The murine leukemia virus (MLV) envelope protein (Env) plays an important role in inducing membrane fusion during viral entry and cell-to-cell fusion. The ecotropic Friend MLV (FMLV) Env is oligomerized into a trimer in the endoplasmic reticulum, cleaved into the surface protein (SU, gp70) and the transmembrane protein (TM, p15E) in the Golgi apparatus, transported to the cell surface, and incorporated into the virion (1). At the time of virus budding or shortly thereafter, p15E is cleaved into p12E and the C-terminal 16-amino-acid long R-peptide (2). Expression of the wild-type Env does not induce syncytia in NIH 3T3 cells, whereas expression of the R-deleted Env does (3,4). No syncytia are induced by the R-deleted FMLV Env in HeLa cells lacking the FMLV receptor.

Env-mediated membrane fusion is initiated by the binding of SU to the receptor followed by postbinding fusion (1). The receptor-binding domain (RBD) resides in the N-terminal half of SU (5,6). Receptor-binding and postbinding fusion functions can be contributed by two different molecules respectively (7,8). In a previous report (7), a binding-deficient Env with a point mutation in RBD and a fusion-deficient Env with a point mutation in TM functioned in a complementary fashion to induce membrane fusion.

We previously obtained a chimeric FMLV Env, CD4-Env, the RBD of which was completely replaced with a surface domain of human CD4 (9). The CD4-Env chimera was processed, expressed on the cell surface, and incorporated into the virion. When coexpressed, CD4-Env bound to the wild-type FMLV Env to form hetero-oligomers. Further, CD4-Env contributed the postbinding fusion function and complemented a fusion-deficient TM mutant to induce membrane fusion (10). Because the CD4-Env and the TM mutant are required to form hetero-oligomers in the complementation, the fusion efficiency may be dependent on the molar ratio of the former to the latter. In our previous study, however, we examined the complementation between two molecules only at the molar ratio of 1:1. In this study, the optimal ratio for the complementation to induce syncytia was determined.

pCXN2 plasmids expressing FE (a wild-type ecotropic FMLV Env), FE.T470H (a fusion-deficient FMLV Env with a point mutation in the extracellular domain of TM), R-deleted FE.D86K (a binding-deficient FMLV Env with a point mutation in RBD), and R-deleted CD4-Env, respectively, were used (10,11). Expression of R-deleted FE.D86K did not induce syncytia by itself, but did by co-expression with FE in NIH 3T3 cells. Similar complementation in inducing syncytia was observed in experiments using R-deleted CD4-Env instead of R-deleted FE.D86K. Also, complementation was observed...
between FE.T470H and R-deleted FE.D86K or R-deleted CD4-Env. In those complementations, the effect of the molar ratio of the two molecules on induction of syncytia was examined.

First, NIH 3T3 cells were co-transfected with different molar ratios of R-deleted CD4-Env- and FE-expression plasmids, pCXN2R(-)CD4-Env and pCXN2FE, and syncytia induction was compared. Because the nucleotide length of pCXN2R(-)CD4-Env is 8.3 kb and that of pCXN2FE is 8.1 kb, the DNA weight ratio approximates the DNA molar ratio. In NIH 3T3 cells, a pCXN2R(-)CD4-Env-to-pCXN2FE DNA ratio of 1:2 induced syncytia most efficiently (data not shown).

To evaluate the fusion more quantitatively, the syncytia level was examined by co-culture of NIH 3T3 cells with HeLa cells cotransfected with different molar ratios of pCXN2R(-)CD4-Env and pCXN2FE. Co-expression of R-deleted CD4-Env and FE at a ratio of 1:2 induced syncytia most efficiently (Fig. 1A). When R-deleted FE.D86K, instead of R-deleted CD4-Env, was expressed together with FE, the optimal ratio was slightly shifted to the ratio of 1:1 (Fig. 1C), suggesting that R-deleted CD4-Env required more normal RBD in trans than did R-deleted FE.D86K for inducing syncytia. When FE.T470H, instead of FE, was expressed together with R-deleted CD4-Env or R-deleted FE.D86K, the syncytia level was greatly reduced and the optimal ratio was shifted to the higher p12E/SU ratio (Fig. 1B and 1D).

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