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LETTER TO THE EDITOR

The Possible Role of Prion-Like Viral Protein Domains on the Emergence of Novel Viruses as SARS-CoV-2

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Abstract
Self-replicating proteins or prions deviate from the central dogma of replication. The discovery of prion-like domains in coronavirus SARS-CoV-2 suggests their possible role in viral evolution. Here, we have outlined the possible role of self-replicating protein-like domains in the emergence of novel viruses. Further studies are needed to understand the function of these viral self-replicating protein-like domains and whether they could be antiviral target(s) for the development of effective antiviral agents in the future.

Keywords Prion-like domains (PrDs) · Viral evolution · Novel viruses · Persistent infection · Immune modulation

Brief Introduction About Prions

Transmission of non-Mendelian phenotypic characteristics (Cox 1965; Lacroute 1971) perplexed researchers until the discovery of a conformational change in the translation suppressor sup35 that could lead to suppression of nonsense codons and modification of the translational of proteins (Wickner 1994).The prion Het-s can induce programmed cell death after fusion of genotypically different strains of the fungus *Podospora anserina* (Coustou et al. 1997; Maddelein et al. 2002). Amyloid-like fibrils are similar to prions and are seen in the brains of Alzheimer's disease patients (Nelson et al. 2005; Ritter et al. 2005). On the other hand, a self-replicating prion is associated with the development and conservation of long-term memory (Si et al. 2003).

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Discovery of Prion-Like Domains in Viruses Including SARS-CoV-2

Prion-like domains (PrDs) have been identified among proteins of several unrelated viruses that replicate in different hosts. Some families of viruses (papillomaviruses, hepatitis viruses A, E and D, and members of Orthomyxoviridae) have few or no PrDs, but in human pathogenic viruses, the highest number of PrDs has been detected in the Herpesvirales Epstein–Barr virus and cytomegalovirus, as well as HIV-1 (Retroviridae family, unassigned order) (Tetz and Tetz 2018).

PrDs have been found in proteins involved in interactions with host proteins during viral adhesion and entry to cells, as well as proteins associated with viral biosynthesis (for example, regulatory proteins and nucleic acid binding proteins) (Tetz and Tetz 2018). Most PrDs have been found in RNA and DNA polymerases and helicases (Tetz and Tetz 2018). However, the exact role of PrDs in proteins associated with viral biosynthesis is still unclear (Tetz and Tetz 2018). Nevertheless, the domains may be required for nucleic acid-protein interactions as high levels of PrDs have been observed in eukaryotic proteins binding nucleic acids (Kushnirov et al. 2007).

PrDs commonly contain glutamine-asparagine-rich regions (QNRs) (Michelitsch and Weissman 2000) and these have been found frequently among regulatory molecules and RNA-binding proteins (Kawaguchi et al. 2013). QNRs are associated with proteins responsible for neurodegenerative

disorders such as Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS). These QNRs are epicentres for misfolding of proteins (Michelitsch and Weissman 2000; Hennig et al. 2015). The identification of multiple PrDs in HSV-1 proteins (including surface proteins and nucleic acid binding proteins) has been suggested to be a reason that HSV infection has been associated with the development of Alzheimer's (Tetz and Tetz 2018; Itzhaki 2014; Harris and Harris 2015).

PrDs have been identified in the spike proteins of SARS-CoV-2 (Tetz and Tetz 2020). The spike protein binds to receptors on human cells. Although SARS-CoV and SARS-CoV-2 both bind to angiotensin-converting enzyme-2 (ACE-2), SARS-CoV-2 binds at 10 to 20 times higher affinity (Tetz and Tetz 2020).SARS-CoV-2 has been reported to be the only coronavirus with such a unique distribution of PrDs in its spike protein, which occur at the receptor-binding domain at S1 region. Therefore, it has been suggested that the PrDs identified in SARS-CoV-2 receptor-binding domain may have an important role in its function during adhesion and entry of SARS-CoV-2 to the host cell (Tetz and Tetz 2020).

Interestingly, PrDs may form a subset of protein domains called intrinsically disordered regions (IDRs) (Chen et al. 2020). IDRs found in the viral N-protein can associate with single-stranded RNA in liquid–liquid phase separation (LLPs) (Chen et al. 2020) which leads to RNA compaction (Chen et al. 2020). This presents a mechanism for viral genome packaging induced by IDRs in LLPS of the viral N-protein which may restrict assemblies into single-stranded RNA condensates (Cubuk et al. 2021).

In addition to the possible role of PrDs for viral adhesion, replication and synthesis, they might be responsible, in viruses like HSV, for suppression of host complement activation and host cell death through apoptosis (Molliex et al. 2015; Fraser and Valyi-Nagy 1993). Furthermore, the preponderance of PrDs among human and insect viruses such as *Herpesviridae* (HSV-1, cytomegalovirus, oncogenic HSV-8 and Epstein-Barr virus), *Baculoviridae* and HIV-1 (Fraser and Valyi-Nagy 1993; Fuller et al. 2012; Carroll et al. 2018) indicates that PrDs in the viral proteins may be involved in the induction of latency and persistent infections (Tetz and Tetz 2018).

The Possible Role of PrDs on Viral Evolution and Infectivity

We suggest that PrDs can modify viral infectivity by interacting with viral proteins leading to modification in structural and functional properties. PrDs may interact with viral expression factor proteins or can independently act as viral expression factor(s) regulating gene expression directly leading to synthesis of novel peptides composed of parent viral proteins. Moreover, PrDs many significantly promote the ability of persistent viral infection by induction of rapid and unusual modifications in the structure(s) of viral particle(s).

RNA viruses tend to have higher mutation rates because RNA polymerase is more permissive of error-based mutations than DNA polymerase. However, interestingly SARS-CoV-2, like other coronaviruses, tends to make few mistakes during RNA synthesis compared to other RNA viruses because they have proof reading mechanisms that recognize and correct mistakes. (Denison et al. 2011) Therefore, the rapid and evolving variations in SARS-CoV-2 are unusual (Pachetti et al. 2020). The recent discovery of PrDs in SARS-CoV-2(Tetz and Tetz 2020) suggests these may make a major contribution to this process. PrDs may interact with the viral peptides, for example, spike proteins, and may cause significant modification in structure of proteins which is in fact responsible for functions of proteins ultimately leading to increase in infectivity or virulence of SARS-2 virus.

PrDs are a subset of intrinsically disordered regions (IDRs) in proteins. The variations in SARS-CoV-2 could be due to co-evolution of RNA and PrDs, as a result of the interactions of PrDs through the IDRs identified in certain SARS-CoV-2 proteins. A dynamic combinatorial study suggests a possible mechanism (Liu et al. 2020). Firstly, the annealing of peptide and nucleotide base building blocks gives rise to chimeric ring structures of high specificity. Secondly, the formation of peptide and nucleic acid building blocks containing nucleobases linked with the amino acids can continuously form new combination(s) by self-assembly leading to spontaneous creation of one-dimensional arrangements of nucleobase in the form of nanostructures (Liu et al. 2020). Moreover**,** the unique variations might be the outcome of stereoselective and regioselective interactions between PrDs simulating the condensation of amino acids, which is demonstrated among amyloids by which the short peptide can selectively direct amino acid arrangement. The addition of activated DL-phenylalanine and activated DL-arginine to the peptide RFRFR-NH_2in the presence of a complementary template peptide Ac-FEFEFEFE- NH_2 can lead to the synthesis of an isotacticpeptideFRFRFRFR-NH_2 (Rout et al. 2018).

A nanomechanical analysis of SARS-Cov-2 and its variant D614G(2021) demonstrated more flexibility of the S-proteins in the receptor-binding domain than other SARS-CoV viruses(Hu and Buehler 2021). Another study on nanomechanics and dynamics of the S-protein of SARS-CoV-2 found that these had a very flexible and rapid motion which was resistant to temperature changes. The S-protein's approach to its receptor triggers the receptors to open a channel allowing viral penetration into the cell, and a certain amount of flexibility may facilitate the lock and key interactions between receptor and S-protein

(Hu and Buehler 2021). These structural mobility and flexibility differences might be associated with the greater infinity of SARS-CoV-2 spikes to ACE-2 receptors compared to other coronaviruses (Hu and Buehler 2021) and its increased infectivity (Kiss et al. 2021). It is suggested that the fluctuations in viral S-proteins could be compared with epidemiological data to determine the infectivity and severity of SARS-2 infection (Hu and Buehler 2021).

Interestingly, a study (Tetz and Tetz 2022) has identified unique distributions of PrDs in SARS-CoV-2 such that SARS-CoV-2 has PrDs in its spike protein at the S1 receptor-binding domain. This may be a mechanism for the finding that SARS-CoV-2 exhibits a 10–20-fold higher affinity to its ACE-2 receptor than SARS-CoV (Tetz and Tetz 2022). Substantial differences in the PrDs of spike proteins among emerging SARS-CoV-2 variants including the B.1.1.529 (Omicron) variant have also been found (Kannan et al. 2022; Venkatakrishnan et al. 2021). It might be possible that these unique variations were induced by PrDs, and the change in infectivity might be associated with the unique distribution of PrDsin S-protein at receptor-binding domain of SARS-CoV-2.

The occurrence of PrDs in assembly proteins suggests they may be crucial for the assembly of the nucleocapsid (N-protein) in enveloped and non-enveloped viruses. Precursor polyproteins of positive sense RNA viruses, which are responsible for the fate of virion throughout the infectious cycle, contain PrDs (Tetz and Tetz 2018). These precursor proteins are in an inactive form until they are cleaved and modified by viral or cellular proteases in a highly regulated manner (Yost and Marcotrigiano 2013). IDRs present in the viral N-protein of SARS-CoV-2 are involved in liquid–liquid phase separation with the virus's single-stranded RNA (Chen et al. 2020). The separation of the SARS-CoV-2N-protein in the presence of RNA as well as human RNA-binding proteins can lead to RNA compaction (Perdikari et al. 2020). This compaction maybe a mechanism for viral genome packaging induced by IDRs in liquid–liquid phase separation of the N-protein by restricting assemblies into single-stranded RNA condensates (Cubuk et al. 2021).

The N-protein of SARS-CoV-2 is rich in IDRs and these can undergo single-stranded liquid–liquid phase separation (LLPS) with viral single-stranded RNA (Chen et al. 2020). This interaction might be the mediator of the recruitment of transforming growth factor-β-activated kinase 1 (TAK1) and IκB kinase (IKK) which are the key kinases for NF-κB activation during infection (Wu et al. 2021). Therefore, IDRs within the N-protein could be key factors responsible for the immune response and cytokine storm induced by SARS-CoV-2 infection.

Future Perspective

Interestingly during the recent COVID-19 pandemic, PrDs have been discovered in proteins of SARS-CoV-2 (Tetz and Tetz 2020). As viral proteins are a major player in viral persistence (Roeth et al. 2004; Mankouri et al. 2009; Rossi and Colin 2015; Kane and Golovkina 2010), we suggest that the discovery of PrDs among viral proteins implicates these in evolution among viruses. Therefore, understanding about the role of PrDs might be a way of managing such emerging infections in the future. Further studies are needed to fully understand the role of PrDs in viral proteins.

Conclusion

Self-replicating proteins or prions are involved in non-Mendelian inheritance by de novo replication. Moreover, they are able to interact nucleic acids and can modulate gene expression and ultimately cellular function. The existence of PrDs and their possible role in viral infection and replication are a new revolution in the field of virology. These may have roles in the emergence of novel strains of viruses. Future studies are needed to understand more about PrDs in viral proteins. These domains and their biological functions might be antiviral target(s) and vaccine candidates in the future.

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