Review

Key Factors in the Multiple Mechanisms of Virus Entry

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Received: 22 October 2024; Revised: 12 November 2024; Accepted: 19 December 2024; Published: 2 January 2025

Abstract: Viruses are non-cellular organisms that must parasitize and multiply within living cells to achieve their replicative procedures. Viral assaults can affect bacteria, eukaryotes, and archaea. Well-known viral illnesses in human history include smallpox, Ebola, the black death, the Spanish flu, human immunodeficiency virus (HIV), rabies, SARS, etc. Each of these diseases has caused countless deaths and severe consequences, greatly hindering the progress of human civilization and economic growth. Invasion of host cells by viruses can be broadly divided into several steps: adhesion, entry, replication, assembly, and release. Viral entry is particularly essential for viral invasion of host cells to cause infection. Different methods are employed by enveloped and non-enveloped viruses to mediate virus entry. Whichever entry technique is used, a few essential proteins (virus membrane proteins and cell receptor proteins) play crucial rules. Our knowledge of the structures of important proteins is also essential since it can inform us of the precise steps involved in this procedure. This review discusses the various methods of virus entry (such as Clathrin/Caveolae-mediated endocytosis, Lipid raft, and Macropinocytosis), lists a few typical fusion proteins in virus entry, and offers brief information on the structural characteristics of virus entry for diseases caused by the HIV and the recently discovered virus SARS-CoV-2. The intention of this page is to provide readers with an overall overview of virus entry pathways and to serve as a theoretical foundation for pertinent researches.

Keywords: virus entry; endocytosis; membrane fusion; HIV; SARS-CoV-2; protein structure

1. Introduction of Virus Entry

Viral entry is a prerequisite for viral infection of host cells. Most viruses can enter cells through endocytosis which is triggered by interaction of virus capsid proteins or spike protein and cell surface receptors. Animal viruses can be broadly classified structurally into two categories: enveloped viruses and non-enveloped viruses. Figure 1 depicts the entry patterns of both enveloped and non-enveloped viruses into cells as well as their final destination.

Enveloped viruses enter cells by fusing membranes through endocytosis (Figure 1A). Glycoproteins, proteins, lipids, and glycolipids in the host cell membrane function as viral receptors to concentrate the virus on the cell surface for attachment and to start cellular endocytosis. Chondroitin sulfate proteoglycan and recombinant heparan sulfate proteoglycan (CSPG and HSPG, respectively) are typical attachment receptors for the majority of enveloped viruses [1]. Viral receptors initiate low-affinity, high-avidity interactions that facilitate binding between the viral membrane and cell membranes, resulting in significant conformational changes [1,2]. However, in certain cases, conformational changes may not be noticeable, such as in the high-affinity binding of adenovirus fibers to the coxsackievirus adenovirus receptor (CAR) [2]. When a virus binds to a receptor, a signaling cascade for endocytosis is triggered [2], leading to the production of endosomes that contain viral particles. Early endosomes become late endosomes by fusing with lysosomes in the cytoplasm under the transport of microtubule-based cytoplasmic dyneins. Dynein proteins are involved in the movement of late endosomes to specific sites. The viral core particles are released into the cytoplasm or nucleus where they are required for replication, resulting in viral replication and packaging, through the fusion of the viral envelope with the endosomal membrane. Viral membrane protein conformational changes are caused by interactions between fusion proteins from the viral envelope and receptors in response to cues from the host cell, such as high affinity, low affinity, pH, and Ca^{2+}. Viral membrane protein conformational changes result in the insertion of exposed viral fusion peptide or internal fusion loop into

the endosomal membrane (about 10~20 nm gap). Viral insertion increases membrane curvature and repulsion, leading the viral membrane to fuse with the endosomal membrane and produce fusion pores. The viral genome is then discharged from the endosome and transported from the fusion pore to its final location.

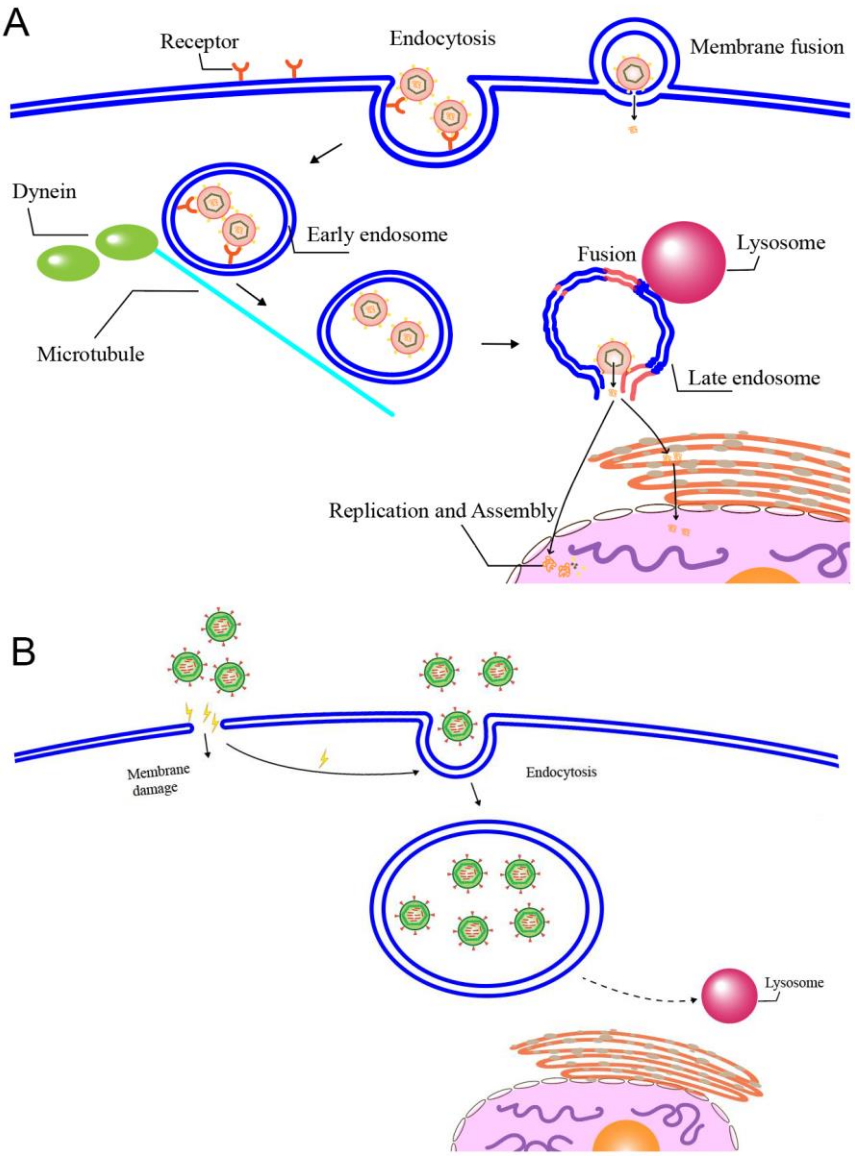

Figure 1. Enveloped and Non-enveloped Virus entry. (**A**): Enveloped viruses enter cells by endocytosis or membrane fusion. Viruses enter cells through endocytosis, forming endosomes containing contents such as viruses. Dynein transport endosomes formed by endocytosis along microtubules, late endosomes membrane fuse viral membrane to release viral nucleic acid into the cytoplasm, then viral nucleic acids enter into nucleus to enable replication and assembly. (**B**): Non-enveloped virus entry. Non-enveloped viruses disrupt the structural integrity of the cell membrane, causing cell membrane pores. Virus enters into cytoplasm through the membrane pores. Non-enveloped viruses can also enter the cell by forming endocytosed vesicles.

Non-enveloped viruses detach part of the viral capsid before entering the host cell, exposing the lipopolysaccharide (Figure 1B). Non-enveloped viruses often enter cells by changes in cell membrane surface conformation caused by viral protein interactions with cellular receptors or by disturbing the structural integrity of the membrane, resulting in cell membrane holes.

2. Endocytosis as a Path of Virus Entry

2.1. Clathrin-Mediated Endocytosis (CME)

The majority of cell surface and extracellular molecules are internalized primarily through clathrin-mediated endocytosis (CME), which is also a crucial method of viral entrance through which multiple cargoes are carried from the extracellular into the intracellular compartment. Multiple enveloped and non-enveloped viruses, however, cleverly take advantage of this ability of host cells and use it to infiltrate cells and subsequently translocate to sites where they need to engage in nucleic acid replication. It has been shown that the influenza A virus (IAV) is capable of entering cells by clathrin-mediated endocytosis and that this entry mechanism depends on cholesterol that has been sequestered in sphingomyelin (SM) [3]. Additionally, it has been demonstrated that clathrin recruitment is necessary for both the spread of the infectious pseudorabies virus (PRV) and the transmission of the poxvirus [4,5]. A dimeric envelope made up of three clathin light chains (CLC) and three clathrin heavy chains (CHC) makes up the fundamental structural component of clathrin . Dynein functions to break off the neck at the recess during clathrin-mediated endocytosis by recruiting clathrin close to the endocytic site. The Heterotetrameric adaptor 2 (AP2) core, a few auxiliary adaptor proteins, and the cell membrane are then bound by clathrin to produce clathrin-coated vesicles (CCVs), which are used to transport virus and extracellular cargo into the cell (Figure 2).

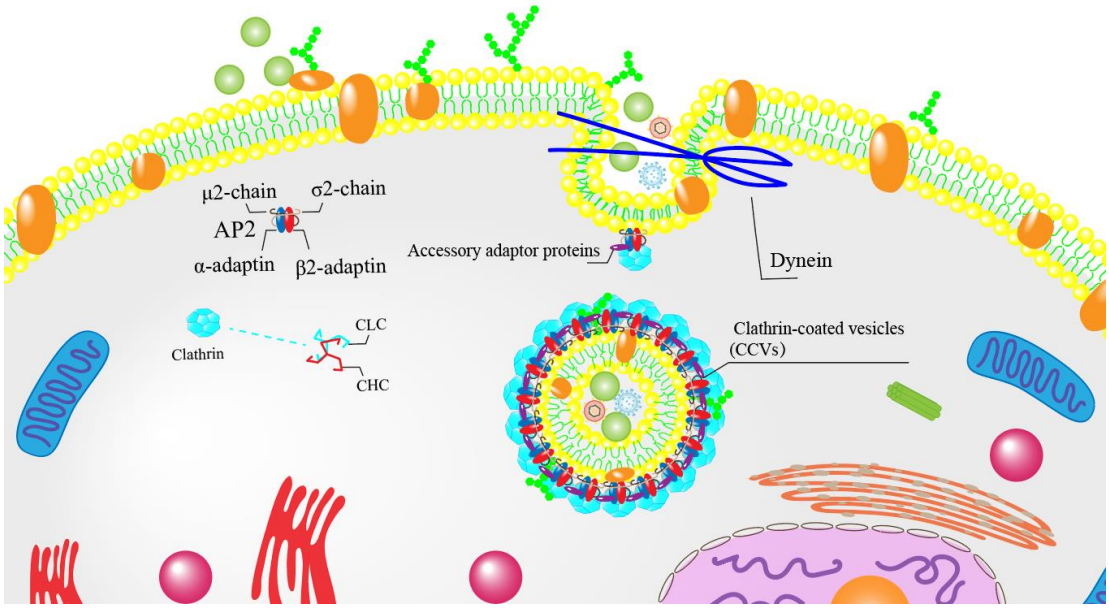

Figure 2. Virus Entry through Clathrin-mediated Endocytosis (CME). Dynein recruit clathrin close to the endocytic site then break off the neck at the recess. The AP2 core, a few auxiliary adaptor proteins, and the cell membrane are then bound by clathrin to produce clathrin-coated vesicles (CCVs), which incorporate virus and extracellular cargo into cytoplasm. Dynein are represented by a scissor pattern.

Many adaptor proteins, including AP-2, Eps-15, Epsin1, and AP180/CALM, are crucial for clathrin-mediated endocytosis, which is required for viral entry [6]. By connecting clathrin to the plasma membrane via clathrin interaction motifs and membrane-bound structural domains, adaptor proteins help clathrin assemble at endocytic sites. AP2 complex, which has a molecular weight of 300 kDa and is made up of subunits, 2, and 2, is the most prevalent clathrin adaptor (Figure 3A,B). By attaching to the plasma membrane phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2], the two most popular cargo motifs (YxxΦ and [ED]xxxL[LI]), clathrin, and other regulatory/auxiliary proteins, AP2 assembles clathrin plays a significant part in CME (Figure 3C). Through many interfaces that bind cargos and membranes carrying PtdIns(4,5)P2 (phosphatidylinositol 4,5-bisphosphate), the AP2 core undergoes conformational modifications from a cytosolic state [7]. As a result of the conformational shift, the clathrin binding domain on its β_2 hinge is exposed. Clathrin is then recruited to endocytic sites to form clathrin-coated pits (CCPs), that subsequently germinate intracellularly to produce clathrin-coated vesicles (CCVs) [8,9]. The dynamin protein, which can cut the neck of developing vesicles on the cell membrane, is another functional protein that is crucial to CME. Vesicle formation is primarily fueled by the super twist created by helical synergy action upon dynamin's GTP hydrolysis [10].

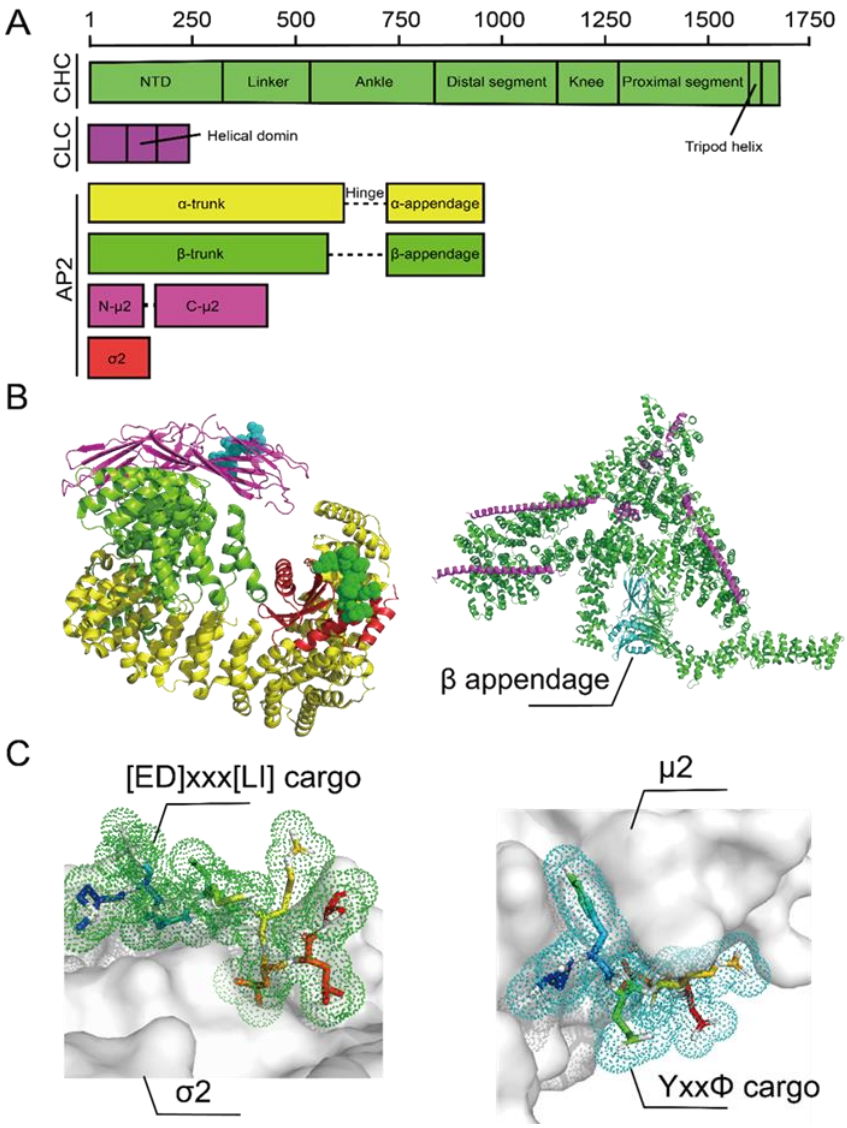

Figure 3. Structural features of Clathrin and adaptor AP2. A: Domain organization of clathrin and adaptor AP2. Three CHC heavy chains and three CLC light chains make up clathrin. (**A**) heterotetramer of four subunits makes up adaptor AP2. (**B**): Cargo-bound adaptor AP2 structure (**left**, PDB: 6QH7). α-yellow; β-green; σ-red; μ-magenta; [ED]xxxL[LI] cargo-green; YxxΦ cargo-cygan. Clathrin combined with AP2 β appendage (**right**, PDB: 6YAI), CHC chain-green; CLC chain-magenta; β appendage-cygan. (**C**): Diagram of two cargo binding pockets of AP2, [ED]xxxL[LI] cargo binds to σ_2 subunit and YxxΦ cargo binds to the μ_2 subunit.

Virus entry may also be affect by other factors involved in clathrin mediated endocytosis, for exAP2 does not drive membrane outgrowth alone; it initially contacts the membrane in a closed conformation through PtdIns(4,5) P2-binding sites on α and β_2 subunits. The equilibrium will change to an open state, where the cargo-binding site is unimpeded, upon the binding of two C-terminal domains of μ_2 ($C\mu_2$) and PtdIns(4,5) P2-binding sites. The YxxΦ cargo-binding pocket, whose density matches that of the bound cargo peptide, is released from the $C\mu_2$ of AP2 relative to the N-terminal of β_2, creating a flat membrane-binding surface. The [ED] xxxL [LI] cargo motif-binding site on σ_2 can access membrane-embedded cargo due to relative movement between the N-terminal of β_2 and σ (Figure 3C). Then, AP2 can "scan" the local membrane for cargo, and its combination will further stabilize the open form on the membrane. As a result, the cargo binding to PtdIns(4,5) P2 is metastably linked. The transition of AP2 from cytoplasmic to single cargo membrane-bound, and from single to double cargo membrane-bound, involves conformational bending of α and β_2 solenoids. As a crucial stage in generating membrane curvature, this procedure does not seem to necessitate AP2 going through oligomerization. When clathrin is recruited by adaptors, their curvature sensitivity is significantly increased [11]. The function of β_2 attachments in regulating membrane curvature, assembly, and/or disassembly in vivo may be reflected in their binding to clathrin scaffolds [7].

2.2. Caveolae-Mediated Endocytosis Pathway (CavME)

Caveolae is a membrane invagination that is well known for its important role in endocytosis. Enveloped viruses can enter host cells via the Caveolae-mediated endocytosis pathway. Caveolin-1 (CAV-1) participates in numerous signaling modalities and is crucial for Caveolae-mediated viral entry (Figure 4A). The EGFR-PI3K-RhoA-ROCK-CFL1 signaling pathway is first activated when the viral envelope protein binds to cellular receptors, which causes conformational changes that cause F-actin polymerization and phosphorylation of CAV-1. Subsequently, phosphorylation of CAV-1 triggers the activation of Rac1, initiating the actin polymerization mediated by PAK1-CFL1, which then initiates the process of viral entry through dynamically dependent Caveolae-mediated endocytosis [12]. Japanese encephalitis virus (JEV), an enveloped virus spread by mosquitoes, has been shown to enter human neuroblastoma SK-N-SH cells and neurogenic rat neuroblastoma B104 cells through a Caveolin-1-dependent route [13,14]. Primitive human brain microvascular endothelial cells (HBMEC) ability to internalize JEV is mostly dependent on host factor ezrin-mediated polymerization of the actin cytoskeleton. JEV internalization is facilitated by Ezrin through Src-mediated vesicular protein-1 phosphorylation [15]. Peste des petits ruminants virus (PPRV), an enveloped virus, can also enter caprine endometrial epithelial cells (EECs) via a caveolae-mediated uptake process that is pH-dependent, cholesterol-dependent, and necessitates kinesin and PI3K but not clathrin [16]. Coronavirus infections, such as those caused by human coronavirus 229E (HCoV-229E) [17] and human coronavirus OC43 (HCoV-OC43) [18,19], are also significantly influenced by caveolae-mediated viral entry.

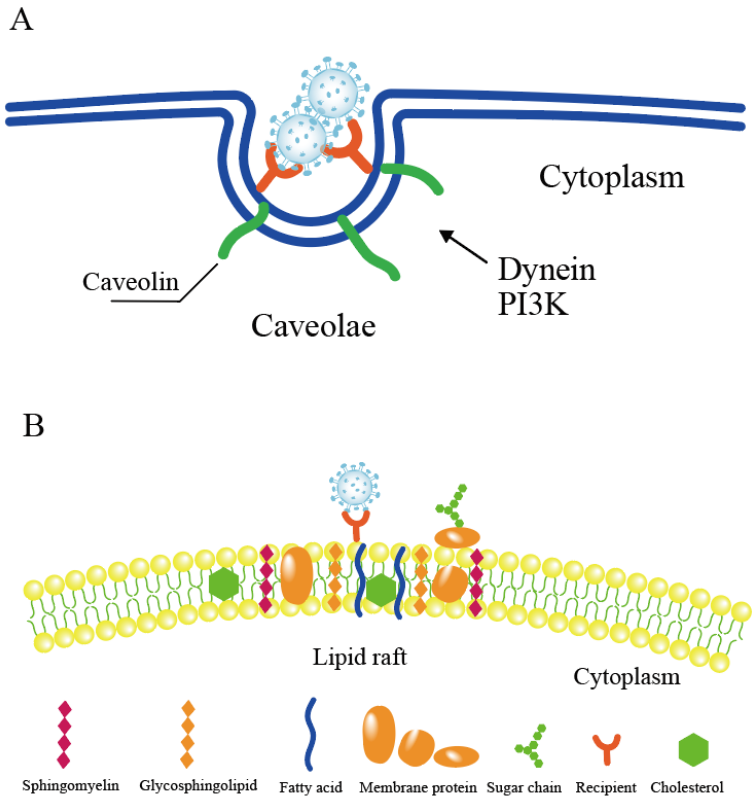

Figure 4. Diagram of the Caveolae-mediated endocytosis and lipid raft composition. (**A**): Caveolae-mediated endocytosis site. (**B**): Lipid raft microstructural domains.

CavME is rarely used by non-enveloped viruses to enter cells. Porcine sapelovirus (PSV), a tiny RNA virus that can enter IPEC-J2 cells via fossa-dependent pH-dependent endocytosis, is an example of Caveolae-mediated non-enveloped virus entry [20]. This process requires kinesin and PI3K but is independent of clathrin and micropinocytosis.

2.3. Lipid Raft-Mediated Endocytosis Pathway

Numerous physiological signaling pathways linked to viral entrance have been revealed to be mediated by lipid raft microstructural domains (Figure 4B) in plasma membranes rich in sphingolipids and cholesterol [21]. Lipid raft microstructural domains are abundant in viral receptors and co-receptors, which may facilitate viral entry

into host cells through attachment and membrane fusion. Both enveloped viruses (such as the hepatitis C virus [22], filoviruses [23], Ebola virus (EBOV) [24], influenza A virus IVA [25], human metapneumovirus (HMPV) [26], and HIV-1 virus [27]) and non-enveloped viruses (such as the EV71 virus [28]) are internalized as a result of lipid rafts. Since there is yet no concrete proof of the presence of lipid raft microstructural domains, many people continue to question the validity of the lipid raft hypothesis. Furthermore, the mechanisms underlying lipid rafts' fundamental structure remain a mystery [29].

2.4. Macropinocytosis Mediated Virus Entry

Macropinocytosis is a type of endocytosis that is clathrin-independent but kinesin-dependent [30,31]. The name "macropinocytosis" refers to the irregular primitive endocytic vesicles (usually 0.5–2 μm, but occasionally up to 5 μm) that, in response to certain stimuli, create huge membrane folds. Fluorescently tagged extracellular material of dextrose can be used to demonstrate macropinocytosis of large-scale non-selective endocytosis. Spectrofluorometry and flow cytometry can also be used to figure out how effectively it occurs [32–35]. Macropinocytosis is also a mechanism by which cancer cells take up nutrients [30,36,37]. Furthermore, viruses frequently use this route to infect host cells [38]. Many viruses that cause human viral diseases, including non-enveloped viruses like adenovirus [39] and echovirus 1 (E-1) [40] and enveloped viruses like Chikungunya virus (CHIKV) [41], AIDS virus (AID) [42], influenza virus [43], respiratory syncytial virus (RSV) [44], Ebola virus (EBOV) [45] and Puumala virus (PUUV) [46], can infect cells through macropinocytosis. Small GTPases of the Ras superfamily, which include Ras, Rho, ADP ribosylation factor (Arf), and Rab GTPases, function in Macropinocytosis as key signaling pathway regulators. Membrane folding and the production of macropinosomes are facilitated by Rho family GTPases, which include Rho, Rac, and Cdc42. By recruiting actin to generate membrane folds in a PI-3K-dependent manner, both Ras/Src protein kinases can promote macropinosome growth [47]. Actin polymerization and membrane ruffling are the results of a signaling cascade that is initiated by active Ras and is also activated by CDC42, Rac1, and Pak1 [48]. Phospholipase Cγ(PLCγ) hydrolyzes PI(4,5)p2 to IP3 and DAG during the membrane ruffle generation stage. The closing of macropinocytic cups is accompanied by PI(4,5)p2 being hydrolyzed by PI3K to PI(3,4,5)p3 [49] (Figure 5). In the macropinosome that is produced after the closing of macropinocytic cups, viruses are included alongside lipids, amino acids, and other nutrients. Viruses can thus invade host cells in this manner. It is still unclear how the macromyosome forms, matures and follows its course at the precise molecular level. To make significant progress in the study of cellular nutrition absorption and virus defense mechanisms, biologists are required to investigate these.

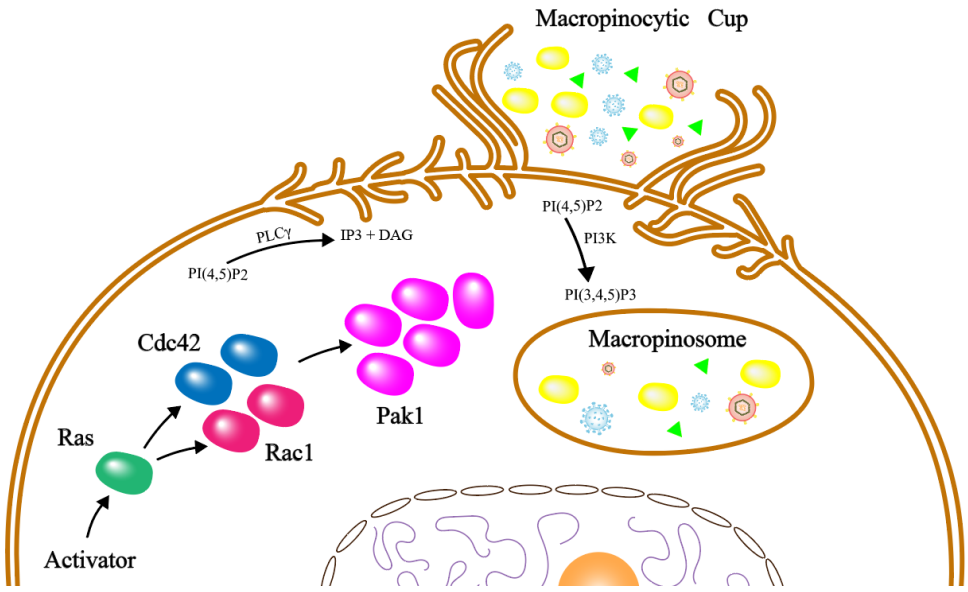

Figure 5. Macropinocytosis Meadiated Virus Entry. Under some certain stimuli, the cell membrane creates huge folds leading to the formation of Macropinocytic cups that contain substances such as viruses to generate macropinosome into the cytoplasm. Thus, viruses are able to enter the cell via this endocytosis pathway.

3. Fusion Proteins as Key Factors during Virus Entry

Envelope proteins, as a crucial component in the infection process, have become a significant target for the development of antiviral medicines and vaccines. Fusion proteins from viruses that have an envelope are divided

into class I (Featuring a fused conformation with a central curly spiral structure α- The characteristic trimer of spiral hair clips), class II (These proteins lack a central coiled-coil helix and have β-folding structural features, forming an extended extracellular domain that refolds to form a hairpin trimer.), and class III (combining structural signatures found in classes I and II.) [50,51]. Table 1 lists some fusion proteins of different envelope virus families [52]. Class I fusion proteins, which are primarily proteins derived from Elastin-Like Polymers (ELPs) and Silk-Like Polymers (SLPs), need host cell proteases for normal function [51]. Furin is the most prevalent host protease involved in the fusing of viral membrane and plasma membrane in Class I fusion proteins. The Env glycoprotein of HIV-1 is synthesized as a trimer by the gp160 precursor in the endoplasmic reticulum, and this process necessitates the intervention of cellular furin proteases for virus-cell fusion [53]. In addition, the furin protease (furin) is required for the fusion of the influenza viruses of the Orthomyxoviridae [54], RSV of the Paramyxoviridae [55], Ebola of the Filoviridae [56] and SARS CoV-2 of the Coronaviridae [57] with the host cell membrane. Correspondingly, the Env glycoprotein (gp120) of HIV, the Fusion glycoprotein (F) of the RSV virus, the Glycoprotein (GP) of the EBOV virus, the Spike glycoprotein (S) of the SARS CoV-2 virus, and the Hemagglutinin (HA) of the influenza virus are the viral fusinogens. E proteins from TBE/dengue viruses [58,59] and E1 proteins from alphaviruses [60] are examples of class II fusion protein. Herpesvirus glycoprotein B (gB), rhabdoviridae G protein, and baculovirus glycoprotein 64 (gp64) are examples of class III fusion proteins. Despite its absence of sequence homology at the amino acid level, these proteins have similar structural characteristics and molecular structures, indicating that they may have originated from a common ancestor, except for evolutionary convergence [61]. The pre-fusion [62] and post-fusion [63] phases of the Vesicular Stomatitis Virus (VSV) fusion protein G protein have been determined. For gB and gp64, only post-fusion structures are known; their mechanisms of conformational changes with membrane fusion are still unresolved [64].

Table 1. Fusion proteins from different families of enveloped viruses [52].

Family	Presentative Virus	Fusion Protein	Class
Orthomyxoviridae	Influenza	HA	Class I
Retroviridae	HIV-1	Env	Class I
	RSV	F/HN	Class I
Paramyxoviridae	Measles Virus	H	Class I
	Henipaviruses	G	Class I
Filoviridae	EBOV	GP	Class I
Coronaviridae	SARS CoV-2	S	Class I
Arenaviridae	LCMV	GP/SSP	Class I
Togaviridae	Rubella Virus	E1/E2	Class II
Flaviviridae	TBEV/DENV/HCV	E/E1/E2	Class II
Bunyaviridae	Hantaan virus	GN/GC	Class II
Herpesviridae	HSV	gB, gH/gL	Class III
Rhabdoviridae	RABV	G	Class III
Baculoviridae	AcMNPV	gp64	Class III
Poxviridae	Orthopoxvirus	8 proteins	Not Classified
Hepadnaviridae	HBV	S/L	Not Classified

4. The Mechanism of HIV Infect Cells

Human immunodeficiency virus (HIV) is the pathogen responsible for Acquired Immune Deficiency Syndrome (AIDS). It is generally believed that membrane fusion is how HIV enters cells. Numerous mechanisms, including clathrin-, Caveolae/lipid raft-mediated endocytosis, and macropinocytosis, can lead to the internalization of HIV [42]. HIV is an enveloped virus, and the envelope spike [Env; trimeric $(gp160)_3$ (Figure 6B), cleaved to $(gp120/gp41)_3$] is belong to class I fusion proteins mentioned in advance for virus entry. The only antigens present on the surface of mature Env spikes, $(gp120/gp41)_3$, are known to trigger potent antibody reactions in infected individuals [65].

Env undergoes a significant conformational shift after binding to its primary receptor CD4 and co-receptors (such as the chemokine receptors CCR5 or CXCR4) Sequentially. This change provides free energy to overcome the kinetic barrier caused mainly by repulsive hydration, triggering membrane fusion and enabling viral entry. This mechanism, which usually involves a cellular receptor and proteolytic cleavage to facilitate conformational rearrangement, transforms a high-energy sub-stable pre-fusion conformation into a low-energy stable post-fusion conformation [66].

To create the receptor-binding fragment gp120 and the fusion fragment gp41, host furin cleaves the precursor protein gp160 after it undergoes trimerization (Figure 6A). Protein is in a sub-stable condition concerning post-

fusion conformation after cleavage between gp120 and gp41. While gp41 directs the fusion of the viral membrane with the host cell membrane, gp120 functions to bind to the receptor. The viral membrane contains the C-terminal transmembrane segment of Gp41, which takes on a pre-fusion shape in the gp160 precursor. The hydrophobic N-terminal fusion peptide (FP) of gp41 is exposed and inserted into the host cell membrane as a result of co-receptor interaction. As a result, the viral and host membranes are bound, and each gp41 fusion peptide in the trimer folds at the hinge region, binding with the amino- and carboxy-terminal helix regions of each gp41 subunit to form the post-fusion conformation, which is a hairpin conformation with a stable six-helix bundle (6HB). To create fusion holes that result in membrane fusion, viral and cellular membranes move in concert when 6HB is formed. The viral components are then transferred into the host cell's cytoplasm [67,68].

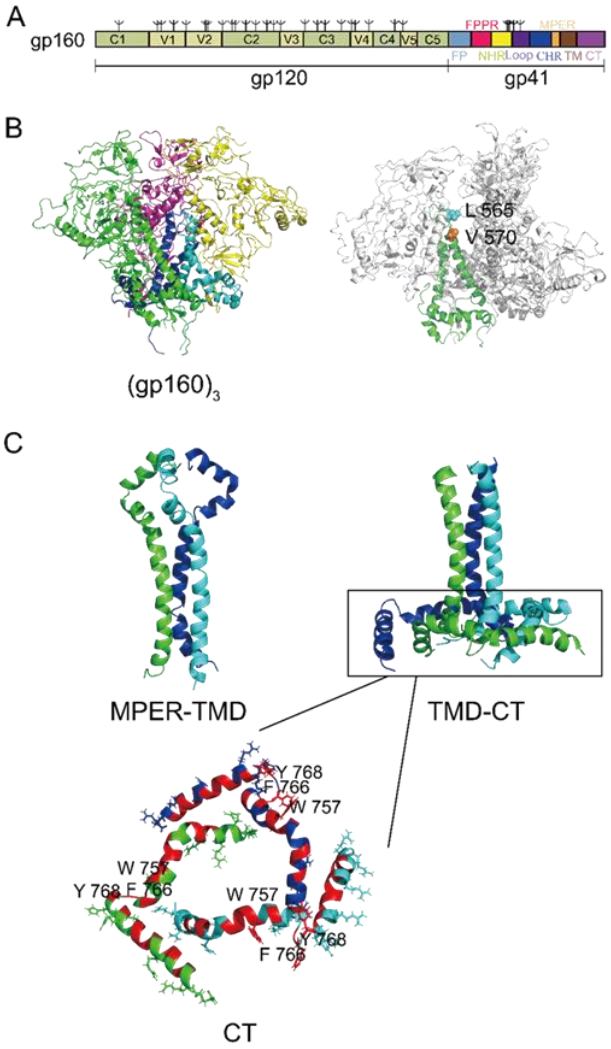

Figure 6. Structures of HIV-1 Envelope protein as a key fact in Virus Entry. (**A**): Domain organization of HIV-1 gp160. (**B**): The $(gp160)_3$ structure (**left**, PDB:7SKA). Position of Leu-565 and Val-570 residues on individual strands in $(gp160)_3$ (**right**). (**C**): Diagrams of structures of MPER-TMD (PDB:6E8W), TMD-CT (PDB:6UJU), and CT. Hydrophobic amino acids (no label, cartoon style) are marked in red, and aromatic amino acids (with label) and basic amino acids (without label) are shown in stick style in the diagram of the CT structure.

Conserved interhelical packing interactions in the gp41 core are crucial regulators of HIV-1 entrance and its inhibition [69]. Additionally, the residues Leu-565 and Val-570 play a significant role in determining the conservation of packing interactions between the gp41 amino- and carboxy-terminal helices (Figure 6B) [70]. Currently, the NMR structures of the transmembrane domain (TMD), the membrane-proximal external region (MPER), and the cyplasmic tail (CT) close to the viral membrane have been determined [71] (Figure 6C). Gp41CT's N-terminal 45 residues are unstructured and do not bind to the membrane. 105 residues at its C-terminus, however, assemble into three membrane-bound amphiphilic -helices with various structural traits, including hydrophobic and basic group surfaces, clusters of aromatic residues, etc. [72]. The C-terminus of TMD is wrapped in CT as an amphiphilic helix, creating a support substrate for the remaining Env. This interaction appears to be

the mechanism by which CT can change the antigenic characteristics of Env [73]. It primarily impacts the antigenic structure close to the top of the Env trimer.

To understand membrane fusion, numerous researchers have concentrated on revealing the molecular structural specifics of virus and receptor interaction at the cellular level. A four-stranded β-fold known as the bridging fold connects the Gp120 core's two distinct structural domains, which are referred to as the internal structural domain and the exter-nal structural domain. Env conformational changes include V1-V2 flipping, V3 exposure, bridging sheet formation, and repositioning of fusion peptide in gp41. Gp41 folding events are thought to be induced by the sequence interaction of gp120 with the principal receptor CD4 and co-receptors (such as CCR5 or CXCR4) [66] (Figure 7A,B). The core of interaction is between F43 and R59 of CD4 and D366, E368, and V425 of gp120. D366 and V425 interact with R59. Additionally, the binding of CD4 to gp120 involves three lysine residues (residues 29, 35, and 46). N280 and K29, S363 and K46, and R59 and D366 all form hydrogen bonds (Figure 7C). All 7-TM helices are in touch with the crown of the V3 ring of gp120, which is embedded in CRS2 of CCR5. Ecl2 of CCR5 contacts with the residues of the V3 stem and crown to form a roughly semicircular handle around the V3 ring. E172 in ECL2 and R304 in V3 in particular might form a salt bond [74]. Additionally, to make contact with the surface of the gp120 bridging sheet, the N-terminal segment of the co-receptor CCR5 adopts an extended configuration with several sharp bends [66]. Sulfated Tyr10, Sulfated Tyr14, and Sulfated Tyr15 of CCR5 all make the most intimate contact with gp120. Sulfated tyrosine aids in CCR5 binding to the HIV-1 gp120/CD4 complex and HIV-1 entry into cells that express CCR5 and CD4 [75]. The inflexible hydrophobic Pro 8, glycosylation site Ser 7, and the disulfide bond between Cys20 and Cys269 all help to maintain the CCR5 N-terminal conformation as well [76] (Figure 7D).

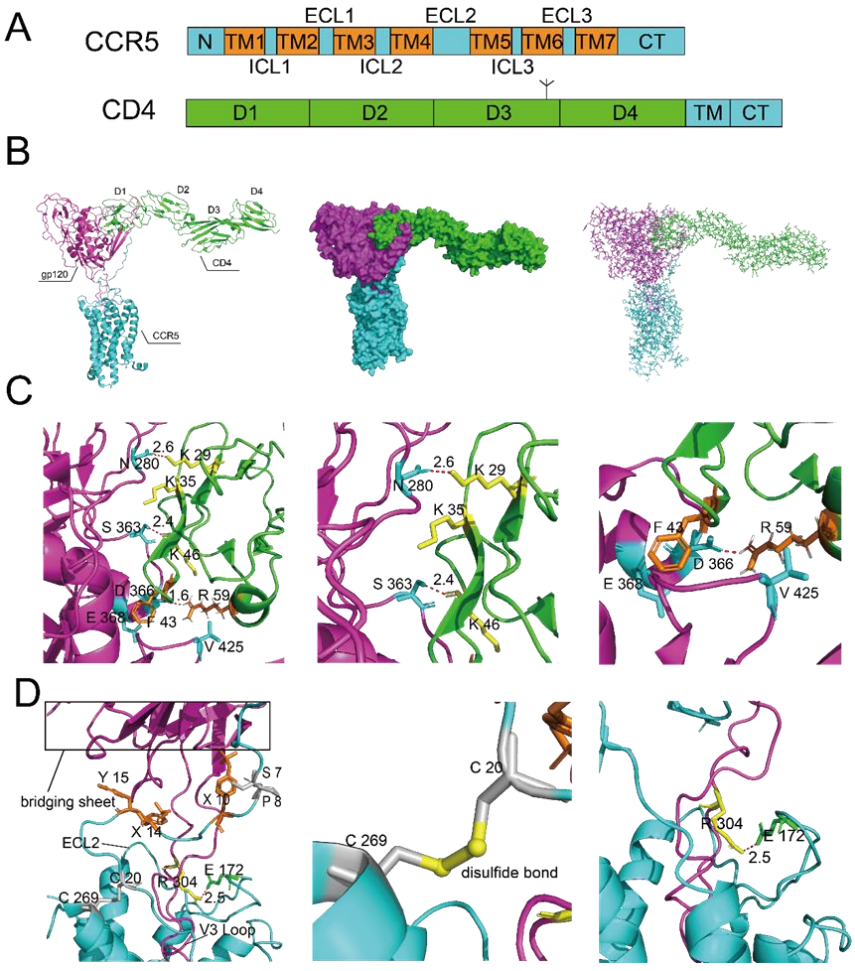

Figure 7. HIV-1 gp120 interacts with receptor CD4 and co-receptor CCR5 [PDB:6MET] during Virus Entry. (**A**): Domain organization of receptor CD4 and co-receptor CCR5. (**B**): Schematic representation of the interaction of gp120 with receptor CD4 and co-receptor CCR5 in different styles in Pymol (from left to right, cartoon, surface, line). (**C**): Contact surface of gp120 with receptor CD4. Key amino acids are indicated by sticks, and hydrogen or salt bonds between them interacting with each other are indicated by red dashed lines. (**D**): Contact surface of gp120 with co-receptor CCR5. Disulfide bonds between Cys20 and Cys269 are indicated by yellow sticks. The black-boxed region is a bridging sheet of gp120.

5. SARS-CoV-2 S-Protein as a Key to Invade Human Cells

SARS-CoV-2 is a recently identified enveloped, positive-sense, single-stranded RNA virus that causes severe acute respiratory syndrome (Corona Virus Disease 2019, COVID-19), which has had a substantial impact on public health and the economy [77]. Fortunately, after their prolonged and persistent efforts, academics and related researchers have unearthed a series of data referring to its viral structure and cellular penetration. This coronavirus virion is made up of four structural proteins: the nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins [78]. The primary processes in its cellular entry does not depend on dynein, clathrin, caveolin and macropinocytosis, but on lipid rafts, endocytosis and membrane fusion (on the cell surface or on endosome) [79,80]. SARS-CoV-2 is capable of attaching to cells via a common receptor angiotensin-converting enzyme 2 (ACE2) [81]. Conformational changes are required in both the S protein and ACE2, as well as interactions between the two.

Prior to SARS-CoV-2 entering host cells, host serine protease furin and other possible preprotein convertases (PCSKs) cleave S protein into two parts—S1 and S2 subunits (Figure 8A). The S2 protein serves as an anchor in membrane fusion, and the S1 protein's function is to bind to ACE2 [81]. The S1 subunit forms the prefusion conformation of SARS-CoV-2, which is the upper half of the central helical bundle, by folding into four domains: the N-terminal structural domain (NTD), the receptor binding domain (RBD), and two carboxy-terminal domains (CTD1 and CTD2). Three RBDs form the head of an S-trimer, which has two perfusion states: 'up' for a receptor-accessible state and 'down' for a receptor-inaccessible state (Figure 8A). The RBD in S1 is exposed in the protomer's "open" conformation and contains a receptor binding motif (RBM) that interacts with ACE2 directly. A D614G mutation in a common SARS-CoV-2 variation in S1 favors "open" conformation, making RBD accessible [82] (Figure 8C). The D614G mutation stabilizes the S protein and is present in all currently circulating variations [81]. Additionally, the upward "open" configuration of RBD is favored by the glycosylation sites N165 and N234 [83] (Figure 8C). Recent studies shows that furin and TMPRSS2 act synergistically in viral entry and infectivity, supporting the combination of furin and TMPRSS2 inhibitors as potent antivirals against SARS-CoV-2 [84–86].

The interaction between the SARS-CoV-2 spike receptor-binding domain (RBD) and ACE2 has been modeled in three dimensions [87]. By presenting the cryo-EM structure of the firmly closed SARS-CoV-2 S trimer and the S trimer bound to ACE2, the molecular intricacies of this membrane fusion mechanism have been elucidated. Y41 of the human ACE-2 (hACE2) receptor is close to H498 in the structure of the human SARS-CoV-2 spike S1 in complex with the human ACE-2 (hACE2) receptor, K353 is close to Y505, Y83/M82 are close to F486 and S19 is close to N477 in the RBD region of the S1 protein (Figure 8C). The presence of ACE2 causes an incredibly drastic shift in the conformational landscape of SARS-CoV-2 from a mostly closed state to an open conformation. S2 subunit-mediated membrane fusion is triggered by a significant conformational change brought by ACE2 binding to S1. The central helix of the S2 subunit rotates counterclockwise, which significantly unravels the tightly closed condition of the S trimer. S1 has significant clockwise rotations of 9.4, 11.2°, and 12.9° in the NTD of protomers 1, 2, and 3, respectively. A 10° downward/outward shift of NTDs is linked to this S1 untwisting [88]. These combined untwisting motions can weaken protomeric interactions, which favors a momentary increase in RBD's ability to bind ACE2 receptors. According to Figure 8B, the S trimer bundled to the ACE2 receptor causes the viral membrane to fusion with the cell membrane. Numerous other host factors, such as the arginine-glycine-aspartate motif (RGD) and DPP4, which are subjects of extensive research, are also required for SARS-CoV-2 infection in addition to ACE2. RGD may play a function in new coronavirus binding to host cells. New coronaviruses' ability to bind to host cells may be influenced by RGD [89].

Comprehending the membrane fusion details between a virus and a cell aid in the creation of disease-prevention and -treatment strategies. The S protein's RBD trimer interacting with the host cell membrane receptor ACE2 triggers membrane fusion. One trimer's RBD changes the trimer's state from "down" to "up," enabling binding between the two. S protein and ACE2 both experience substantial structural change. According to Figure 8A, the S2 protein of SARS-CoV-2 is composed of a fusion peptide (FP), a fusion peptide proximal region (FPPR), a heptapeptide repeat 1 (HR1), a central helix (CH), a junction domain (CD), a heptad peptide repeat 2 (HR2), transmembrane fragment (TM) and cytoplasmic tail (CT) [90]. HR1 and HR2 consist of a duplicated heptapeptide: HPPHCPC, where H is a hydrophobic or conventional bulk residue, P is a polar or hydrophilic residue and C is another charged residue [91]. HR1 is proximal to FP, while HR2 is close to the transmembrane anchor. After the S1 structural domain binds to ACE2, FP enters into the cell membrane. Three HR pairs of the S trimer go through conformational changes to create the six-helix bundle (6HB) motif, in which the HR1 helix forms a central helical coil fusion core that is encircled by three HR2 helices arranged in an antiparallel configuration. Its configuration is exceedingly stable and is expected to assist membrane fusion in getting over a significant energy barrier [92]. Finally, the mobility of the protein complex causes the virus membrane and cell membrane to approach and fuse, allowing the virus genome to enter the cell.

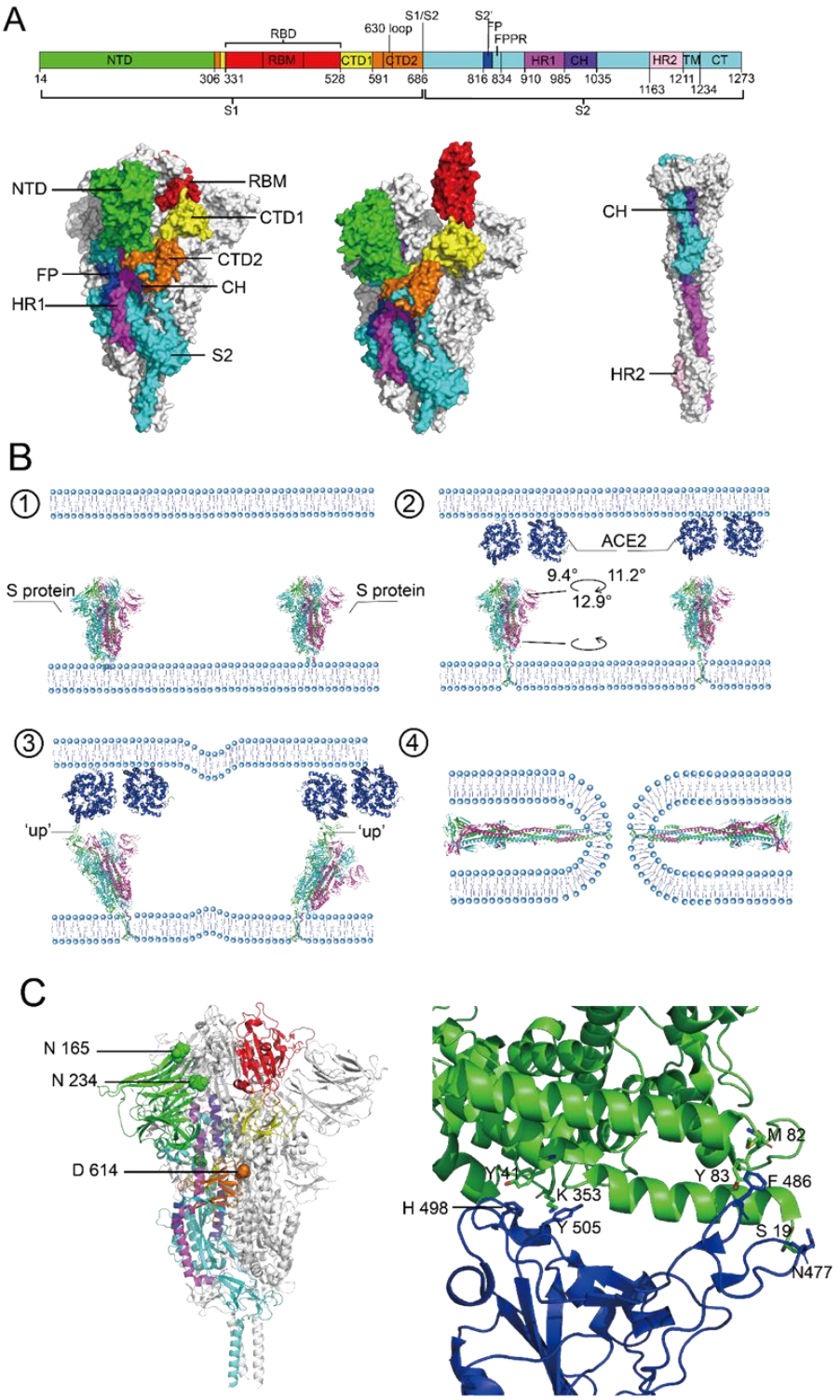

Figure 8. Diagram of SARS-CoV-2 S protein structure and its role in Virus Entry. (**A**): Domain organization of SARS-Cov-2 S protein. The color of the gene fragment in the S protein is consistent with the relevant region in the protein's 3D structure. S protein's RBD is in the "down" state in the pre-fusion state on the left [PDB: 6XR8], and it can bind to the ACE2 receptor to cause membrane fusion, while its RBD is in the "up" state in the center [PDB: 6VYB], as seen in the image on the right. The post-fusion state of the S protein is shown on the right [PDB: 6XRA]. (**B**): Diagram of SARS-CoV-2 Membrane Fusion Process. (**C**): The single strand of the S protein trimer's critical mutation site D614 and glycosylation sites N165 and N234 are depicted as spheres (left, [PDB: 6XR8]). In the right figure (PDB: 7ZDQ), the specifics of the interaction between S1 RBD (green) and ACE2 (blue) are displayed.

6. Entry of Non-Enveloped Viruses

Comparatively less research has been done on non-enveloped virus cell entrance than enveloped viruses. Due to the absence of an envelope, non-enveloped viruses cannot enter the host cell by membrane fusion, but can enter

via endocytosis and subsequently penetrate the endosomal membrane to reach the cytoplasm [93]. Only cellular endocytosis of non-enveloped viruses via cell membrane is insufficient to initiate infection. Infection requires that non-enveloped viruses enter organelle for further transport within host cells to reach their destination for subsequent viral exposure and eventual transport of viral genome to its destination to trigger infection [94]. Each virus's physical characteristics give it a distinct way to infect cells through host membrane penetration and intracellular transit pathways. Non-enveloped viruses can spread their genomes to their target cells by rupturing the host cell membrane with the help of the protein in their viral outer coat [95]. For instance, the "membrane perforation" function of the rotavirus outer protein VP4 can be seen using electron cryomicroscopy, which also allows for the visualization of conformational alterations elements involved in this process [96]. Cleavage of VP4 into the N-terminal fragment VP8* and the C-terminal fragment VP5* is necessary for rotavirus infection [97]. The viral particle is attached to the host cell receptor (often a glycolipid) via VP8*. After initial absorption into the target cell, VP5* penetrates the lipid bilayer surrounding the vesicle membrane into the particle [96]. It is crucial to emphasize that rotavirus entry is accompanied by changes in VP4 conformation and reorganization of the spines, albeit the precise kinetics of this process are unknown.

Adenovirus, a major cause of acute respiratory infections, is another example of a non-enveloped virus entrance method. It is a medium-sized, envelope-free virus with a capsid diameter of approximately 90 nm [98]. Adenovirus cellular entrance is accompanied by membrane damage brought on by protein VI. Initial mechanical cues from cell surface viral receptor, primary receptor CAR, and secondary receptor integrin reveal membrane-soluble protein VI. Initial wounds are created on the host cell surface by protein VI's amphiphilic alpha helix; nevertheless, these first wounds are too tiny for direct mass viral transit. However, these tiny incisions make it easier to trigger Ca^{2+}-dependent lysosomal cytosol vomiting, which raises the amount of ceramide lipid species on the plasma membrane and improves viral endocytosis [99].

7. Cell-to-Cell Transport

Intercellular junctions serve as the protective layer of cells and a vital conduit for information and material transfer between cells and the cells in their immediate surroundings or the extracellular environment [100]. To facilitate infection and reproduction easier, viruses have developed a variety of ways to change intercellular junctions. Infection and replication of viruses are made easier by direct viral intercellular transport, which helps viruses avoid attaching to inhibitors [2]. Tight junctions, anchoring junctions (adherens junctions, desmosomes, and hemidesmosomes), and communicating (gap) junctions are three types of functions for mammalian intercellular junctions. Adherens junctions connect the actin of surrounding cells via related cytoskeleton proteins. Rotaviruses and hepatitis C viruses alter the tight junctions' structure and function, allowing for intercellular transmission [101,102]. By destroying intercellular adhesion connections and inactivating adhesion-linked proteins, the human papillomavirus transmits cells to cells [103]. By hijacking intercellular gap junctions, HIV can spread virulence agents and damaged signals to nearby uninfected cells [104].

Eukaryotic cells are connected by long, bridge-like structures called tunneling nanotubes (TNTs), which facilitate intercellular communication. Through TNTs containing stable (acetylated and de-tyrosinylated) tubulin generated by conserved alphaherpesvirus US3 protein kinase, cells can communicate information (for example, green fluorescent protein [GFP]). Membrane vesicles allow PRV virus particles to move between infected and uninfected cells along US3-induced TNTs [105]. According to reports, the retrovirus HIV-1 uses TNTs to move from infected to uninfected T cells more effectively through cell-to-cell contact [106].

In addition, the Seneca Valley virus (SVV) can be transported between infected and non-infected cells via exosomes [107]. Molecular mechanisms underlying various cell-to-cell connections are still unknown, however, a large number of studies exist to demonstrate that viruses can undergo cell-to-cell transport, which is an important means of possible viral infection and amplification.

8. Approaches to Inhibit or Block Viral Entry

Blocking virus entry into host cells is a key strategy for preventing viral infections. Scientists and researchers have developed various approaches to inhibit or block virus entry. Some of the common strategies are as follows: 1. Neutralizing antibodies specifically bind to the viral attachment proteins or receptors, preventing the virus from entering host cells; 2. Vaccines stimulate the immune system to produce antibodies against the virus's attachment proteins or other critical components involved in entry; 3. Interfering Peptides can mimic the host cell receptors and compete with the virus for attachment; 4. Small molecule inhibitors are developed to block the function of viral proteins required for entry; 5. RNA interference is a molecular technique that can be used to target and silence specific genes, including those encoding viral receptors or attachment proteins; 6. Nanoparticles coated with

molecules that mimic host cell receptors can bind to and sequester viruses, preventing them from attaching to real host cells; 7. In the context of some viral infections, such as SARS-CoV-2, hydroxychloroquine, and zinc have been studied for their potential to interfere with virus entry and replication. However, the efficacy of these treatments remains a subject of ongoing research and debate. It's important to note that the effectiveness of these strategies can vary depending on the virus, and not all approaches will work for every viral infection. Finally, detailed structures of virus envelope proteins and host cell receptors are still needed to elucidate the mechanisms of virus entry to support more in the virus control and treatments.

Virus entry into host cells is a crucial step in the viral life cycle and is essential for the virus to replicate and cause infection. It's important to note that different viruses use various strategies and mechanisms for cell entry (For example, the endocytosis mechanisms of different animal viruses are shown in Table 2), and these processes can be quite complex and specific to the virus type. Understanding these mechanisms is essential for developing antiviral drugs and vaccines to combat viral infections. Additionally, the specific receptors and entry mechanisms are often targets for therapeutic interventions to block viral entry into host cells and prevent infection. Solving the structure of viral fusion proteins, host cell receptors, and the areas of action between them can help us clarify the mechanisms behind viral infection.

In a word, all the structural and interacting information of virus and human cell receptors are still not enough in explaining all phenomena during virus entry. The detailed mechanisms of virus-host interplay need further studies both on structural and dynamic information as well as their interaction with cell membrane during the path virus infect into cells.

Table 2. Main mechanisms of animal virus endocytosis [108].

Virus	Enveloped/Non-Enveloped	Common Entry Mechanisms
IAV	enveloped	membrane fusion (cell surface/endosome); clathrin- dependent endocytosis
HIV-1	enveloped	membrane fusion (cell surface/endosome); clathrin-/caveolae–dependent endocytosis; macropinocytosis; lipid raft
RSV	enveloped	membrane fusion (cell surface/endosome); clathrin/caveolae- dependent endocytosis; macropinocytosis
EBOV	enveloped	membrane fusion (cell surface/endosome); clathrin-dependent endocytosis; macropinocytosis
SARS CoV-2	enveloped	membrane fusion (cell surface/endosome); clathrin- dependent endocytosis; micropinocytosis; lipid raft
JEV	enveloped	membrane fusion (cell surface/endosome); clathrin/caveolae- dependent endocytosis; lipid raft
PRV	enveloped	membrane fusion (cell surface/endosome); clathrin- dependent endocytosis; micropinocytosis
PPRV	enveloped	membrane fusion (cell surface/endosome); caveolae-dependent endocytosis; macropinocytosis
PSV	non-enveloped	caveolae- dependent endocytosis
EV71	non-enveloped	clathrin/caveolae- dependent; lipid raft
AD	non-enveloped	membrane damage
HRV	non-enveloped	membrane pore

Author Contributions: Y.W.: conceptualization, writing-original draft preparation; Q.Y., S.L., W.J., Z.Q., Lina W., Lian W., R.M., K.Z., S.C., J.X., L.Z., M.Z.: visualization, validation; Q.B.F.: supervision, funding acquisition, writing-reviewing and editing.

Funding: This work was fundeded by National Natural Science Foundation of China (32271320) and Creative Research Group of Zhongshan City (Lingnan Pharmaceutical Research and Innovation team CXTD2022011).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Please state "Written informed consent has been obtained from the patient(s) to publish this paper" if applicable.

Data Availability Statement: The authors declare that data published in this paper are all available for nonprofit uses.

Acknowledgments: We thank Donghai Lin from Xiamen University for the rigorous reading and useful suggestions on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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