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Adnaviria: a new realm for archaeal filamentous viruses with linear A-form double-stranded DNA genomes

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Abstract

The International Committee on Taxonomy of Viruses (ICTV) has recently adopted a comprehensive, hierarchical system of virus taxa. The highest rank in this hierarchy are realms, each of which is considered monophyletic but apparently originated independently of other realms. Here we announce the creation of a new realm, *Adnaviria*, which unifies archaeal filamentous viruses with linear A-form double-stranded DNA genomes and characteristic major capsid proteins unrelated to those encoded by other known viruses.

Text

In 2018, the International Committee on Taxonomy of Viruses (ICTV) expanded the number of taxonomic ranks available for virus classification from 5 to 15 (1, 2). This development enabled formal recognition of the evolutionary kinships among distantly related viruses and led to creation of four realms—virus taxa that are roughly equivalent to the domain rank in cellular taxonomy (3): The realm *Riboviria* encompasses all RNA viruses and reverse-transcribing viruses that encode homologous RNA-directed RNA polymerases and reverse transcriptases, respectively; *Monodnaviria* comprises viruses with predominantly single-stranded (ss) DNA genomes and encoding rolling-circle replication initiation endonucleases of the HUH superfamily; *Duplodnaviria* unifies viruses with double-stranded DNA genomes that produce virions with icosahedral capsids formed by major capsid proteins (MCPs) with the HK97 fold; and *Varidnaviria* comprises dsDNA viruses with icosahedral capsids built from MCPs with double-jelly roll fold (4). However, many virus families remained unassigned to higher taxa due to the lack of demonstrable evolutionary relationships to viruses assigned to the four realms.

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Here we announce the creation, and recent official acceptance by the ICTV (https://talk.ictvonline.org/taxonomy/), of a new realm, *Adnaviria*, which encompasses structurally related archaeal filamentous viruses with dsDNA genomes that adopt the A-form conformation within their virions.

Filamentous viruses infect hosts of all cellular domains but, despite overall similar morphology, virions of eukaryotic, bacterial, and archaeal filamentous viruses are built from capsid proteins with different structural folds (5). Furthermore, the types of the viral genomes that are protected by the corresponding capsid proteins are radically different among viruses infecting hosts from the different domains of life (6-11): All known eukaryotic filamentous viruses have linear ssRNA genomes and are classified in realm *Riboviria* (4); all known bacterial filamentous viruses have circular ssDNA genomes and belong to realm *Monodnaviria* (4, 12); and all known archaeal filamentous viruses have dsDNA genomes and, until recently, have remained unassigned to taxa ranked higher than order.

Archaeal viruses that form filamentous virions are currently classified into four families: *Clavaviridae*, *Lipothrixviridae*, *Rudiviridae*, and *Tristromaviridae* (13). Based on the shared gene content, structural similarity of their virions, and homology of the MCPs, families *Lipothrixviridae* and *Rudiviridae* were included in order *Ligamenvirales* (14). In virions of both families, the nucleoprotein helix is composed of asymmetric units containing two MCP molecules, a homodimer in the case of rudivirids and a heterodimer of paralogous MCPs in the case of lipothrixvirids (Figure 1) (11, 15). The MCPs of ligamenviral particles have a unique α-helical fold first found in the MCP of rudivirid Sulfolobus islandicus rod-shaped virus 2 (SIRV2) (16). Lipothrixvirids and rudivirids share a characteristic feature in that the interaction between

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the MCP dimer and the linear dsDNA genome maintains the DNA in the A form. Consequently, the entire genome adopts the A-form in virions (15-18).

Clavavirids (19, 20) and tristromavirids (21) do not encode proteins with recognizable sequence similarity to proteins of ligamenvirals (22). Hence, the two families were not included in *Ligamenvirales*. Recently, the structure of particles produced by tristromavirid Pyrobaculum filamentous virus 2 (PFV2) has been characterized by cryogenic electron microscopy (cryo-EM) (23). Unexpectedly, the two nucleocapsid proteins of tristromavirids were found to be structurally related to those of ligamenvirals, and the virion organizations were discovered to be remarkably similar, including the A-form conformation of the genomic dsDNA (23) (Figure 1). Similar to lipothrixvirids, virions of tristromavirids are enveloped by a lipid membrane (24). Based on the structural similarity between members of *Ligamenvirales* and *Tristromaviridae*, we inferred that they share a common origin (23). To formalize this evolutionary relationship, *Tristromaviridae* was assigned to a new order, *Primavirales*, which, along with *Ligamenvirales*, was included in a new class, *Tokiviricetes*.

Members of the *Tokiviricetes* represent one of the most commonly detected virus groups in terrestrial hot springs and are globally distributed (25-27). Rudivirids have been extensively studied in particular, and many aspects of their life cycles are understood in considerable detail (28). Notably, the mechanisms of genome replication do not appear to be conserved among tokiviricetes. It has been proposed that rudivirus SIRV2 uses a combination of strand-displacement, rolling-circle, and strand-coupled genome replication mechanisms, which generate multimeric, highly branched, “brush-like” intermediates reaching lengths of >1,200 kb (~34 genome units) (29). The latter are then processed into unit-length, linear genomes with covalently linked hairpin ends. The genome replication of the lipothixvirid Acidianus

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filamentous virus 1 has been suggested to start by the formation of a D loop and to progress by the strand displacement replication mechanism, whereas termination relies on recombination events through the formation of terminal loop-like structures, though the genes involved in this unique mechanism of replication remain unknown (30). Similar to rudivirid genomes, tristromavirid genomes have terminal inverted repeats (21), but the replication mechanism of these viruses has not been investigated.

High-resolution structures are now available for virions of six distinct tokiviricetes (11, 15). Like many structurally related viruses in the two other realms of dsDNA viruses (*Duplodnaviria* and *Varidnaviria*), there is no detectable sequence similarity among the capsid proteins of viruses from different tokiviricete families, suggesting a vast undescribed diversity of viruses in this part of the virosphere. Indeed, it has been suggested that tokiviricetes were present in the last archaeal common ancestor, and possibly even in the last universal cellular ancestor (LUCA) (31). Regardless, the available data unequivocally show that archaeal filamentous viruses do not fall into any of the four established realms. Thus, given the lack of detectable relationship with other viruses and officially acknowledging the uniqueness of the three families of archaeal filamentous viruses that contain A-form DNA in their virions, a new taxon of the highest rank, realm *Adnaviria*, was created for their classification. To bridge the gap between the class and realm taxa, intermediate kingdom and phylum taxa, named *Zilligvirae* and *Taleaviricota*, respectively, were established.

In contrast, structural characterization of clavavirid Aeropyrum pernix bacilliform virus 1 (APBV1) particles by cryo-EM confirmed that the fold of its MCP and its overall virion organization are unrelated to that of ligamenviral, tristromavirid, or other characterized virus

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particles (32). Therefore, notwithstanding their filamentous virions, clavavirids are not included in realm *Adnaviria* and likely represent another realm to be established in the future.

Etymology of new taxa recently accepted by the ICTV:

- *Adnaviria*; from A-form DNA characteristic of viruses in this realm and suffix *-viria* for realm taxa.
- *Zilligvirae*; after Wolfram Zillig (1925–2005), a pioneer of research on hyperthermophilic archaeal viruses, and the suffix *-virae* for kingdom taxa.
- *Taleaviricota*; from Latin *talea*, meaning "rod" (referring to the virion morphology), and suffix *-viricota* for phylum taxa.
- *Tokiviricetes*; from Georgian თოკი (*toki*), meaning "thread", and suffix *-viricetes* for class taxa.
- *Primavirales*; from Latin *prima*, meaning "first", referring to the fact that Thermoproteus tenax virus 1, classified in this order, was the first hyperthermophilic archaeal virus to be isolated in 1983 (33).

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146 **Figure legend**

147 **Figure 1. Taxonomic organization of realm *Adnaviria*. A.** Taxonomic ranks from family to
148 realm are rendered with different colors. **B-D.** Cryogenic electron microscopy shows structures
149 of the major capsid protein (MCP) homodimer (characteristic of rudivirids) and heterodimer
150 (characteristic of lipothrixvirids and tristromavirids) (**B**), MCPs bound to viral double-stranded
151 (ds) DNA genome in A-form conformation (**C**), and side views of the viral nucleocapsid are
152 shown for each constituent family (**D**). *Rudivirdae*, *Lipothrixviridae*, and *Tristromaviridae* are
153 represented by structures of Sulfolobus islandicus rod-shaped virus 2 (PDB entry: 3J9X),
154 Acidianus filamentous virus 1 (PDB entry: 5WTG), and Pyrobaculum filamentous virus 2 (PDB
155 entry: 6VTB), respectively. The figure is modified from ref (11).

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156 **References**

157 1. Gorbalenya AE, Krupovic M, Mushegian A, Kropinski AM, Siddell SG, Varsani A,
158 Adams MJ, Davison AJ, Dutilh BE, Harrach B, Harrison RL, Junglen S, King AMQ,
159 Knowles NJ, Lefkowitz EJ, Nibert ML, Rubino L, Sabanadzovic S, Sanfaçon H,
160 Simmonds P, Walker PJ, Zerbini FM, Kuhn JH. 2020. The new scope of virus taxonomy:
161 partitioning the virosphere into 15 hierarchical ranks. Nat Microbiol 5:668-674.
162 2. Siddell SG, Walker PJ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE,
163 Gorbalenya AE, Harrach Bz, Harrison RL, Junglen S, Knowles NJ, Kropinski AM,
164 Krupovic M, Kuhn JH, Nibert M, Rubino L, Sabanadzovic S, Sanfaçon H, Simmonds P,
165 Varsani A, Zerbini FM, Davison AJ. 2019. Additional changes to taxonomy ratified in a
166 special vote by the International Committee on Taxonomy of Viruses (October 2018).
167 Arch Virol 164:943-946.
168 3. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Dempsey DM, Dutilh BE, Harrach
169 B, Harrison RL, Hendrickson RC, Junglen S, Knowles NJ, Kropinski AM, Krupovic M,
170 Kuhn JH, Nibert M, Rubino L, Sabanadzovic S, Simmonds P, Varsani A, Zerbini FM,
171 Davison AJ. 2019. Changes to virus taxonomy and the International Code of Virus
172 Classification and Nomenclature ratified by the International Committee on Taxonomy of
173 Viruses (2019). Arch Virol 164:2417-2429.
174 4. Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI, Yutin N, Zerbini FM, Kuhn
175 JH. 2020. Global organization and proposed megataxonomy of the virus world. Microbiol
176 Mol Biol Rev 84:e00061-19.
177 5. Krupovic M, Koonin EV. 2017. Multiple origins of viral capsid proteins from cellular
178 ancestors. Proc Natl Acad Sci U S A 114:E2401-E2410.

Downloaded from https://journals.asm.org/journal/jvi on 02 July 2021 by 157.99.174.72.

Accepted Manuscript Posted Online

6. DiMaio F, Chen C-C, Yu X, Frenz B, Hsu Y-H, Lin N-S, Egelman EH. 2015. The molecular basis for flexibility in the flexible filamentous plant viruses. Nat Struct Mol Biol 22:642-4.

7. Klug A. 1999. The tobacco mosaic virus particle: structure and assembly. Philos Trans R Soc Lond B Biol Sci 354:531-5.

8. Stubbs G, Kendall A. 2012. Helical viruses. Adv Exp Med Biol 726:631-58.

9. Tarafder AK, von Kügelgen A, Mellul AJ, Schulze U, Aarts DGAL, Bharat TAM. 2020. Phage liquid crystalline droplets form occlusive sheaths that encapsulate and protect infectious rod-shaped bacteria. Proc Natl Acad Sci U S A 117:4724-4731.

10. Zamora M, Méndez-López E, Agirrezabala X, Cuesta R, Lavín JL, Sánchez-Pina MA, Aranda MA, Valle M. 2017. Potyvirus virion structure shows conserved protein fold and RNA binding site in ssRNA viruses. Sci Adv 3:eaao2182.

11. Baquero DP, Liu Y, Wang F, Egelman EH, Prangishvili D, Krupovic M. 2020. Structure and assembly of archaeal viruses. Adv Virus Res 108:127-164.

12. Roux S, Krupovic M, Daly RA, Borges AL, Nayfach S, Schulz F, Sharrar A, Matheus Carnevali PB, Cheng J-F, Ivanova NN, Bondy-Denomy J, Wrighton KC, Woyke T, Visel A, Kyrpides NC, Eloe-Fadrosh EA. 2019. Cryptic inoviruses revealed as pervasive in bacteria and archaea across Earth's biomes. Nat Microbiol 4:1895-1906.

13. Prangishvili D, Bamford DH, Forterre P, Iranzo J, Koonin EV, Krupovic M. 2017. The enigmatic archaeal virosphere. Nat Rev Microbiol 15:724-739.

14. Prangishvili D, Krupovic M. 2012. A new proposed taxon for double-stranded DNA viruses, the order "Ligamenvirales". Arch Virol 157:791-5.

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Downloaded from https://journals.asm.org/journal/jvi on 02 July 2021 by 157.99.174.72.

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15. Wang F, Baquero DP, Beltran LC, Su Z, Osinski T, Zheng W, Prangishvili D, Krupovic M, Egelman EH. 2020. Structures of filamentous viruses infecting hyperthermophilic archaea explain DNA stabilization in extreme environments. Proc Natl Acad Sci U S A 117:19643-19652.

16. DiMaio F, Yu X, Rensen E, Krupovic M, Prangishvili D, Egelman EH. 2015. A virus that infects a hyperthermophile encapsidates A-form DNA. Science 348:914-7.

17. Kasson P, DiMaio F, Yu X, Lucas-Staat S, Krupovic M, Schouten S, Prangishvili D, Egelman EH. 2017. Model for a novel membrane envelope in a filamentous hyperthermophilic virus. Elife 6:e26268.

18. Liu Y, Osinski T, Wang F, Krupovic M, Schouten S, Kasson P, Prangishvili D, Egelman EH. 2018. Structural conservation in a membrane-enveloped filamentous virus infecting a hyperthermophilic acidophile. Nat Commun 9:3360.

19. Prangishvili D, Mochizuki T, Liu Y, Krupovic M, ICTV Report Consortium. 2019. ICTV Virus Taxonomy Profile: *Clavaviridae*. J Gen Virol 100:1267-1268.

20. Mochizuki T, Yoshida T, Tanaka R, Forterre P, Sako Y, Prangishvili D. 2010. Diversity of viruses of the hyperthermophilic archaeal genus *Aeropyrum*, and isolation of the Aeropyrum pernix bacilliform virus 1, APBV1, the first representative of the family *Clavaviridae*. Virology 402:347-54.

21. Prangishvili D, Rensen E, Mochizuki T, Krupovic M, ICTV Report Consortium. 2019. ICTV Virus Taxonomy Profile: *Tristromaviridae*. J Gen Virol 100:135-136.

22. Iranzo J, Koonin EV, Prangishvili D, Krupovic M. 2016. Bipartite network analysis of the archaeal virosphere: evolutionary connections between viruses and capsidless mobile elements. J Virol 90:11043-11055.

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Accepted Manuscript Posted Online

23. Wang F, Baquero DP, Su Z, Osinski T, Prangishvili D, Egelman EH, Krupovic M. 2020. Structure of a filamentous virus uncovers familial ties within the archaeal virosphere. Virus Evol 6:veaa023.

24. Rensen EI, Mochizuki T, Quemin E, Schouten S, Krupovic M, Prangishvili D. 2016. A virus of hyperthermophilic archaea with a unique architecture among DNA viruses. Proc Natl Acad Sci U S A 113:2478-83.

25. Baquero DP, Contursi P, Piochi M, Bartolucci S, Liu Y, Cvirkaite-Krupovic V, Prangishvili D, Krupovic M. 2020. New virus isolates from Italian hydrothermal environments underscore the biogeographic pattern in archaeal virus communities. ISME J 14:1821-1833.

26. Bautista MA, Black JA, Youngblut ND, Whitaker RJ. 2017. Differentiation and structure in Sulfolobus islandicus rod-shaped virus populations. Viruses 9:120.

27. Prangishvili D, Arnold HP, Gotz D, Ziese U, Holz I, Kristjansson JK, Zillig W. 1999. A novel virus family, the *Rudiviridae*: Structure, virus-host interactions and genome variability of the sulfolobus viruses SIRV1 and SIRV2. Genetics 152:1387-96.

28. Prangishvili D, Koonin EV, Krupovic M. 2013. Genomics and biology of rudiviruses, a model for the study of virus-host interactions in Archaea. Biochem Soc Trans 41:443-50.

29. Martínez-Alvarez L, Bell SD, Peng X. 2016. Multiple consecutive initiation of replication producing novel brush-like intermediates at the termini of linear viral dsDNA genomes with hairpin ends. Nucleic Acids Res 44:8799-8809.

30. Pina M, Basta T, Quax TE, Joubert A, Baconnais S, Cortez D, Lambert S, Le Cam E, Bell SD, Forterre P, Prangishvili D. 2014. Unique genome replication mechanism of the archaeal virus AFV1. Mol Microbiol 92:1313-25.

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247 31. Krupovic M, Dolja VV, Koonin EV. 2020. The LUCA and its complex virome. Nat Rev
248 Microbiol 18:661-670.
249 32. Ptchelkine D, Gillum A, Mochizuki T, Lucas-Staat S, Liu Y, Krupovic M, Phillips SEV,
250 Prangishvili D, Huiskonen JT. 2017. Unique architecture of thermophilic archaeal virus
251 APBV1 and its genome packaging. Nat Commun 8:1436.
252 33. Janekovic D, Wunderl S, Holz I, Zillig W, Gierl A, Neumann H. 1983. TTV1, TTV2 and
253 TTV3, a family of viruses of the extremely thermophilic, anaerobic, sulfur reducing
254 archaebacterium *Thermoproteus tenax*. Mol Gen Genet 192:39-45.

255

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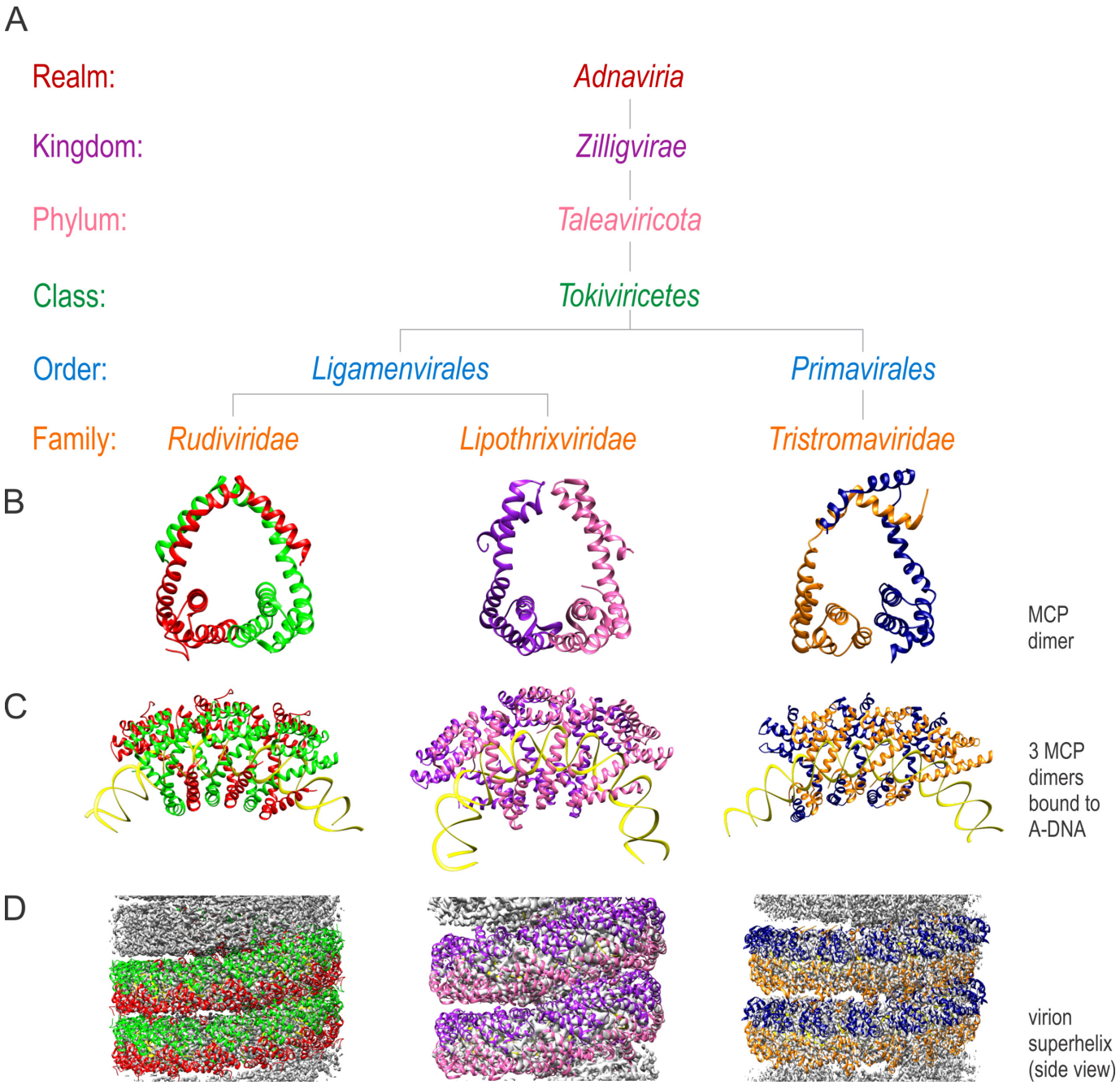
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