Evidence for Breathing of a Class I Fusion Protein at the Cell Surface

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Respiratory syncytial virus (RSV) causes a substantial disease burden in infants and the elderly worldwide. Of the two glycoproteins present on the viral surface—the fusion protein (F) and the attachment protein (G)—only F is absolutely required for infection. RSV F is a class I fusion protein, which exists in a metastable conformation (prefusion F) that undergoes dramatic conformational changes to mediate viral entry. We isolated an antibody, called CR9501, that potently neutralizes RSV and prevents the rearrangements of prefusion F required for membrane fusion. Surprisingly, an RSV F–CR9501 complex crystallized as a monomer, despite being fused to a trimerization motif. Additional structural and biochemical studies demonstrated that CR9501 accelerates the disassembly of prefusion F trimers into component monomers and exhibits distinct competition profiles on trimeric and monomeric F. We utilized the unique properties of CR9501 to demonstrate that full-length F trimers sample a monomeric conformation on the surface of transfected cells. In order to reduce flexibility and facilitate crystal packing, a monomeric variant of prefusion F was complexed with CR9501 and crystallized, resulting in a 3.3 Å crystal structure of the antibody–F complex. Analysis of this structure, as well as over 30 previously determined prefusion F structures, revealed a breathing motion that may precede the opening of F at the cell surface. Collectively, these findings suggest that the prefusion F trimer breathes on the surface of cells and have implications for understanding the role of oligomerization in class I-mediated membrane fusion.