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QUASISPECIES FEATURE IN SARS-CoV-2

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ABSTRACT

Since the identification of the SARS-CoV-2, genus Beta- Coronavirus, in January 2020, the virus quickly spread in less than 3 months to all continents with a susceptible human population of about a 7.9 billion, and still in active circulation. In the process, it has accumulated mutations leading to genetic diversity. Regular emergence of variants of concern/significance in different ecology shows genetic heterogeneity in the base population of SARS-CoV-2 that is continuously expanding with the passage of the virus in the vast susceptible human population. Natural selection of mutant occurs frequently in a positive sense (+) single-stranded (ss) RNA virus upon replication in the host. The Pressure of sub-optimal levels of virus-neutralizing antibodies and also innate immunity influence the process of genetic/ antigenic selection. The fittest of the mutants, that could be more than one, propagate and emerge as variants. The existence of different lineages, clades, and strains, as well as genetic heterogeneity of plaque purified virus population, justifies SARS-CoV-2 as 'Quasispecies' that refers to swarms of mutant sequences generated during replication of the viral genome, and all mutant sequences may not lead to virion. Viruses having a quasispecies nature may end up with progressive antigenic changes leading to antigenic plurality that is driven by ecology, and this phenomenon challenges vaccination-based control programs.

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1 Introduction

'Quasispecies' is described as mutant clouds comprising of a population of a virus comprising of a large number of genomic variants (Domingo et al, 2012; Domingo & Perales, 2019). Inside infected cells, a parent virion RNA molecule seldom produces daughter RNA of identical nucleotide sequence, and this occurs as part of the biology of mRNA sense viruses. This variation in the nucleotide sequence in form of substitution/deletion is consequent to error(s) during copying of template/parent RNA molecule by viral RNA-dependent-RNA-polymerase. The significance of a mutant genome sequence, beyond phylogenetic and evolutionary analyses, lies with the existence of matching mutant virions. Mutant virus populations with non-identical genome continuously generate due to error-prone viral RNA genome replication, and the spectrum of mutants in a virus population, either plaque purified or not, vary with the progress in virus replication and subsequent selection process that is influenced by ecology comprising of host, environment/ landscape and the virus itself (Domingo & Perales, 2019). Viruses restrict themselves by either eliminating the host or immunizing the host following primary infection. Such natural restriction in controlling the virus population is usually breached by the emergence of variants that vary in major immunogenic domains. This has been the reason for the second wave of Covid-19 in India and elsewhere when many variant virus populations with antigenic dissimilarity have been identified.

2 Human CoVs

Human respiratory sickness caused by coronavirus was identified in 1962 (Habas et al., 2020). There are seven species of hCoV, which include α -CoVs of HCoV-NL63 & 229E, β -CoVs of HCoV-OC43, HKU1, SARS-CoV-1 and 2, and MERS-CoV. HCoV-229E, -OC43, -NL63, and HKU1 are distributed globally. Even though HCoVs have been identified to create epidemics every 2–3 years with a significant risk of reinfection, there is a scarcity of data on the epidemiology and clinical symptoms of these four HCoVs around the world (Phelan et al., 2020; Ye et al., 2020).

3 The Coronavirus disease- 2019 (Covid-19)

Consequent to the steady transboundary transmission of an emerging Coronavirus, SARS-CoV-2, since December 2019 from Wuhan, PRC, causing highly transmissible respiratory disease, named as Covid-2019, in humans across the world (first Covid-19 case in India was detected on 30 January 2020), the World Health Organization (WHO of the UN) declared 'Covid-19 pandemic on March 12, 2020 (Li et al., 2020a; Pattnaik & Yadav, 2020; Platto et al., 2021). The real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) targeting multiple virus genes (E, N, RdRp) is the gold standard method for detection of SARS-CoV-2 with variable sensitivity that primarily depends on virus load in the

clinical sample, and duration and severity of clinical disease (Corman et al., 2020; Wang et al., 2020a; Li et al., 2020b). During sampling from nostrils and throat or elsewhere, the stage of infection in the individual is largely unknown. Spot antigen test is also being used as a point-of-care (POC) test, and negative samples are subjected to rRT-PCR for the nucleic acid test (NAT). Antibody (IgM and IgG) tests are also in use for retrospective diagnosis. NAT positivity is taken as an active infection. There has been NAT positivity with no clinical sickness; also, NAT negativity with respiratory sickness. Radiological examination of the chest by computerized tomography (CT) of patients with suspected SARS-CoV-2 infection is highly sensitive in arriving at a clinical decision based on the degree of lung pathology (Inui et al., 2020; Yang et al., 2020). The Dutch Radiological Society developed the 'COVID-19 Reporting and Data System' (CO-RADS) that combines chest CT results, clinical data, and NAT results (Prokop et al., 2020; Zayed et al., 2021). Pulmonary pathology is not exclusive to Covid-19, and immunohistochemistry is required to diagnose SARS-CoV-2 infection.

There is no specific treatment for Covid-19, however chemotherapeutic compounds like Remdesvir/ Veklury (ATP analog), Flavipiravir (pyrazine), aminoquinoline, etc., have been used selectively (Majumder & Minko, 2021). In treatment, though ACE2 enzyme inhibitors and receptor blockers (ARBs) have been used in cardiovascular diseases; but increase in expression of ACE2 receptors on cells following treatment with inhibitor/blocker has been observed (Albini et al., 2020). The use of live attenuated vaccines of Polio, MMR, and BCG (bacillus Calmette-Guerin) could boost non-specific immunity (NSI)/ innate immunity and help in reducing the severity of Covid-19 (Majumder & Minko, 2021).

4 The virus

The SARS-CoV-2 is a *Sarbecovirus* in the Genus beta-Coronavirus (β -CoV). Like any other CoV, the genome of SARS-CoV-2 is (+) ssRNA (mRNA sense) of < 30 kb with 5' - cap, 3' - poly(A) tail (Pattnaik & Yadav, 2020). The organization of the viral RNA genome is 5' cap-L-UTRs-polymerase-Spike-Env-Membrane-Nucleocapsid-3'UTR-poly(A) (Lu et al., 2020). There are 14 open reading frames (ORFs) in the viral RNA genome, that encode 4 structural proteins of the spike (S), envelope (E), membrane/matrix (M), and nucleocapsid (N), and 16 non-structural proteins (NSPs; from proteolytic processing of polyproteins pp1A and 1ab). The structural genes are interspaced with 9 accessory genes; ORFs 3a, 3b, 6, 7a, 7b, 8, 9b, 9c, and 10, and the accessory proteins are essential for virus replication, pathogenesis, and virus- morphogenesis processes (Thomas, 2021). Six of the NSPs, viz., 3, 9, 10, 12, 15, and 16 are essential in virus replication (Krichel et al., 2020). The S glycoprotein is a transmembrane that binds through residues in

the Receptor Binding Domain (RBD; in S1 domain) to ACE2 expressed on cells of most human organs. The host TMPRSS2 facilitates membrane fusion with the involvement of the S2 domain of the S glycoprotein. There is a similarity as well as distinction in amino acid composition in RBD between SARS-CoV-1 and -2 (Wan et al. 2020), as both the virus use ACE2 receptor (Hofmann et al., 2020; Wang et al., 2020b). Six different amino acid residues in the RBD are critical in the attachment of the virus to the ACE2 receptor, viz., Leu⁴⁵⁵, Phe⁴⁸⁶, Gln⁴⁹³, Ser⁴⁹⁴, Asn⁵⁰¹, and Tyr⁵⁰⁵ in SARS-CoV-2, and in contrast Tyr⁴⁴², Leu⁴⁷², Asn⁴⁷⁹, Asp⁴⁸⁰, Thr⁴⁸⁷ and Tyr⁴⁹¹ in SARS-CoV-1 (Mohamadian et al., 2020). Both the SARS-1 and -2 viruses are antigenically distinct and different. Highly antigenic epitopes in RBD had A348V, V367F, and A419S (Singh et al., 2020). There was attenuation of SARS-CoV-2 upon deletion of 30 amino acid residues in the S1-S2 junction of S glycoprotein (Lau et al., 2021). Deletion of 382 nucleotides in the accessory protein ORF8 (Δ 382 variant of SARS-CoV-2) is associated with mild disease (Young et al., 2020). The N protein attaches to the viral RNA during the morphogenesis of new virion particles and also contributes to immune evasion by the virus (Mu et al., 2020). The M protein is conserved and in association with N protein and accessory proteins, 3a and 7a facilitate the budding of new virion particles (Roy et al., 2020; Ysrafil, 2020). The E protein facilitates virion maturation and releases from infected cells (Naqvi et al. 2020). Globally, NSP1/NSP2 and ORF7a/3a are the most mutable genes of SARS-CoV-2 (Roy et al., 2020). Both the NSPs and ORF7a and ORF3a are involved in virus replication (Bianchi et al., 2021; Thomas, 2021).

5 Variations in the genome

The S gene is variable in the nucleotide sequence, and the virion surface S glycoprotein determines host susceptibility. Mutations and evolution of the Spike gene have been elaborated (Winger & Caspari, 2021). The most dominant transition and trans-version in the S gene across the globe were C \rightarrow U and G \rightarrow U (Roy et al., 2020). The S protein substitution D⁶¹⁴ \rightarrow G was the first major event in the mutation and evolution of SARS-CoV-2 in the COVID-19 pandemic (Korber et al., 2020; Plante et al., 2020;

Yurkovetskiy et al. 2020). This substitution is located in a B- cell epitope in the S1 domain that classified the SARS-CoV-2 sequences in 2 subtypes, viz. SARS-CoV-2a and -2b, having amino acid residues D614 and G614 respectively. Lineages of SARS-CoV-2b have spread the world over. Both the subtypes differ in immunogenicity, and subtype 2b is reported to be less immunogenic compared to subtype 2a, the parent virus. This evolution possibly made the host hypo-responsive so that the virus could persist in the human population (Kim et al., 2020). Further G614 increased the stability of the virion and has dominated the global Covid-19 scenario. Mutants with mutations around the ACE2 binding site of the virus are in circulation (<http://cov-glue.cvr.gla.ac.uk>; <https://www.gisaid.org>). Naturally occurring mutations in the S gene at positions E484, F490, Q493, and S494 reduced binding of the virus to the Mabs C121 and C144, and mutations at R346, N439, N440, K444, V445, and G446 lead to reduced affinity to the Mab C135 (Robbiani et al., 2020; Baum et al., 2020). This observation shows the presence of virus populations differing in epitope profile. The selection of neutralization escape mutants in vivo will depend upon the concentration and affinity of the neutralizing antibodies (Weisblum et al., 2020).

Several clades and Spike variants have evolved during the pandemic in different countries, viz., Alpha (B.1.1.7; U.K.), Beta (B.1.351; South Africa), Gamma (P.1; Brazil and Japan), Delta (B.1.617.2; India), Delta Plus (K⁴¹⁷ \rightarrow N), Epsilon (B.1.429 and B.1.427), Kappa (B.1.617.1; India), and Eta (B.1.525), etc. An S variant with substitution N⁵⁰¹ \rightarrow Y and deletion of histidine and valine codons at positions 69 and 70, were identified in the UK (Tang et al., 2020). The worldwide prevalence of clade B.1.617 was followed by the generation of its variant strains of Kappa (κ), Delta (δ), and Delta plus (Figure 2).

Several non-synonymous mutations were detected in the transmembrane and C-terminus domains of the E protein (Hassan et al., 2020). Recurrent non-synonymous mutations are linked to adaptation in humans (vanDorp et al., 2020). Several sites of variation in ORFs 1a, 1b, S, 3a, M, 8, and N indicate elective mutations in the virus (Wang et al., 2020c).

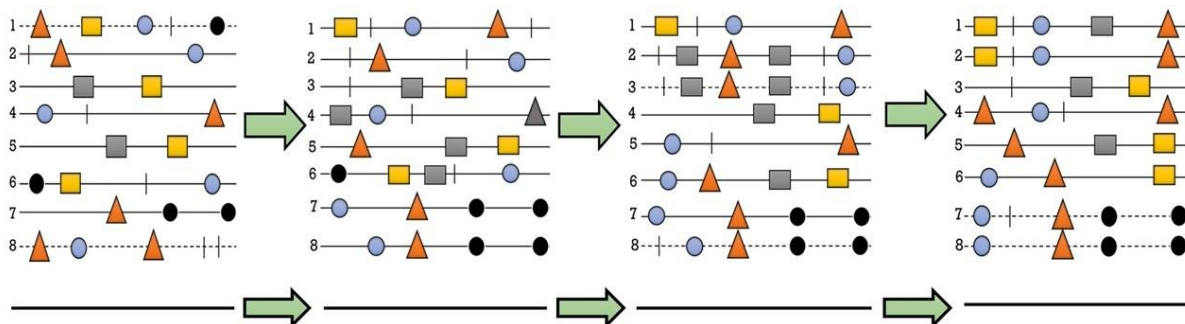


Figure 1 Representation of the evolution (change in composition) of a viral quasispecies (modified from the source: Domingo et al., 2012)

Conclusion and Future Prospects

There is a continuous evolution of SARS-CoV-2 leading to SNP variants and many lineages. SARS-CoV-2 has undergone strong selection pressure over a short period since December 2019. Forces of selection (fitness is the criterion) by the host immune system during replication of the virus and its transmission between hosts play an important role (Roy et al., 2020). The virus also undergoes genetic evolution due to mutations accruing over time and space, producing variants differing from the parent strain(s). The mutation is independent of the fitness of the parent and mutated genome; evolution rate includes time factor. A comprehensive investigation of thousands of SARS-CoV-2 genome sequences identified > 1000 mutations. Virus strain having

less sensitivity to neutralizing antibodies in the immunosuppressed patients was identified. The asymmetric antigenic relationship observed between strains (Yadav et al., 2021) is of epidemiological significance. The phenomenon of quasispecies in foot and mouth disease virus (FMDV; Aphthovirus genus) that has (+) ssRNA (mRNA sense) of about 8.5 kb with 5' -cap, 3'- poly(A) tail has been described, where even virion population in a purified plaque is genetically heterogeneous (Figure 1). CoVs have a higher mutation rate ($0.44 - 2.77 \times 10^{-2}$ per site per year) compared to the Foot-and-mouth disease virus (6×10^{-3} per site per year), though both have (+) ss RNA genome. Plaque purification of SARS-CoV-2 in Vero E6 cells revealed genetic variants in small plaques that could not be detected in the original clinical material. This shows that the variants appeared upon replication of the virus. These

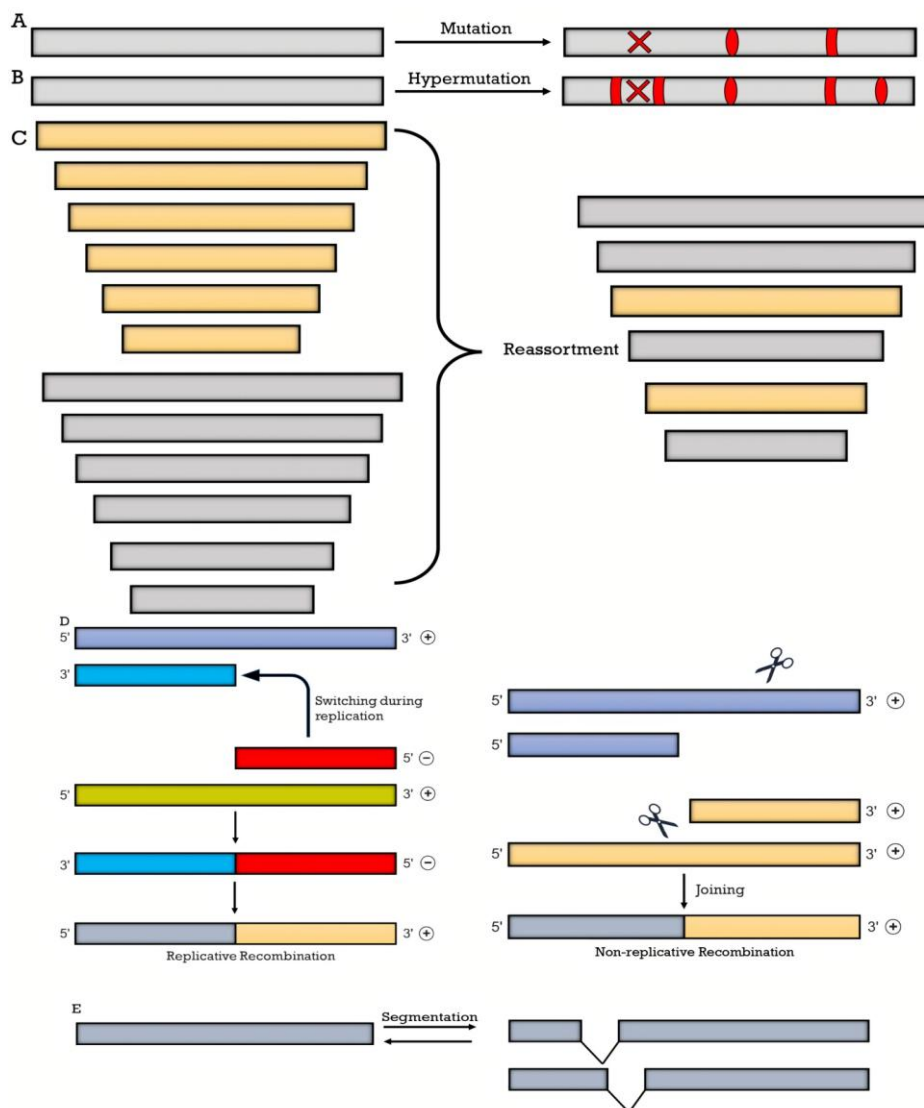


Figure 2 Representation of several types of genetic modifications that can alter the composition of viral quasispecies (modified from the source: Domingo et al., 2012)

variants were either deletion mutants (15-30 nucleotides deleted) or were having point mutations in the S1-S2 interface. Analysis of 55,189 SARS-CoV-2 sequences identified 2175 sequences having non-synonymous mutations in the Spike gene. The frequency of variation varied from 5.48 in coding regions to 6.96 in the noncoding regions (Cao et al., 2021). These observations support quasispecies in SARS-CoV-2. Despite the ongoing global mass vaccination program, the emergence of new a variant virus threatens control of Covid-19. The proposed future directions are (i) random and regular screening of symptomatic patients for SARS-CoV-2 infection is needed, (ii) regular sequencing and analysis of SARS-CoV-2 isolates should be conducted, (iii) mass awareness about the variants and their effect on human health is to be carried out, (iv) more stress should be given to the vaccination of the entire population and other personal hygienic measures.

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Conflict of interest

None

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