How SARS-CoV-2 spike glycoprotein mediates virus to cell fusion

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The first decade of the 21st century has seen three major epidemics caused by zoonotic coronaviruses (CoVs). The ongoing COVID-19 pandemic caused by SARS-CoV-2 is a clear alarm, calling for further research aimed at better understanding of the biology of the coronaviruses. The first step of CoV infection is mediated by the spike (S) protein; the S1 subunit mediates binding to the angiotensin converting enzyme 2 (ACE2) on target cells, whereas the S2 subunit mediates virus-target cell fusion that introduces the viral RNA+ genome into the cytoplasm. Here we propose to focus on the physicochemical mechanism of the S-mediated membrane fusion processes, relying on our extensive experience with studies of fusion proteins of broad evolutionary scope. Characterization of the fusion reaction will be based on *in vitro* fusion assays using ectopic expression of the S glycoprotein and its receptor in mammalian cells, as well as pseudotyped vesicular stomatitis virus (VSV) encoding for S in place of its natural fusion protein (fusogen). Fusion will be characterized for kinetics, fusion intermediates, optimal pH, cofactors requirements, conformational changes, oligomeric states and alternative pathways of fusion at the plasma membrane or inside endosomes. We will also **test the effects of small molecules and peptides to block pseudovirus-cell and cell-cell fusion**. As a second approach, we will adapt a new animal model system that we have recently developed that allows studying the fusion of pseudotyped viruses with different tissues in live *C. elegans*. To this end, we will introduce S-pseudotyped VSV into *C. elegans* expressing hACE2 in intestine or body wall muscles. Based on the power of *C. elegans* as a molecular genetic model organism we will employ RNAi, small molecules and forward genetic screens to search for new players such as coreceptors, enhancers and suppressors of S-mediated membrane fusion. **The proposed study is expected to reveal new players in the SARS-CoV-2-cell fusion process, and has the potential to identify antiviral molecules that may be effective to fight COVID-19** as well as new epidemics of CoVs that will probably emerge from zoonoses in the future.