doi:10.1017/S1431927621005122
Microsc. Microanal. 27 (Suppl 1), 2021

ICAM-1 induced re-arrangements of capsid and genome prime rhinovirus 14 for activation and uncoating

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Rhinoviruses (RVs) are the predominant cause of common colds in humans. RV14 utilize intercellular adhesion molecule-1 (ICAM-1) as a receptor. Genome release is preceded by activation of RV particles, a process that involves formation of pores in the capsid, release of a minor capsid protein VP4, and genome reorganization. It was proposed before that ICAM-1 primes the virus for activation and subsequent genome release.

However, the exact molecular mechanism of this phenomenon remains unknown.

Here we present the mechanism by which the binding of ICAM-1 to rhinovirus 14 primes the virus for activation and genome release.

We show that the binding of ICAM-1 to RV14 does not promote neither activation of the virus nor genome release at pH and ionic composition close to that of the extracellular environment. Instead of, ICAM-1 only induces translocation of C-terminus of VP4 away from a threefold symmetry axis of the capsid to two-fold symmetry axis, where a pore in the capsid will open upon particle activation. Thus, VP4 subunits become optimally positioned for the release from activated particles. The movement of the C-termini of VP4 subunits from the threefold axis reveals a patch of positively charged residues, which attracts negatively charged RNA and induces local re-organization of the virus genome. Moreover, experiments with acidification of the virus proved that binding of ICAM-1 is necessary for activation of the virus and subsequent genome release.

When the virus is exposed to acidic pH which mimics that of an endosome, no changes in the structure of the virus were observed. However, when the virus-receptor complex is formed prior acidification, genome release is initiated.

Furthermore, the cryo-EM reconstruction of RV14 virion contains resolved density for octa-nucleotides from the RNA genome, which are positioned on twofold symmetry axes of the capsid. One base from each octa-nucleotide forms a stacking interaction with Trp38 of VP2. This interaction is disrupted and replaced by C-terminus of VP4 upon binding of RV14 to ICAM-1.

In summary, the binding of RV14 to ICAM-1 brings the virus to a conformation with altered structure of both capsid and genome, which primes the virus for activation and uncoating.

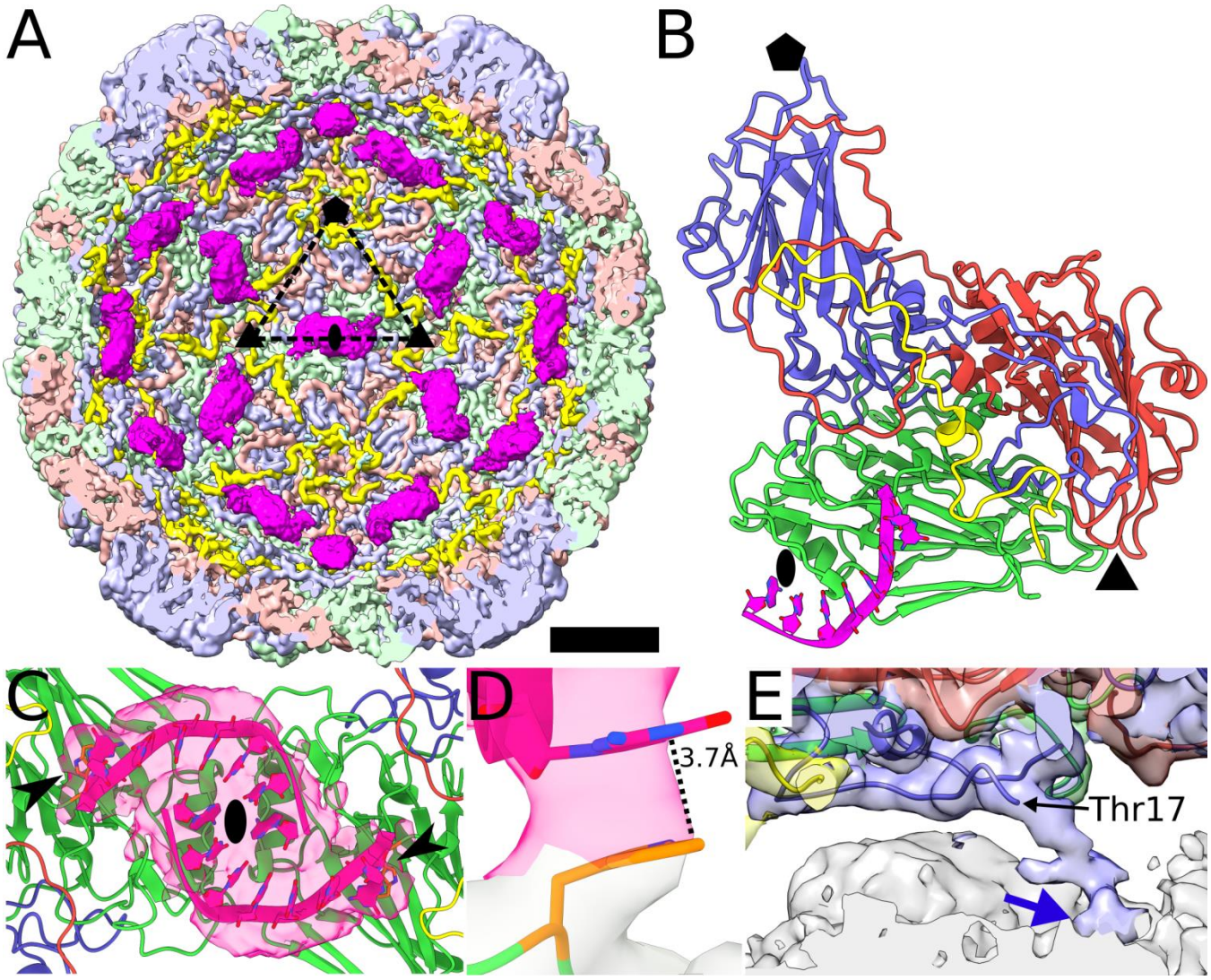

Figure 1. Structure of virion of rhinovirus 14 contains resolved density corresponding to octanucleotides from its RNA genome. (A) Surface representation of cryo-EM of reconstruction of virion of rhinovirus 14 with front half of the particle removed to show internal structure. Density corresponding to VP1 is shown in blue, VP2 in green, VP3 in red, VP4 in yellow, and RNA segments in pink. Borders of a selected icosahedral asymmetric unit are indicated with a dashed triangle and positions of selected twofold, threefold, and fivefold symmetry axes are represented by an oval, triangle, and pentagon, respectively. Scale bar indicates 5 nm. (B) Cartoon representation of icosahedral asymmetric unit of rhinovirus 14 viewed from the inside of the capsid. The color coding of individual virus components is the same as in panel A.Positions of twofold, threefold, and fivefold symmetry axes are represented by an oval, triangle,and pentagon, respectively. (C) Two RNA octa-nucleotides that interact with each other and with VP2 subunits. Protein and RNA coloring is the same as in panel B. The cryo-EM density of the RNA segments is shown as a pink semi-transparent surface. RNA bases and side chains of Trp38 of VP2 are shown in stick representation, in orange, and indicated with black arrowheads.The position of a twofold symmetry axis is indicated with an oval. (D) Detail of stacking interaction between Gua2 from RNA segment and Trp38 of VP2. The cryo-EM densities of RNA and protein are shown as semi-transparent surfaces in pink and grey, respectively. (E)Interaction between N-terminus of VP1 and genome. Capsid proteins are shown in cartoon representation with the same coloring as in panel B. Cryo-EM densities of

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individual proteins are shown as semi-transparent surfaces colored according to the chain they belong to. The density of the RNA genome is shown in grey. The blue arrow indicates the contact between the N-terminus of VP1 and the genome. The position of Thr17, the first modelled residue from the N-terminus of VP1, is indicated.

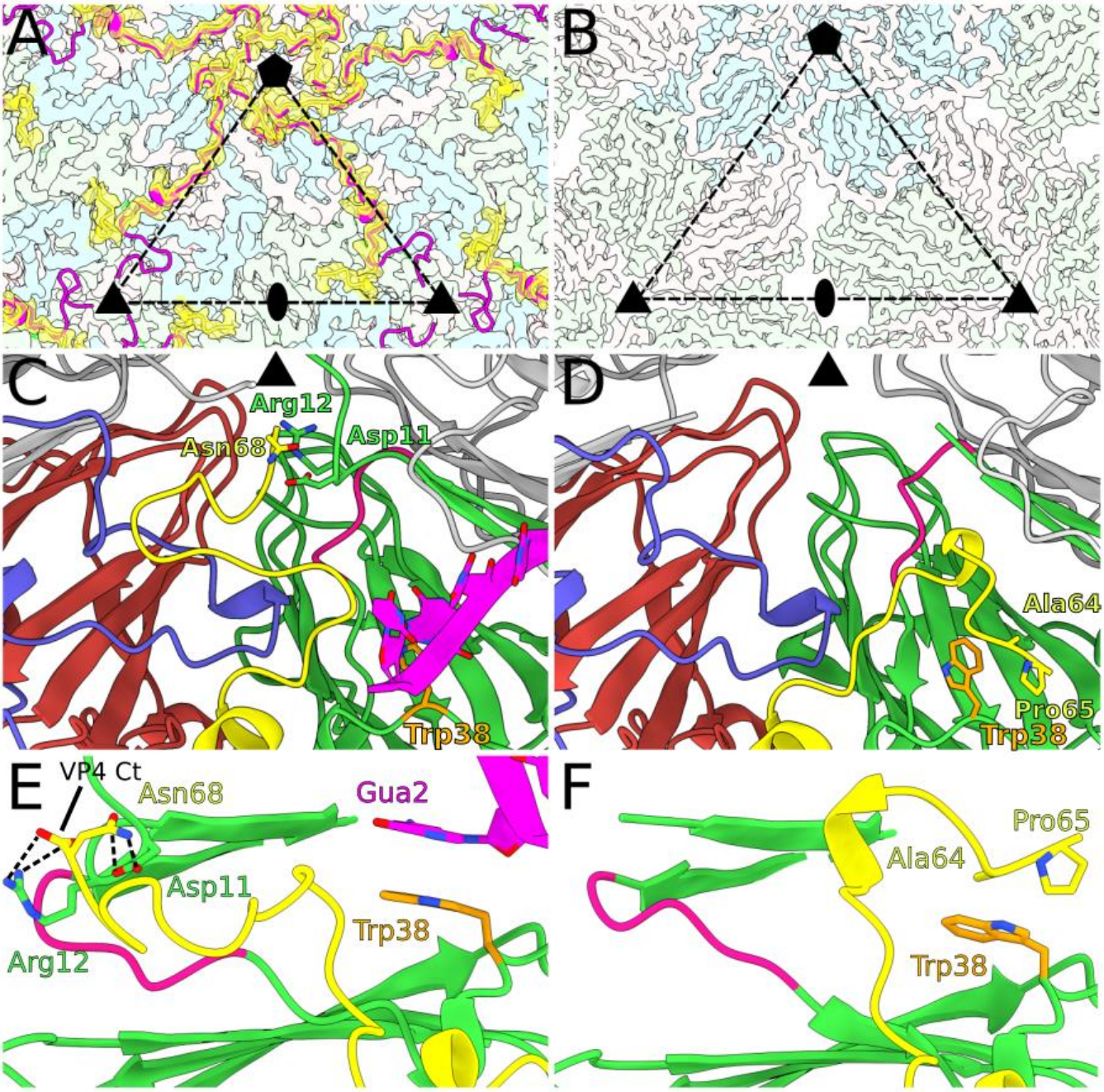

Figure 2. Changes of structure of C-terminus of VP4 induced by ICAM-1 binding to rhinovirus 14.(A) Surface representation of cryo-EM reconstruction of capsid of rhinovirus 14 in complex withICAM-1 viewed from inside the virion. Density corresponding to VP1 is shown in pale blue, VP2 in pale green, VP3 in pale red, and VP4 in semi-transparent yellow. The structure of VP4 in the rhinovirus 14-ICAM-1 complex is shown in cartoon representation in yellow, whereas the structure of VP4 in the virion of rhinovirus 14 is shown in magenta. The positions of selected icosahedral symmetry axes are indicated

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with a pentagon for fivefold, triangle for threefold, and oval for twofold. Borders of a selected icosahedral asymmetric unit are indicated with a dashed triangle. (B) Capsid structure of empty particle of rhinovirus 14 containing pores around twofold symmetry axes and between twofold and fivefold symmetry axes through which VP4 may be released from the particle. (C-F) Differences in structure of VP4 subunits in virion (C,E) and rhinovirus 14-ICAM-1 complex (D,F). Capsid proteins are shown in cartoon representation. VP1 is shown in blue, VP2 in green, VP3 in red, VP4 in yellow, and RNA segments in pink. (CE) Asn68 from C-terminus of VP4 interacts with Asp11 and Arg12 of VP2 in virion of rhinovirus 14. The residues Asp11 and Arg12 are stabilized in position by the underlying loop of VP2 formed by residues 27-32 (highlighted in magenta). The sidechain of Trp38 (highlighted in orange) forms a stacking interaction with Gua2 that is part of the resolved RNA segment positioned next to a twofold axis. (DF) Binding of rhinovirus 14 to ICAM-1 induces conformational changes of virus capsid that include movement of residues 27-32 of VP2 towards particle center, which prevents interaction of C-terminus of VP4 with residues Asp11 and Arg12 of VP2. The C-terminus of VP4acquires a new conformation, which covers the sidechain of Trp38 of VP2 and blocks its interaction with RNA.

References

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