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Rafting through the palms: S-acylation of SARS-CoV-2 spike protein induces lipid reorganization

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The spike protein of SARS-CoV-2 directs binding to the viral receptor, stimulating a membrane fusion reaction that allows virus entry. In this issue of *Developmental Cell*, Mesquita et al. (2021) demonstrate that S-acylation of spike protein protects it from degradation and triggers cholesterol/sphingolipid-rich raft-like domains to form, enabling membrane fusion.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of the ongoing COVID-19 pandemic. Much attention has been focused on the viral spike protein, as it is responsible for binding of the virus to its receptor, angiotensin-converting enzyme 2, and subsequent fusion between viral and cellular membranes during virus entry (Hoffmann et al., 2020). Also of great interest is that the spike protein is the target of neutralizing antibodies elicited by the recently developed SARS-CoV-2 vaccines. The spike proteins are present at the viral membrane as trimers acquired by the particle as it buds into the ER-Golgi intermediate compartment (ERGIC). Each SARS-CoV-2 spike protein monomer is composed of two subunits generated by furin cleavage of the spike protein precursor—an extracellular S1 subunit, which binds the receptor, and a transmembrane S2 subunit, which drives membrane fusion (see Figure 1). The coronavirus S2 subunit bears a cytoplasmic tail that is highly enriched in Cys residues (ten in the case of SARS-CoV-2). It has been shown that these Cys residues are sites for post-translational modification by S-acylation (McBride and Machamer, 2010; Petit et al., 2007; Puthenveetil et al., 2021), a reaction that is mediated by host cell S-acyltransferases belonging to the zinc finger Asp-His-His-Cys domain-containing (ZDHHC) family of transmembrane enzymes. In humans there are around two dozen ZDHHC enzymes that mediate the S-acylation of thousands of proteins. S-acylation results in attachment of long-chain fatty acids, predominantly palmitic acid, to cytosol-facing cysteines by a thioester linkage. Attachment of the 16-carbon palmitic acid to the cytoplasmic tails of targeted proteins increases local hydrophobicity and can modulate protein-protein interactions and stability and modify the lipid microenvironment surrounding the S-acylated protein. Recent work has shown that S-acylation of SARS-CoV-2 spike is required for virus infectivity (Puthenveetil et al., 2021, Wu et al., 2021), underscoring the biological significance of this post-translational modification in the context of COVID-19. In the Mesquita et al. (2021) study, the team set out to elucidate the role of SARS-CoV-2 spike S-acylation in viral fusion and infection and to define the acyl transferases mediating this reaction (Mesquita et al., 2021).

The authors of this report first targeted ZDHHC expression by siRNA to identify the acyltransferases responsible for spike S-acylation (Mesquita et al., 2021). The results suggest that spike S-acylation occurs shortly after spike biosynthesis in the endoplasmic reticulum (ER) and is initially mediated by ZDHHC20; subsequently, additional Cys residues are modified by ZDHHC9 and ZDHHC20. Mutating the palmitoylated Cys residues in the spike cytoplasmic tail, or knocking down ZDHHC9 and/or ZDHHC20 expression by siRNA, decreased spike half-life, suggesting a role for S-acylation in spike protein turnover. Molecular dynamics simulations suggested that S-acylation leads to the collapse of the cytoplasmic tails, bringing the palmitate molecules together close to the spike protein transmembrane domain and increasing the local acyl chain concentration (see Figure 1). Because S-acylation is known to be a determinant in targeting proteins to cholesterol-rich membrane domains (Lorent et al., 2017), the authors hypothesized that S-acylation modifies the lipid microenvironment around the spike. Consistent with this hypothesis, biochemical assays showed that mutation of the cytoplasmic tail Cys residues or depletion of ZDHHC20 reduced the association of spike with so-called detergent-resistant membrane (DRM), often used as a surrogate for measuring lipid raft association. Significantly, while the ER-associated protein ERGIC53 generally does not associate with DRM, when cells were infected with SARS-CoV-2 this ER marker became DRM associated in a ZDHHC20-dependent manner. Spike protein S-acylation was observed to increase the levels of raft-like lipids, e.g., cholesterol, sphingomyelin, and hexosylceramide, in virus particles. Together, the data support the idea that S-acylated spike protein drives the formation of ordered lipid domains in the ERGIC during virus assembly and subsequently in the virus particle itself. Finally, the team showed that a defect in spike acylation led to decreased particle infectivity.

The results of the Mesquita et al. study provide new insights on the impact of S-acylation on the lipid microenvironment of the SARS-CoV-2 spike protein and the consequences for spike function. These results extend prior findings made with SARS-CoV (Petit et al., 2007) and help to

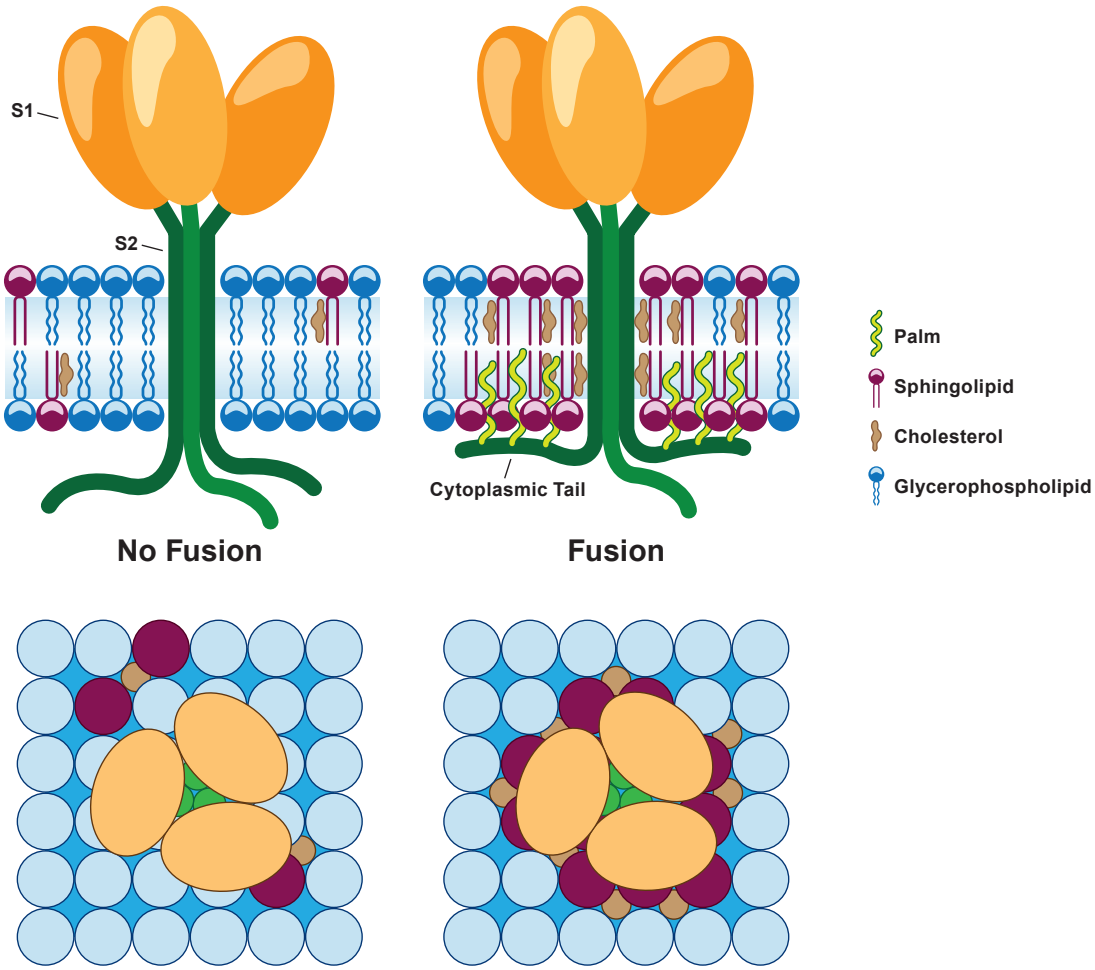

Figure 1. Modification of the SARS-CoV-2 lipid microenvironment by S-acylation of the spike protein
On the left is depicted a non-palmitoylated spike trimer, in both side and top-down view, showing a primarily glycerophospholipid-rich membrane environment. On the right is depicted the clustering of sphingolipids and cholesterol induced by spike protein palmitoylation. As indicated, the non-palmitoylated spike is impaired for membrane fusion and infection, whereas the palmitoylated spike is fusion competent. S1, spike protein S1 subunit; S2, spike protein S2 subunit; palm, palmitic acid.

clarify a previously reported requirement for cholesterol in SARS-CoV-2 fusion and entry (Sanders et al., 2021). In broader terms, it remains to be determined what impact S-acylation has on the conformation of the spike trimer, its mobility in the membrane, and the mechanistic basis for the loss of fusion in the absence of Cys S-acylation. In light of the importance of S-acylation in SARS-CoV-2 infection, it is tempting to consider the possibility of developing selective inhibitors targeting ZDHHC acyltransferases involved in spike S-acylation (Lan et al., 2021). Results described in this manuscript, together with *in vitro* reconstitution experiments described recently (Puthenveetil et al., 2021), point to ZDHHC20 being one of the major players in S-acylation of spike. However, there are some discrepancies regarding which of the other specific ZDHHC family member or members could also be responsible for catalyzing this modification. Therapeutic targeting of S protein acylation will first require definitive identification of which enzyme(s) is responsible in SARS-CoV-2-infected tissues *in vivo* and then the development of highly potent and specific inhibitors of the relevant enzyme(s). Recently, high-resolution structures of two different members of the ZDHHC family, including human ZDHHC20, were solved (Rana et al., 2018). This will likely be an enabling factor in the development of such inhibitors. Nevertheless, as with any attempt to block the activity of an essential host enzyme, circumventing toxicity is a major concern. Because S-acylation is critical to the replication cycle of other pathogenic viruses, there are potentially broad benefits of a successful inhibitor development program. These are early days, but future studies focused on developing a greater understanding of protein S-acylation and therapeutic targeting of this process in the context of viral infection certainly appear warranted.

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