The paramyxovirus family comprises major human and animal pathogens, including measles virus (MeV), mumps virus, respiratory syncytial virus, and the highly pathogenic Hendra and Nipah viruses. Viral attachment and fusion (F) envelope glycoproteins mediate fusion of the envelope membrane with target cell membranes to gain cell entry. This fusion event involves a series of conformational changes in F that ultimately result in refolding of the prefusion F trimer into a stable postfusion conformation and opening of a fusion pore. Structural rearrangements of F are irreversible, mandating that initiation of F refolding must be strongly coordinated with the presence of a target membrane to ensure productive virus entry. This coordination is achieved by the attachment glycoprotein complex, which triggers F refolding upon receptor binding. The known structures of F complexes in both pre- and postfusion conformations help illuminate the F refolding pathway (1); however, insight into the molecular framework that defines productive attachment and connects receptor binding to F triggering remains limited. Here, focusing on MeV as a paramyxovirus of high clinical significance, we define the minimal requirements for productive receptor engagement by the MeV attachment (H) protein and reveal fundamental molecular determinants that link receptor binding by H to F triggering, outlining a molecular triggering mechanism of the MeV entry machinery (Fig. P1).

MeV H glycoproteins, similar to the attachment proteins of other paramyxoviruses, feature a globular head domain connected to a transmembrane domain and short luminal tail through a long, rod-like stalk considered to form a helical bundle. The protein assembles into covalently linked homodimers, and a tetrameric dimer–of–dimer organization represents the physiological oligomer (2, 3). The receptor-binding sites are located in the head domains, whereas the stalks of MeV H (2) and related paramyxovirus attachment proteins (4) harbor discrete contact zones considered to mediate specific interaction with F and to induce F triggering. Receptor binding, F interaction, and F triggering represent discrete functions of H that can be complemented in trans (2). Although head monomers and the monomer–monomer interface remain largely unchanged upon receptor binding, tetrameric ectodomain fragments of H crystallize in different dimer–dimer organizations (3). It was suggested that these structures represent pre– and postreceptor–bound F–triggering conformations, but their physiological relevance and the receptor-binding–induced transitioning of MeV H between distinct tetramer organizations remain to be determined.

To probe directly for conformational changes in H tetramers, we established a biochemical assay that separates MeV H tetramers by size and conformation. This assay revealed distinct structural arrangements of F–binding–competent and F–binding–incompetent H tetramers. Exposure of wild-type H tetramers to physical stress or the addition of proteinaceous ligands that trigger the receptor-binding site induced rearrangement from the initial F–binding–competent form to the F–binding–incompetent conformation. Disulfide-bond engineering in the H stalk demonstrated that F triggering requires structural flexibility between H monomers in the central stalk section: Cysteine substitutions in this region abolished fusion-triggering activity, whereas partial reduction of disulfide bonds reversed the arrest.

To test the significance of these findings in the context of native, membrane-embedded glycoprotein complexes, we identified conformation-dependent monoclonal antibodies directed against H proteins

**AUTHOR SUMMARY**

The paramyxovirus family comprises major human and animal pathogens, including measles virus (MeV), mumps virus, respiratory syncytial virus, and the highly pathogenic Hendra and Nipah viruses. Viral attachment and fusion (F) envelope glycoproteins mediate fusion of the envelope membrane with target cell membranes to gain cell entry. This fusion event involves a series of conformational changes in F that ultimately result in refolding of the prefusion F trimer into a stable postfusion conformation and opening of a fusion pore. Structural rearrangements of F are irreversible, mandating that initiation of F refolding must be strongly coordinated with the presence of a target membrane to ensure productive virus entry. This coordination is achieved by the attachment glycoprotein complex, which triggers F refolding upon receptor binding. The known structures of F complexes in both pre- and postfusion conformations help illuminate the F refolding pathway (1); however, insight into the molecular framework that defines productive attachment and connects receptor binding to F triggering remains limited. Here, focusing on MeV as a paramyxovirus of high clinical significance, we define the minimal requirements for productive receptor engagement by the MeV attachment (H) protein and reveal fundamental molecular determinants that link receptor binding by H to F triggering, outlining a molecular triggering mechanism of the MeV entry machinery (Fig. P1).

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**Fig. P1.** Triggering model of the MeV fusion machinery. Binding of a soluble or membrane-embedded receptor to one dimer in the H tetramer (colored by monomer) initiates H head rearrangements along the dimer–dimer interface, and these rearrangements lead to partial unwinding of the central H stalk region (red arrow). This reorganization converts the H tetramer into an F-binding–incompetent conformation, which separates the preassembled H-F complexes, leading to triggering of the F trimer (dark blue). Productive fusion characterized by opening of a fusion pore and content mixing requires MeV H contact with a membrane-embedded receptor.
against MeV F that allowed us to differentiate the prefusion and triggered conformations of the F trimer. Monitoring the triggering status of native F revealed that exposure to synthetic soluble SLAM receptor (5) is sufficient to initiate F refolding under physiological conditions. This finding underscores the physiological significance of the H tetramer rearrangements, demonstrates that very little external energy is required to trigger the viral entry cascade, and confirms that MeV F triggering is achieved readily at the plasma membrane level at neutral pH.

A real-time quantitative fusion assay revealed that triggering the MeV fusion complex with soluble receptor does not result in productive fusion pore formation. This finding possibly indicates that a defined proximity of donor and target membrane is required for fusion, that receptor clustering in the target membrane coordinates the assembly of multiple activated fusion complexes, or that H-receptor interactions are required locally to bend the opposing donor and target membranes for fusion.

Our current view of the paramyxovirus entry machinery is based largely on static X-ray structures (i.e., refs. 1 and 3) and the analysis of mutated and/or severely modified envelope glycoproteins (i.e., refs. 4 and 5). In the present study, we illuminate the mechanistic framework of the fusion-triggering cascade of physiological MeV envelope glycoprotein complexes. Based on our data, we assert that the native MeV fusion machinery is composed of preassembled H–F complexes in a prereceptor-bound prefusion conformation. Upon ligand binding to at least one of the covalent dimer pairs that constitute the H tetramer, rearrangements of the H head domain initiate partial unwinding/unraveling of the central H stalk section but leave the overall physical integrity of the H tetramer and the tetrameric arrangement of the upper stalk section intact. These conformational changes convert the H tetramer into an F-binding-incompetent form, severing the interaction between the H stalk and the F trimer. The change in microenvironment at the former H–F interface may be sufficient to trigger F refolding directly. Remarkably little energy is required to initiate H tetramer reorganization, because docking of soluble receptor is sufficient to initiate the process efficiently. Our advanced mechanistic understanding of the physiological paramyxovirus entry machinery highlights the contact zone of attachment and fusion proteins as an attractive molecular target for the structure-directed design of innovative paramyxovirus entry inhibitors. Clinically, cost-effective and efficacious paramyxovirus blockers have high potential to contribute to a better control of endemic virus transmission through postexposure prophylaxis and to improve disease management.