Structural basis of dsRNA recognition by the J2 monoclonal antibody

Charles Bou-Nader[1], Ankur Bothra[2], Ilias Skeparnias[1], David N. Garboczi[3], Stephen H.m Leppla[2], Jinwei Zhang[3]

[1]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, [2]Laboratory of Parasitic Diseases, National Institute of Allergy, and Infectious Diseases, [3]Structural Biology Section, Research Technologies Branch, National Institute of Allery and Infectious Diseases

charles.bou-nader@nih.gov

dsRNA is often sensed as a hallmark of viral infection to trigger an innate immune response. In the constant arms race between viruses and the host, some host protein factors are activated upon the recognition of non-self dsRNA to curb the infection[1] (e.g. RIG-I/MDA5, PKR, RNase L, TLRs). Given the importance of dsRNA to trigger an immune response and its prevalence in RNA structures, several methods to detect or to isolate dsRNA have been developed. For instance, the J2 monoclonal antibody is widely used to detect dsRNA in cultured cells, to enrich dsRNA or to identify infections by positive sense RNA viruses[2]. Most recently, the J2 antibody was used to facilitate the discovery of glycoRNAs[3] and to test for dsRNA contaminations in the preparation of mRNA vaccines as quality control[4]. However, despite decades of widespread use, J2's length and sequence preferences for dsRNA and mechanisms of recognition have remained unknown.

Using fluorescence polarization and SEC-MALS we found that J2 recognizes dsRNAs shorter then 23-bp, half the size of the minimal epitope previously proposed (>40-bp), suggesting its usefulness to study small RNAs. To understand how this antibody senses dsRNA, we solved its co-crystal structure bound to dsRNA at 2.8 Å. The structure and attendant mutational analyses revealed that J2 tracks the minor groove of the A-form dsRNA via non- contiguous interactions to its riboses, phosphate backbone, and sugar edges of nucleobases. Recognition of the minor groove is mediated principally by aromatic residues reminiscent of the S9.6 monoclonal antibody used to map R-loops[5]. This strategy contrasts starkly with double-stranded RNA binding domains (dsRBDs) that recognize a linear, contiguous minor-major-minor groove interface along one flat face of the dsRNA mainly using basic residues to contact the phosphate backbone[6]. Together, this work reveals a novel dsRNA-recognition strategy distinct from those of dsRBDs and expands our knowledge of RNA recognition by antibodies.

References

{1} Hur S. Annu Rev Immunol 2019
{2} Schonborn J. et al., NAR 1991
{3} Flynn R. et al., Cell 2021
{4} Baiersdorfer M. et al., Mol Therapy 2019
{5} Bou-Nader C. et al., Nat Commun 2022
{6} Bou-Nader C. et al., NAR 2019