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STRUCTURAL BIOLOGY

A picornavirus unlike the others

Technological breakthroughs in cryo-electron microscopy are allowing the capture of virus structures, leading to advances in the field of viral microbiology almost daily. An innovative structure–function study on Aichi virus reveals that novel entry mechanisms, yet undiscovered, may exist for non-enveloped viruses.

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Recent advances in cryo-electron microscopy (cryo-EM) are producing atomic resolution structures captured in a hydrated near-physiological state. This breakthrough is providing extraordinary detail for the nanomachines we call viruses. Picornaviridae, a family of small RNA-containing viruses, are the common causative agents of human illness. For example, the family includes human rhinovirus, the causative agent of the common cold, a relatively mild disease that nevertheless costs the US economy US$40 billion each year[1]. There are also some severe diseases represented within the family, such as poliomyelitis and myocarditis. Some family members cause deadly disease, like the seasonal epidemics of enterovirus 71 that inflict Asia and Pacific regions, a geographical restriction that is not yet understood. Now writing in *Nature Microbiology*, Stuart and colleagues have solved the structure of a new picornavirus family member, Aichi virus (AiV), which has the potential to cause extreme disease and exhibits some interesting differences from other known picornaviruses[2].

This relative newcomer to the picornavirus family was first identified in the Aichi Prefecture, Japan, after a 1989 outbreak of acute gastroenteritis that was linked to consumption of raw oysters. So far, it is the only virus in the Kobuvirus genus that has been found in humans. Picornaviruses also have a place in the history of structural biology, as they were the first animal viruses for which the 3D structures were solved in 1985[3,4], when the four capsid structural proteins (VP1, 2, 3 and 4) first acquired the blue, green, red and yellow colour code. When these first structures were revealed, there was tremendous excitement mapping antigenic sites and predicting locations for receptor binding. However, subsequent picornavirus structures seemed remarkably similar, so much so that with something of a 'ho-hum' attitude, antigenic sites for coxsackieviruses were assumed to be the same as the ones established for rhinovirus. But now, with more recent structure solutions, excitement is again in the air as we see how diverse the members of the picornavirus family truly are[5–8]. Interestingly, AiV structure exhibits distinctive and singular structural features, which the authors have tied to function.

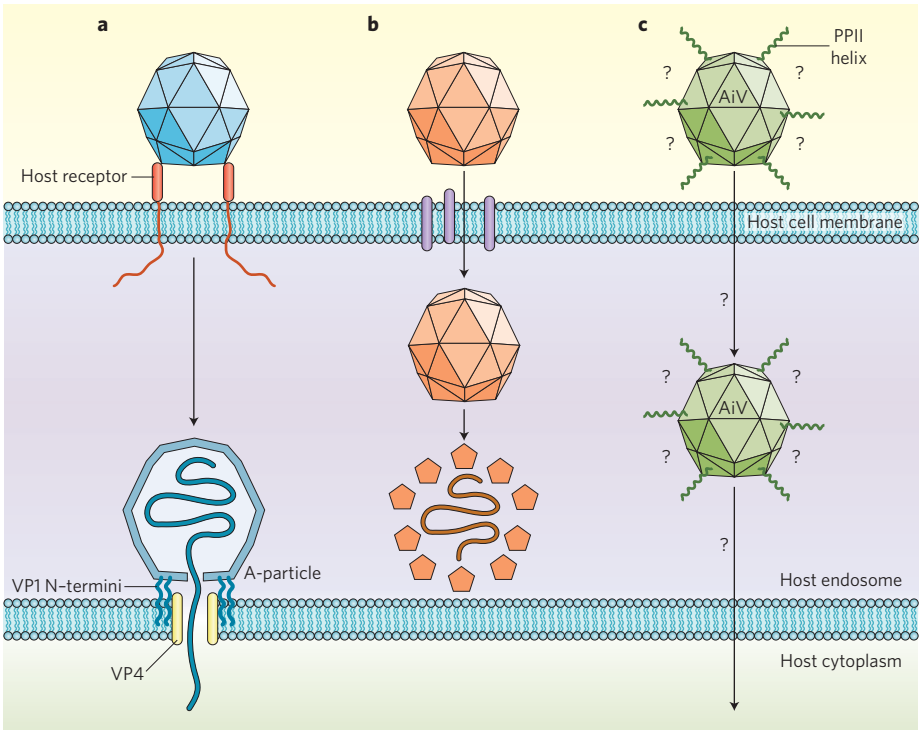

Figure 1 | Picornavirus genome release. **a**, Many picornaviruses bind a receptor that initiates global and local conformational changes and formation of the A-particle. At the attachment site there is loss of pocket factor, extension of the VP1 N-termini that anchor the particle to the membrane, and extrusion of an unknown number of VP4 molecules, forming a pore through which the viral RNA genome is released. **b**, Some picornaviruses like FMDV and cardioviruses have no pocket factor and there is no cleavage of VP0 (into VP4 and VP2) upon incorporation of the genome into the capsid during assembly. These viruses dissociate into 12S pentameric units at low pH, which allows the release of the genome. **c**, For AiV, no receptor is known and attachment to host might be mediated by a PPII helix. The method of genome uncoating is unknown but is probably not through the formation of the A-particle (as in **a**) because there is no pocket factor and no VP4. AiV also does not dissociate into pentamers at low pH (as in **b**). Therefore, AiV probably uses a different, yet undiscovered, mechanism.

Like the parechoviruses, AiV has an extremely long VP0 (370 residues) that does not undergo the final maturation cleavage to VP2 and VP4, which is assumed to be RNA-catalysed. The AiV genome has the highest degree of RNA secondary structure of any picornavirus and contains an RNA encapsidation signal at the 5′-terminal RNA stem-loop.

The 3.68 Å resolution 3D reconstruction allowed atomic modelling and AiV capsids were found to have pores similar to those seen in foot-and-mouth disease virus (FMDV), another picornavirus. There are distinctive 'cooling tower' structures around the fivefold axes formed by extended loops. Notably, in AiV, the uncleaved portion of VP0 that corresponds to VP4 is significantly longer than the VP4 protein in other picornaviruses, and the structure folds back on itself to form a 'bow and arrow' shape that interacts with other protomers.

Numerous structural studies have sought to understand how picornaviruses release their genomes[9–12]. One basic route of uncoating proceeds through an entry intermediate called the 'altered particle', or A-particle. The current model suggests that receptor binding induces loss of pocket factor, leading to global and local conformational changes to form a unique pore through which the genome is released (Fig. 1). Alternatively, for FMDV and cardioviruses, the pentameric subunits dissociate at low pH, presumably releasing the genome. Like a typical enterovirus, AiV became unstable at lower pH; however, the capsid did not dissociate into pentamers. This suggests AiV releases its genome in a different way, but since AiV has no pocket factor, following the path through formation of an A-particle seems unlikely. So, by what mechanism does AiV release the genome? Although this is currently unclear, perhaps there is another mechanism of genome release that has yet to be discovered.

These findings led the authors to correlate the viral structure to function by systematically analysing the interactions between any one subunit and its environment within the protomer of a pentamer. They found that interactions between pentamers determine the particle stability with a compelling correlation. Thus, increasing the interactions between virus pentamers will make for a sturdier, more heat-resistant virus. These findings have translational potential, as there is a distinct need for such stable viral capsids for vaccine efforts in our ongoing fights to eradicate poliovirus and control FMDV. The next step might be to combine this result with the authors' previous success in developing a molecular-dynamics-based strategy for the evaluation of mutations designed to increase capsid stability by increased noncovalent interactions[13].

The last surprise associated with the AiV structure came when a poly-L-proline type II (PPII) helix was discovered on the external surface of AiV at the integrin recognition site. Aside from the well-known α-helix and β-strand structures, PPII helices are the only other regularly occurring secondary structures in folded proteins. Also known to maintain local order in unfolded proteins, PPII contributes to a range of functions including protein–protein interactions[14,15]. Thus, this PPII helix might serve as a recognition signal for cellular receptors or to recruit virulence factors to manipulate the host's cellular machinery. The structure of a PPII helix has not been found previously in picornaviruses and suggests a novel interaction with the host.

Using high-resolution cryo-EM technology, the authors have solved the structure of a new picornavirus family member that has some interesting differences, which have been linked to function. The innovative study shows that there may be yet another unknown mechanism for genome release as the AiV capsid does not dissociate at low pH to release the genome into the infected host. In addition, there is no pocket factor to control particle expansion and the VP0 does not cleave to form VP4 for insertion into the endosome membrane to allow the genome to be extruded through a pore. AiV attachment to the host is also still a mystery. Finally, the discovery of a PPII helix is novel and exciting. Although found more rarely than α-helices and β-strands, this study underscores a need to start examining other virus structures for PPII, which is non-trivial as PPII helices are not regularly assigned by most model-building software. ❐

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