Structural study of viral RNA detection by Rig-I-like helicases in innate immune system

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The innate immune response is the first defense line against the pathogens in the cell. This response plays a key role in infections with negative strand RNA viruses (e.g. Ebola hemorrhagic fever, influenza, mumps, and measles). Most of the detection of the negative strand RNA viruses is performed by the so-called Rig-I-like helicases. The three vertebrate Rig-I-like helicases exist (Rig-I, MDA5, and LGP2) and each of them functions differently. For example, the LGP2 helicase is especially interesting as it does not have the domain that usually triggers the signaling. Does this mean that LGP2 must necessarily cooperate with the other helicases to evoke an immune response? Cooperation between these helicases is indeed important and it happens when the helicases coat the viral RNA to form nanometer-sized RNA-protein filaments. The filament formation allows a conformational change to occur which in turn exposes signaling domains that trigger the downstream signaling. To fully understand the process of detection and response to negative strand RNA viruses, it is necessary to obtain structural information for all three helicases, their RNA-binding specificities and the conditions for the cooperative filament formation that eventually triggers the immune response.

Here we present the first and unusually high-resolution crystal structures of full-length chicken LGP2 (*ch*LGP2) bound to various dsRNAs in closed and semi-closed states. These *ch*LGP2 structures capture two different states from the ATP hydrolysis cycle. The comparison of these states highlights the ATP-dependent structural transition that is necessary for the function and also filament formation. Both structures and the affinity measurements are showing that *ch*LGP2 can bind with high affinity to any short dsRNA (LGP2 binds 5’ mono/triphosphate blunt ends and also 5’ and 3’ overhang dsRNAs). This is in stark contrast with the behavior of Rig-I which accommodates specifically the 5’ triphosphate dsRNA only. We also present several high-resolution structures of an another Rig-I-like helicase, the *ch*MDA5. With *ch*LGP2 and *ch*MDA5 in hands, we proceed to use electron microscopy to show that *ch*LGP2 forms filaments on dsRNA that structurally resemble the filaments of MDA5. Surprisingly, we observe a synergistic filament formation when a mixture of chMDA5 and *ch*LGP2 is incubated with RNA. Furthermore, we manage to map the domains responsible for synergistic filament formation to the CTD and helicase domains of *ch*LGP2 using functional assays. With all the collected data, we propose a model where the synergistic cooperativity of LGP2 promotes MDA5 oligomerization on dsRNA by end-binding and filament nucleation. Last but not least, we have now taken the first steps to study the structure of mixed MDA5 and LGP2 filaments using cryo-electron microscopy and super-resolution microscopy.