Mini-Review

A Family of Novel Cyclophilins, Conserved in the Mimivirus Genus of the Giant DNA Viruses

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Abstract

The cyclophilin (abbreviated here as CYN) family represents a large group of protein prolyl isomerase (PPIase), many of which are also chaperones that promote proper folding of a large variety of client proteins. Over the past few years, megaviruses with giant DNA genomes were discovered and placed in the order Megavirales. Recently, the first complete genome sequence of Acanthamoeba polyphaga mimivirus, a member of the Mimiviridae family of the Megavirales order, revealed a novel CYN that lacked PPIase activity and contained unique peptide insertions. To examine the universality of this unique CYN, I have reviewed and compared all CYN sequences found in the Megavirales genomes that are currently available. The results showed that multiple unique sequence features are indeed highly conserved in CYNs of all members of the Mimivirus genus, whereas viruses of the other genera of this family encode canonical CYNs only. Overall, the primary structures of all Mimivirus CYNs were highly similar, but different from those in the other genera, although the higher order structures were conserved across genera. In summary, this review establishes a family of novel but conserved cyclophilins that occur in a single viral genus.

Keywords: Megavirus, Mimivirus, Klosneuvirus, Chaperone, Protein prolyl isomerase, Cyclophilin, Amoeba

1. Introduction

Cyclophilins are a major class of immunophilins, possessing protein prolyl isomerase (PPIase) activity that accelerates the cis-trans isomerization of the proline peptide bonds in the unstructured polypeptide [1–7]. They also facilitate native folding of their polypeptide substrates through the PPIase activity as well as chaperone function [8, 9]. Of note, I have used the recently accepted root, CYN, for cyclophilin (instead of the older, CyP), as described previously [10, 11]. The cyclophilins (CYNs) are ubiquitously present in all eukaryotes ranging from advanced metazoans to unicellular protists such as fungi, protozoan parasites and amoeba; in contrast, they have been absent in traditional viral genomes, which are much smaller than cellular genomes. However, the recent discovery of very large viruses (often broadly called “megaviruses”), which contain double-stranded DNA genomes in the megabase range, has obfuscated the difference between viruses.
and cells [12–14]. The new and expanding Megavirales order, created to accommodate these viruses (Fig. 1), houses at least four established megavirus families, which are further divided into several genera. Many of these giant viruses encode genes that were formerly in the cellular realm, such as those related to translation [12, 17–22]. Pioneering study of one of the first sequenced megavirus genomes, that of the Acanthamoeba polyphaga Mimivirus [21], led to the discovery of a mimiviral protein that was structurally similar to CYN but contained several unique sequence features and properties, notably, peptide insertions, lack of PPIase activity, CsA insensitivity, and formation of multimers [23]. In view of the potentially critical role of CYN in megavirus life cycle and host-virus interaction, I considered it important to investigate the occurrence and nature of cyclophilins in these newly discovered viruses. To this end, I have explored the available genome sequences of all megaviruses for their CYN orthologs, which revealed conserved genus-specific CYN subsets. Specifically, the unique CYN sequences were found exclusively in the Mimivirus genus, whereas the sister Klosneuvirus genus encoded canonical CYN sequences only. These findings may have implications in the mechanism of evolution and functional specificity of viral cyclophilins.

2. The Megavirales Order of Viruses

Since the giant viruses are relatively new, in order to have a roadmap for this study I first collected information on all major viruses in Megavirales order, and here, I present them in their currently accepted taxonomic ranks (Fig. 1). Only selected families of the parent order are shown, of which Mimiviridae is the most populated and best studied. All genomes were mined for CYN-like sequences in multiple ways, as described in Methods. The three other families (Iridoviridae, Phycodnaviridae, Pandoraviridae) contained no identifiable CYN sequence. The Mimiviridae family is divided into four Genera, although the recent trend is to view Tupanvirus as a subset of Mimivirus genus. The two most populated genera are Klosneuvirus and Mimivirus, both of which are further subdivided into several “lineages”, as shown (Fig. 1). No CYN-like sequence was found in the single known member of the Cafeteriaviridae genus [24] and in the Hokovirus and Courdovirus lineages. It should be mentioned that new megaviruses continue to be discovered, and as such, this is an emerging field in which the taxonomic position of many viruses remains to be finalized. Also to note that the extended Mimiviridae family is sometimes referred to as Megaviridae, although the latter has not been recognized by ICTV (International Committee on Taxonomy of Viruses). Thus, to avoid confusion and redundancy, I elected the Mimivirus genus to accommodate three recent viruses that have been called “Megavirus” (Fig. 1) [17, 25].

3. Cyclophilin Sequences in the Mimiviridae Family of Giant Viruses

As observed above, Mimiviridae is the only family in the Megavirales order that encode CYNs (Fig. 1). Within this family, and if we accept Tupanviruses as essentially Mimiviruses, there are only two genera that encode CYNs, namely Klosneuvirus and Mimivirus. As seen by the GenBank accession numbers, these two genera contain, respectively, 4 and 11 viruses, leading to a total of 15 CYN orthologs in the Megavirales order. Regarding the Klosneuvirus KNV1, its 101-amino acid long cyclophilin sequence in the GenBank (Accession ARF11440) lacks approximately 183 residues at the C-terminus that is highly conserved in this family. This appears to be due to an extra T in the homopolymeric stretch of 8 T’s in the coding sequence, leading to a premature stop codon. We do not know whether this is a sequencing error, common in homopolymeric nucleotide runs, or a naturally occurring truncated CYN. Nevertheless, deletion of this extra T from the Klosneuvirus genome sequence (GenBank: KY684108.1) generated a coding sequence, the conceptual translation of which produced a 284-residue CYN protein sequence (Fig. 2) that is highly similar to the other Mimivirus CYNs and has been used in the rest of the review.

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**Fig. 1.** Classification of select viruses in the Megavirales order. This chart is not meant to be exhaustive, but represents only a subset of families, relevant to this study. Thus, Poxviridae and Asfarviridae were excluded, and so was the recently discovered Marseilevirus, which likely represents a new family of giant viruses [15]. The corrected sequence of KNV1 CYN has been described (Fig. 2). Each GenBank number represents a nonredundant CYN; absence of a GenBank number under a virus name indicates that no CYN homolog was found in that viral genome. CYN sequences from Mimivirus Bombay (AMZ30348.1) and Hirudovirus strain Strangue (AH45239.1) were also excluded, because they were essentially identical (100% and 99% amino acid identity, respectively) to that of the Acanthamoeba polyphaga mimivirus (AAV50868.1), isolates that were originally called Tupanvirus and Megavirus have been placed under the Mimivirus designation. As stated earlier, new megaviruses are being discovered and classified [13, 14, 16], some of which may eventually be assigned into a lineage of Mimiviridae. Examples of such viruses, not placed in this classification tree, include: Terraz, Aureococcus anophagefferens virus (AAV) (“brown tide virus”), Pyramimonas orientalis virus, Phaeocystis globosa virus (PgV) (represented by PgV-16 T strain), Organic Lake Phycodnavirus 1 and 2 (hosts of Organic Lake virophage), Yellowstone Lake Phycodnavirus 4 (YSLGV4), and Chrysochromulina ericina virus (CeV) (e.g. CeV 01).
KNV1 CYN protein sequence (284 amino acids):

MGDMNRVFLPFLVILVLLDLYALKYNQ IFGCNTVYPSXPrQPIKQPPRPLKHYQPPRSPKTITADNISQVIBLKN
DGGQDNGNEHLYKNDLSGLTGSQSLNLENGNSETIPSSFSDKQUNNPWFIFEGKMKRGRISIZIZZDSVYTVF
NFSALETKQGVRKKCVRRI RGYQMNYGRDPSKHHYKIDFEDRLKMKDQGLSLNAMANGPNINISQ
FITTDFTPHLDGHKVVKVINGWVKRLKFETGDQPRKVLKTVDCNA

Fig. 2. Nucleotide sequence and conceptually translated protein sequence of Klosneuvirus KNV1 cyclophilin. The correction of the sequence has been described in the text. This is an intronless gene, and the start and stop codons are underlined.

4. Distinctive Primary Structures of Klosneuvirus and Mimivirus Cyclophilins

Next, I examined the relationships among the CYNs by multiple sequence alignment; to conserve space, only the enzymatically important area is presented here (Fig. 3). For comparison, I also included an animal (human) and a plant (Arabidopsis thaliana) CYN as prototypes of canonical CYN sequences. It was immediately obvious (Fig. 3) that all Mimivirus CYN sequences are highly similar, and as a result, clustered together. The Klosneuvirus CYN sequences were also highly similar, but differed significantly from the Mimivirus group. Within each group, small subsets were also obvious, as shown in a phenogram plot of sequence similarity, where they are clustered together (Fig. 4). For the sake of brevity, I will often refer to these two groups as the Klosneuvirus CYN (klosCYN for short) and Mimivirus CYN (mimiCYN for short).

In general, the mimiCYN group followed the uniqueness noted in the first Mimivirus CYN characterized [23], whereas the klosCYN group was canonical, resembling human CYN (commonly known as hCyPA) and Arabidopsis CYN (commonly Cp4). As shown (Fig. 3), the 13 major amino acid residues that are essential for the PPlase enzymatic activity of canonical CYN [26] are all conserved in the klosCYN group, with the exception of Indivirus CYN, in which Ala224 is replaced by Cys.

In contrast, with few exceptions, all of the 13 residues in the mimiviral CYN were highly superimposable [23]. These results clearly show that the mimiviral CYNs retain CYN-like structural folds in spite of their significant departure from the canonical amino acid sequence, which recapitulates justifies their cyclophilin designation. The large insertions in the mimiviral CYNs apparently did not affect the structural folds because they were located in the flexible linker areas (green lines in Fig. 3), as the crystal structure of A. polyphaga CYN also revealed [23]. Insert #3 was the only insert that was not located in a canonical CYN linker, but it created a short (-)-strand of unknown significance and with no obvious effect on the rest of the molecule.

6. Methods

All sequences were obtained from GenBank, and homology searches with various CYN query sequences, including those of the large DNA viruses, were conducted with the appropriate protein BLAST program, including PSI-BLAST, PHI-BLAST and DELTA-BLAST using default parameters. Searches were performed genomewide as well as within a subset by specifying the organism in the pull-down list, such as “Mimiviridae”, “Mimivirus”. Host organism names, such as “Acanthamoeba” were also used, to search for any cellular homolog (which were not found). Multiple sequence alignments were performed by Clustal Omega [27] at the EMBL-EBI web server [28], as described before [10].

7. Discussion, Summary and Outlook

The central conclusion in this short review is that the cyclophilin (CYN) clades of the two genera in the Mimivirales order of giant viruses, namely Klosneuvirus and Mimivirus, possess distinctive differences. Whereas the Klosneuviral CYNs resemble the classical CYN family, the mimiviral CYNs are unique in multiple ways, largely in line with the first mimiviral CYN characterized earlier [23]. The unique sequence features that distinguish the mimiviral CYNs from canonical CYNs are the lack of PPlase residues and activity, and major sequence insertions in the central catalytic region. Of note, the relationship between PPlase activity and chaperone function of CYN has been debated, since PPlase-defective CYNs may exhibit wild type levels of protein folding activity [9]. For example, recombinant human CYN, in which the catalytically important residues R55, F60, Q63, and H126 (Fig. 3) were changed to
Neither group, however, contains any recognizable C-terminal extensions, while the mimivCYNs are extended towards the N-terminus. Outside of their catalytic cores, the larger members of the cellular cyclophilins are found to contain 7–8 amino acid long insertions in the same loop [30]—8 amino acid long insertions in the same loop [30]—those that are different from consensus are in teal colour. Mutation of the green highlighted residues in human CYNA (R55, F60, Q63, H126) into Ala destroyed PPIase activity but not chaperone activity [9]. The two red triangles are common sites of peptide insertion in a variety of cyclophilins (detailed under Discussion). Asn (N) and Tyr (Y), relatively abundant in sequences outside helices and strands. Two Mimivirus-specific short helices are shown as white arrows. The 13 consensus residues, important for PPIase activity [26], are in red colour; those that are different from consensus are in teal colour. Mutation of the green highlighted residues in human CYNA (R55, F60, Q63, H126) into Ala destroyed PPIase activity but not chaperone activity [9]. The two red triangles are common sites of peptide insertion in a variety of cyclophilins (detailed under Discussion). Asn (N) and Tyr (Y), relatively abundant in sequences outside helices and strands. Two Mimivirus-specific short helices are shown as white arrows. The 13 consensus residues, important for PPIase activity [26], are in red colour; those that are different from consensus are in teal colour. Mutation of the green highlighted residues in human CYNA (R55, F60, Q63, H126) into Ala destroyed PPIase activity but not chaperone activity [9]. The two red triangles are common sites of peptide insertion in a variety of cyclophilins (detailed under Discussion). Asn (N) and Tyr (Y), relatively abundant in sequences outside helices and strands. Two Mimivirus-specific short helices are shown as white arrows. The 13 consensus residues, important for PPIase activity [26], are in red colour; those that are different from consensus are in teal colour. Mutation of the green highlighted residues in human CYNA (R55, F60, Q63, H126) into Ala destroyed PPIase activity but not chaperone activity [9]. The two red triangles are common sites of peptide insertion in a variety of cyclophilins (detailed under Discussion). Asn (N) and Tyr (Y), relatively abundant in sequences outside helices and strands. Two Mimivirus-specific short helices are shown as white arrows. The 13 consensus residues, important for PPIase activity [26], are in red colour; those that are different from consensus are in teal colour.
protists (C. elegans P52011.1, T. cruzi O76990, B. malayi Q27450.1, Onchocerca volvulus AAD09564.1), and plants (A. thaliana P34790.1, Zea mays P21569.1), to large mammals (Bos taurus P26882.6). Of these, the T. cruzi CYN also contains another insertion where mimiCYN insert #2 is located.

In view of the many novel features of the mimiCYN family, as discussed above, I embarked on an intense search for homology with the hope of gaining an insight into their origin and/or function. However, use of whole or overlapping parts of the mimiCYNs as query sequences and various BLAST programs and parameters failed to retrieve any significant homolog from all non-mimivirus sequence databases. Pathogens and parasites often function as gene transmission vectors through genetic recombination, and this has also been implicated for giant viruses [19, 20]. Specifically, the amoeba serves as the host for many mimiviruses, both in nature and in the laboratory, and therefore, efforts were made to determine if the mimiCYN genes may have been derived from various lineages of amoeba, such as Acanthamoeba, Amoeba, Dictyostelium, and Entamoeba. Again, no obvious host CYN homolog resembling the unique features of mimivirus would be found; in other words, the amoeba cyclophilins were found to possess the canonical features. One search returned the GenBank entry WP_082173706.1, described as a hypothetical, partial protein from the bacterium Microvira massiliensis (the corresponding whole genome shotgun sequence is NZ_CAHM01000427.1). I ignored this entry because it was 100% identical to the Powai lake megavirus CYN (ANBS0819.1), which may have resulted from contamination during entry because it was 100% identical to the Powai lake megavirus CYN from the bacterium of amoeba cyclophilins were found to possess the canonical features. One search returned the GenBank entry WP_082173706.1, described as a hypothetical, partial protein from the bacterium Microvira massiliensis (the corresponding whole genome shotgun sequence is NZ_CAHM01000427.1). I ignored this entry because it was 100% identical to the Powai lake megavirus CYN (ANBS0819.1), which may have resulted from contamination during entry because it was 100% identical to the Powai lake megavirus CYN from the bacterium.

**Conflict of Interest**

None.

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