



FIG. 1. RSV and SeV fusion proteins have different requirements for cell-cell fusion. (A) Schematic diagram of RSV fusion (F_{RSV}) and SeV fusion (F_{SeV}) proteins. The three principal hydrophobic regions found in paramyxovirus F proteins are shown in black: the N-terminal signal peptide (SP), the fusion peptide (FP), located adjacent to the cleavage site at the N terminus of F1, and the C-terminal transmembrane region (TM). Heptad repeat sequences HRA and HRB (hatched boxes) are located adjacent to the FP and TM regions, respectively. Cleavage sites of F_{RSV} (site I, R₁₀₉, and site II, R₁₃₆) and F_{SeV} (R₁₁₆) are indicated by arrows. Cleavage results in the formation of disulfide-linked F2 and F1 polypeptides, and in the case of F_{RSV} , cleavage at both sites results in the removal of the intervening segment pep27, shown in light gray. (B) BSR-T7/5 cells were cotransfected in microchamber culture slides with 0.25 μ g of pTM1 plasmids encoding RSV or SeV F genes and 0.25 μ g pTM1-G or pTM1-HN plasmids, as indicated, or 0.25 μ g empty vector pTM1 (not indicated). The transfection mixture was removed 7 h posttransfection and cells were incubated in serum-free medium with (+) or without (–) trypsin. Cells were fixed and immunostained as indicated in Materials and Methods. Immunofluorescence of cells transfected with the empty pTM1 plasmid was conducted in parallel, and the absence of fluorescence confirmed specific antibody staining (data not shown). (C) BSR-T7/5 cells were transfected in 48-well plates with plasmids encoding RSV or SeV genes. BHK cells were simultaneously transfected with a plasmid encoding the luciferase gene (pTM1-Luc). At 7 h posttransfection, BHK cells were detached using 1 mM EDTA, resuspended at a density of 1×10^6 cells/ml in serum-free medium, and overlaid at a 1:1 ratio onto BSR cells, either in the presence or absence of trypsin. At 30 h posttransfection (or 48 h posttransfection for experiments involving F_{RSV}), cells were lysed and analyzed for luciferase activity. Results (relative light units) are expressed as a percentage of the wild-type (wt) fusion (F_{SeV} plus HN plus trypsin, or F_{RSV} plus G plus trypsin), with background activity subtracted (determined by overlaying BHK cells transfected with pTM1-Luc onto BSR cells transfected with the empty pTM1 plasmid). Mean values from three independent experiments are shown.

forms in approximately equal proportions. A band migrating at a lower molecular weight than F0 was observed for the mutant Fc Δ _{103–117}. Although the identity of this band has not been thoroughly investigated, it may represent the F0 precursor of the Fc Δ _{103–117} mutant, which lacks an N-glycosylation site that has previously been shown to be used in the Z strain of SeV F protein (N104) (35).

When trypsin was added to the medium of the transfected cells (Fig. 3B, lower panel), cleavage of the F0 precursor of

both wild-type F_{SeV} and F_{RKR} to F1 was clearly enhanced. In contrast, only a slight increase in F0 processing was detected when trypsin was added to cells expressing the Fc mutant. However, the extent of F0-to-F1 cleavage was enhanced by trypsin in all four F_{SeV} mutants containing both F_{RSV} cleavage sites, and Fc Δ _{103–130} appeared fully cleaved to F1 in the presence of trypsin. Thus, insertion of an F_{RSV} cleavage site II in the SeV F protein increases the extent of cleavage at this site in the absence of trypsin. This cleavage is further enhanced by