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Engineered "restriction RNases" for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids

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Ribonucleases (RNases) are valuable tools applied in the analysis of RNA sequence, structure and function. Their substrate specificity is limited to recognition of single bases or distinct secondary structures in the substrate. Thus far, there have been no RNases available for purely sequence-dependent fragmentation of RNA, analogous to restriction enzymes for DNA. We have therefore searched for existing RNases that could be engineered to become sequence-specific. Using a combination of bioinformatics methods and experimental protein engineering we have obtained prototypes of two sequence-specific "restriction RNases" (RRNases): First, we identified members of the RNase III super family that exhibit sequence specificity towards dsRNA: They recognize a specific tetranucleotide target sequence and are capable of cleaving individual sites in long dsRNA molecules. For one of such enzymes we solved the crystal structure and constructed a structural model of a protein-RNA complex. Second, a prototype RRNase that cleaves the RNA strand in DNA-RNA hybrids five nucleotides from a nonanucleotide recognition sequence was constructed by fusing two functionally distinct domains: A non-specific RNase HI and a zinc finger that recognizes a sequence in DNA-RNA hybrids. The optimization of the fusion enzyme specificity was guided by a structural model of the protein-substrate complex and involved a number of steps including site-directed mutagenesis of the RNase moiety and optimization of the inter domain linker length. Potentially, RRNases may be used *in vitro* for production of RNA molecules with defined length and termini which may be a cheaper alternative to chemical synthesis; they may be also used *in vivo* for targeted RNA degradation.

Biography

Janusz M Bujnicki is a Professor of Biology and Head of a Research Group at IIMCB in Warsaw and at Adam Mickiewicz University, Poland. He has graduated from the Faculty of Biology, University of Warsaw in 1998 and defended his PhD in 2001. He was awarded with Habilitation in 2005 and with the Professor title in 2009. His research combines bioinformatics, structural and synthetic biology. He is an author of >280 publications which have been cited >5300 times (self-citations excluded), Hirsch index 37 (according to Scopus). He has received numerous awards, prizes, fellowships and grants including EMBO/HHMI Young Investigator Program award, ERC Starting Grant, Award of the Ministry of Science and Award of the Prime Minister and was decorated with the Knight's Cross of the Order of Polonia Restituta by the President of the Republic of Poland. In 2013 he has won the national plebiscite "Poles with Verve" in the Science category. He is involved in various scientific organizations and bodies including the Polish Young Academy, Citizens of Science, Science Europe and Scientific Policy Committee. He is also an Executive Editor of the *Journal Nucleic Acids Research*.

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