglobin intermediate. By annealing this compound the oxygen-oxygen bond is broken and the reaction propagates to the ferryl compound II intermediate [3], [4].

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Keywords: haem proteins, radiation damage, spectroscopy

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Structural basis of double-stranded RNA recognition by RIG-I

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Innate immunity requires sensory molecules to detect pathogens. RIG-I-like receptors (RLRs: retinoic acid-inducible gene I, RIG-I; melanoma differentiation-associated gene 5, MDA5; and laboratory of genetics and physiology 2, LGP2) sense viral RNAs and result in immunological responses against viral infection. RLRs belong to a family of cytoplasmic DExD/H box RNA helicases. The helicase domain of RIG-I and MDA-5 is connected to two caspase activation and recruitment domains (CARDs) at the N terminus and a Zn ion binding regulatory domain at the C terminus**.** Upon binding and activation by viral dsRNA or triphosphated RNA, RIG-I and MDA-5 recruit the adaptor IPS-1 (also known as MAVS, CARDIF or VISA) on the outer membrane of the mitochondria through the CARDs domain. This leads to the activation of several transcription factors including IRF3, IRF7 and NF- κ B, and the production of type I interferon (IFN) and inflammatory cytokines.

Several crystal structures of the regulatory domains and their complexes with duplex RNA (dsRNA) are available, providing structural insights into RNA recognition by the RD domain. The role of the helicase domain in RNA sensing and CARDs activation is still largly unknown. To understand the mechanistic basis of RIG-I activation, we determined the crystal structure of RIG-I dsRNA complex. In this structure, the dsRNA interacts extensively with both the helicase domain and the regulatory domain, forming a "hotdog" like complex. The linker region between the two domains adopts a lever-like conformation, suggesting the coupling between the two domains upon activation by dsRNA. Within the helicase domain, Rec-A like domain 1 dominates the interaction with the dsRNA, and this interaction may be responsible for activation of the CARDs domain. The two Rec-A like domains adopt an open conformation in the absence of ATP. This suggests additional conformational changes may occur upon ATP hydrolysis, providing a means of switching a signal on and off, to allow tight regulation of the host immune response. Structural and biochemical studies of full length RIG-I will give more insights into the process of RIG-I activation.

To our knowledge, this is the first structure of a super-family 2 protein (SF2, which are RNA-dependent ATPases, and often helicases) in complex with duplex RNA. The structural and functional diversity of the "helicase" family is now expanded.

Keywords: RIG-I helicase, virus RNA, innate immunity

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Crystallographic insights into the structure of spliceosomal snRNPs

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The protein-coding regions of most eukaryotic genes are interrupted by non-protein-coding sequences called introns. The entire gene, including the introns, is transcribed as precursor mRNA (pre-mRNA) from which introns are removed and protein-coding regions ligated together by the spliceosome, a dynamic, multisubunit assembly [1]. The five splicesomal snRNPs (U1, U2, U4, U5 and U6) are its primary components. The snRNPs, along with many *trans*-acting protein factors, recognise the intron boundaries, catalyse intron excision and the subsequent ligation of the exons.

We have reconstituted snRNP particles and sub-particles, crystallized them and solved their structures in order to gain insight into spliceosomal snRNP structure.

The ten subunit, functional core of U1 snRNP was reconstituted from an *in vitro* transcribed RNA along with the seven Sm core proteins and the U1-specific U1-70k and U1-C proteins, all recombinantly expressed in *Escherichia coli*. The particle's crystal structure was solved at 5.5 Å resolution [2]. This was the first crystal structure of a spliceosomal snRNP. A striking feature is the N-terminal polypeptide of U1-70k, which extends over 180 Å from its RNA binding domain, wraps around the Sm protein core domain, and finally contacts the U1-C protein on the far side of the particle. The interaction of U1-C with the 5' end of U1 snRNA, which base-pairs with a putative 5' splice site mimic, suggests why U1-C is crucial for 5' splice-site recognition.

We have also assembled the U4 snRNP core domain. The RNA consists of the Sm site and two flanking hairpins, to which are bound the seven Sm proteins: B, D3, D1, D2, F, E and G. The Sm proteins form a heptameric ring through which passes the single-stranded Sm site. The crystal structure was solved at 3.6 Å resolution[3]. A hydrogen-bonding scheme, which explains the recognition and specificity of the Sm proteins for the Sm site is inferred from the structure and this is likely conserved in the U1, U2, U4 and U5 snRNPs. Comparison with the U1 structure suggests that although the core Sm binding site is recognised in a similar way by different snRNPs, there are differences in how the cores interact with other regions of the snRNPs.

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Keywords: splicing, RNA, nucleoprotein

MS.71.3

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Structural studies of a CRISPR RNA processing endonuclease

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The CRISPRs (Clustered Regularly Interspaced Short Palindromic

Repeats) found in prokaryotic genomes confer small RNA-mediated protection against viruses and other invaders. The CRISPR loci are transcribed to precursor RNAs that are subsequently processed to the individual invader-targeting CRISPR RNA (crRNA). Distinct families of CRISPR-associated Cas proteins function to cleave within the repeat sequence of CRISPR transcripts by vastly different mechanisms. Cas6 represents a family of processing endonucleases that recognize and cleave nonstructured RNA. Crystal structures and complementary biochemical studies of Cas6 bound with a repeat RNA suggest a wrap-around model of processing. This model differs from two other known models of crRNA processing in which the endonuclease either depends on a structured RNA substrate or a guide RNA for processing. The discovery of the families of crRNA processing endonuclease has significantly expanded the repertoire of RNA processing endonucleases.

Keywords: CRISPR RNA, riboendonuclease, protein-RNA complex

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Procrustean bed of RNA silencing suppression

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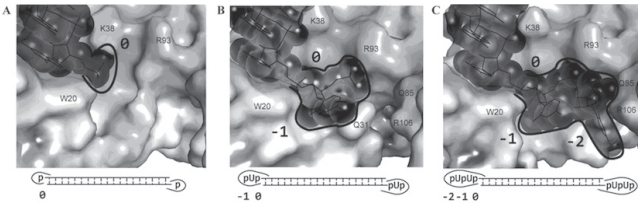
The p19 protein from tombusvirus is a caliper-like machine that recognizes siRNA by length of 19 base pairs [1,2] and blocks RNA silencing through direct binding of siRNA molecules [3]. Here we present four newly solved crystal structures of p19 in complex with (i) 19 bp RNA pGG$(CAG)_5$CC, (ii) 19 bp RNA pUUG$(CUG)_5$CU with 1-nt 5'-overhang, (iii) 19 bp RNA pUUUG$(CUG)_5$CU with 2-nt 5'-overhang, and (iv) 20 bp RNA pG$(CUG)_6$C, that have been studied at 1.73 Å, 1.86 Å, 2.30 Å, and 2.00 Å resolution, respectively.

Comparative analysis of structures (i) to (iii) shows the presence of the 5'-end recognition centre in p19, which can bind 5'P (Fig. A), 1nt 5'-overhang (Fig B) and 2nt 5'-overhang (Fig C). The finding of the recognition center designated for 5'-overhangs of RNA is surprising, as siRNAs are known to contain 3'-overhanging segments rather than 5'-end ones. On the other hand, such a center could facilitate the unwinding of terminal base-pairs in 'longer-than-19bp' RNA fragments. Moreover analysis of 20 bp RNA (iv) revealed the ability of p19 to unwind terminal base pair, hence maintaining the double helical region length of 19 base pairs. Unwound 5'-end nucleotide is stabilized by several direct and water-mediated interactions, whereas unwound 3'end overhang remains invisible in electron density map, in accordance to previously reported data that showed unimportance of 3'overhangs for p19 binding with siRNA [1,2].

As siRNAs are known to be 21-25 nucleotides long the discovered center can be of help for understanding the mechanism of binding of small RNAs of longer length than 19 bp by RNA silencing suppressor p19.



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Keywords: protein, RNA, interaction

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Biochemical studies of the snRNPs core domain formation in Saccharomyces cerevisiae

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The spliceosome is a dynamic molecular machine, which catalyze excision of non-coding sequences (introns) from precursors of messenger RNAs (pre-mRNAs). It is assembled from 4 canonical subunits – small nuclear ribonucleoprotein particles (U1, U2, U4/U6 and U5 snRNPs) and a number of other, non-snRNP proteins. Each round of splicing requires hierarchical *de novo* assembly of the spliceosomal subunits on pre-mRNA, followed by a series of structural rearrangements to form catalytically competent particle. The catalytic core of the spliceosome consists of a highly structured RNA network formed between U2, U5 and U6 snRNAs and the conserved sequences in the pre-mRNA.

Our goal is to get insight into the structure and the function of splicing machinery by X-ray crystallography combined with electron microscopy and biochemical methods. We are particularly interested in proteins and RNAs involved in U5.U4/U6 tri-snRNP formation in *Saccharomyces cerevisiae*. Our approach is based on *in vitro* reconstitution of spliceosomal subcomplexes from recombinant proteins and *in vitro* transcribed snRNAs. This method has been successfully applied to reconstitute human U1 snRNP[1] and the U4 snRNP core domain[2]. I will present our recent progress in core domain reconstitutions of budding yeast tri-snRNP components and a perspective for future biochemical and structural studies.

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MS.72.1

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Graphical tools for structure determination and refinement in PHENIX

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PHENIX [1] is a software package for phasing and refinement of macromolecular crystal structures. Although the individual programs