Structural Insights Into N-Linked Glycan Remodeling of the SARS- Cov-2 Spike Protein

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The spike protein engages cellular receptors and membranes, which enables coronavirus entry into host cells. In infected cells, this spike protein is synthesized in the endoplasmic reticulum (ER), glycosylated in ER and the Golgi network compartments, and then exported to the plasma membrane (PM), where it mediates cell-cell fusion for coronavirus transmission and is recognized by the host immune system. This ER to PM trafficking is accompanied by post-translational modifications such as N-glycosylation of the spike luminal domain in the ER/Golgi lumen. These N-linked glycans are critical modulators of spike stability, immunogenicity, and receptor interactions during SARS-CoV-2 infections.

Furthermore, the spike protein from Pfizer-BioNTech, Moderna, and Astra Zeneca genetic vaccines utilizes the host machinery for biogenesis and N-glycosylation along the same ER and Golgi pathway. Hence, insights into N-glycan biogenesis and remodeling will have implications for the fundamental understanding of SARS-CoV-2 infection mechanisms and genetic vaccine technology. Although ER and the Golgi network provide the compartmentalized enzymatic machinery for spike glycosylation and N-glycan remodeling, surprisingly little is known about the biochemical nature of site-specific N-glycans changes, their dependence on inter-organelle transit and the subsequent modulation of conformational changes in these spike glycoforms. Here we show that trafficking through the ER-Golgi-PM pathway involves extensive remodeling of the global glycan landscape in the spike protein. A panel of spike constructs arrested in distinct stages of trafficking reveals a gradient of site-specific changes in the content and charge of glycans, as evaluated by mass spectrometry. We identify a critical role of the spike trans-membrane and cytosolic domains in this glycan remodeling process. Finally, single-particle cryoEM analyses provide insights into conformational changes in these spike constructs, including in the receptor binding and N-terminal domains. Overall, our investigation elucidates structural details from the early stages of spike biogenesis to the late stages of glycan remodeling in the trans-Golgi, thus opening avenues to understand key details of CoV propagation, assembly, infection, and immunity.