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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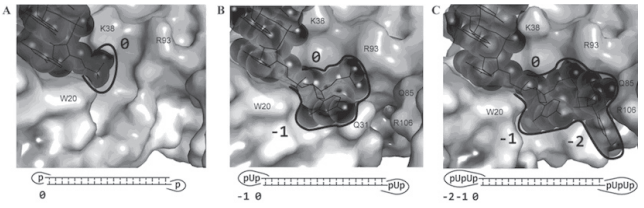
The p19 protein from tombusvirus is a caliper-like machine that recognizes siRNA by length of 19 base pairs [1,2] and blocks RNA silencing through direct binding of siRNA molecules [3]. Here we present four newly solved crystal structures of p19 in complex with (i) 19 bp RNA pGG$(CAG)_5$CC, (ii) 19 bp 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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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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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