158. ROLE OF MA TRIMERIZATION IN HIV-1 ENVELOPE GLYCOPROTEIN INCORPORATION

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ABSTRACT

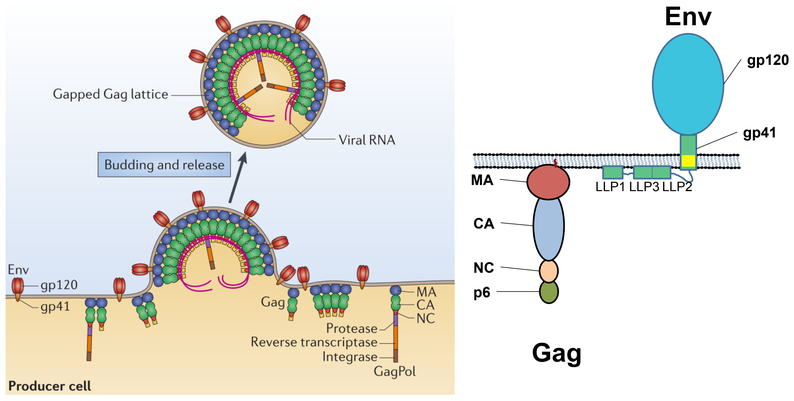
Background: The HIV-1 envelope (Env) glycoprotein complex traffics to the plasma membrane as a heterotrimer containing three molecules each of the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. The gp41 subunit contains a long cytoplasmic tail that interacts with, or is accommodated by, the matrix (MA) domain of Gag during viral assembly. It has been proposed that hexamers of MA trimers form in membrane-bound Gag, overlaying the hexameric capsid lattice. Mutations at the tips of the putative MA trimers (which face the central aperture of the hexamer) prevent the incorporation of Env into particles. The goal of this study was to elucidate the role of MA trimerization in HIV-1 Env glycoprotein incorporation.

Methods: We recently developed a glutaraldehyde cross-linking assay, using a virus with two lysine residues located near the MA trimer interface to analyze MA trimer formation in both immature and mature viral particles. This virus-based MA trimerization assay has enabled us to probe the role of MA trimer formation in Env incorporation.

Results: MA residue L74 is critical for MA trimerization and Env incorporation. This residue is located in the hydrophobic region of the MA trimer interface. Two mutant viruses, L74E and L74G, do not incorporate Env and do not replicate in T cells. MA trimers were also not detected in these viruses. We have selected compensatory mutations that rescue the Env incorporation defect imposed by these mutations. Interestingly, two identified mutations, F43I and F43L, are located in the hydrophobic core of the trimer interface close to L74. We showed that both mutations F43I and F43L, in combination with another mutation, V34I, completely rescued Env incorporation and restored virus replication and infectivity. We are currently using a variety of approaches to monitor the formation of MA trimers in the replication-competent V34I/F43I/L74E and V34I/F43I/L74G mutants.

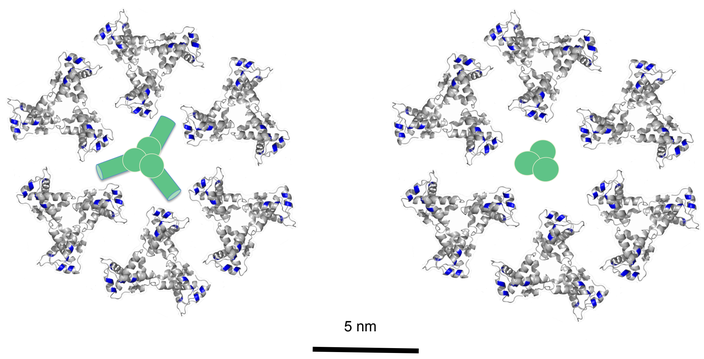
Conclusions: We demonstrated that alterations in the structure of the MA trimer interface affect Env incorporation in assembled virions. Our findings suggest that MA trimer formation is a necessary step for Env incorporation during production of viral particles. These data may ultimately be useful for development of novel therapeutics targeting the MA trimer interface and Env incorporation.

HIV-1 Assembly: Gag and Env



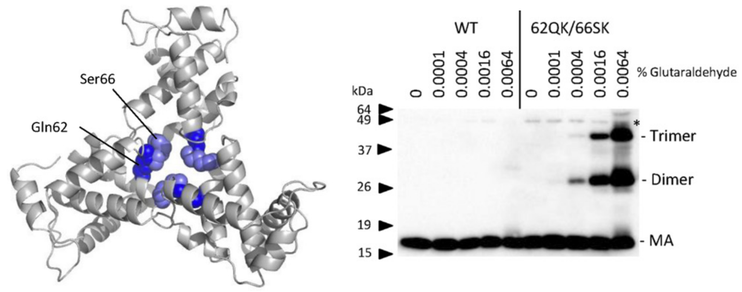
Freed, *Nature Reviews* 2015

MA Lattice and Env Accommodation



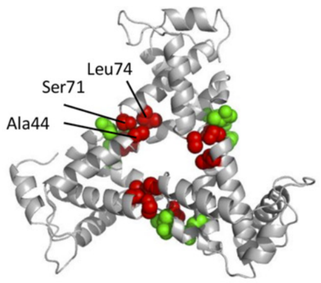
Based on previous structural studies (Alfadhli et al., 2009; Hill et al., 1996) it has been proposed that MA in virions forms hexamers of trimers with a central aperture into which the gp41 cytoplasmic tail (CT) could fit. Mutations in the tips of MA trimers (at regions facing a central hexamer aperture, indicated in blue) prevent the incorporation of Env into particles. The truncation of the long CT restores the Env incorporation level.

MA Trimerization Assay

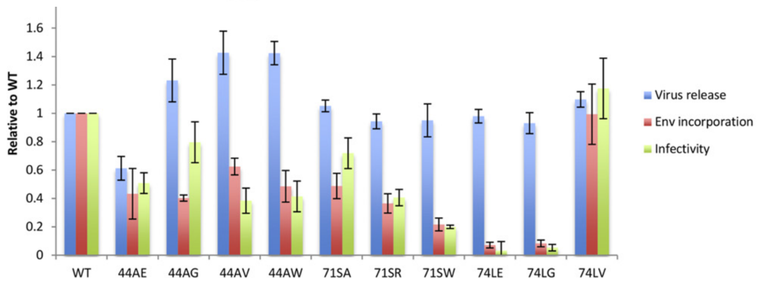


A cross-linking assay using viruses with two lysine residues located at the MA trimer interface. 293T cells were transfected with the HIV-1 MA mutants. At 48 h posttransfetion, viral samples were collected, treated with glutaraldehyde, loaded on the SDS/PAGE gel, and analyzed by western blotting with anti-MA Ab.

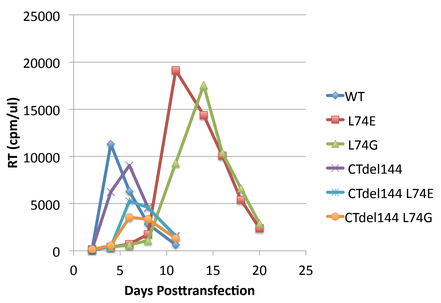
Mutations at the MA Trimer Inteface Impair Env Incorporation and Infectivity



Characterization of viruses with mutations at the MA trimer interface (Tedbury et al., 2016). HeLa cells were transfected with the MA mutants. At 48 h posttransfection, virus particles were pelleted by ultracentrifugation. Cell- and virion-associated samples were analyzed by SDS/PAGE and western blotting for gp41 and Gag. Virus release was calculated as the ratio of virion CA over total Gag levels, and Env incorporation was calculated as the ratio of virion gp41 to virion CA; all numbers were expressed relative to WT. For the single-cycle infectivity assay TZM-bl cells were infected with equivalent amounts of WT and mutant viruses. The luminescence values of WT were normalized to 100%.



L74E and L74G Viruses Do Not Replicate in T Cells Truncation of the gp41 CT Allows the Mutant Viruses to Replicate

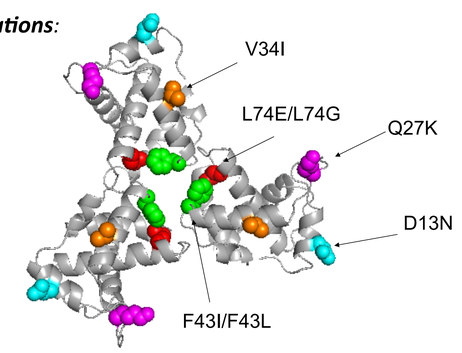


MT4 cells were transfected with WT pNL4-3 and MA mutants. Cells were split every other day. Supernatant was collected for RT analysis. Genomic DNAs were isolated from the cells collected at the RT peak and Gag coding regions were amplified and sequenced to identify compensatory changes.

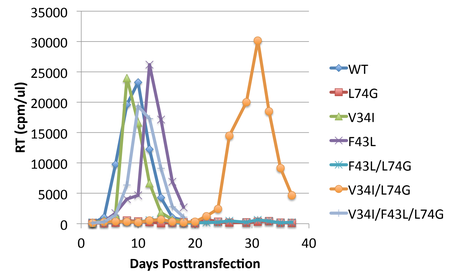
L74E and L74G Viruses Acquire Second-Site Compensatory Mutations

Second-site compensatory mutations:

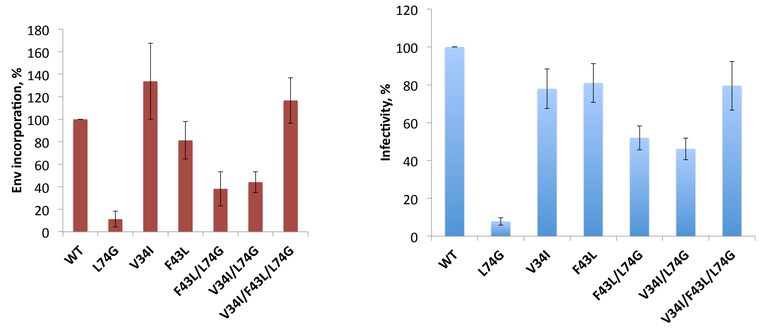
L74E	V34I/F43I
	V34I/E51K
	E51K
L74G	V34I/F43L
	D13N/V34I
	Q27K



Second-Site Compensatory Mutations Restore Replication Activity, Infectivity and Env Incorporation Level of L74E and L74G Mutants

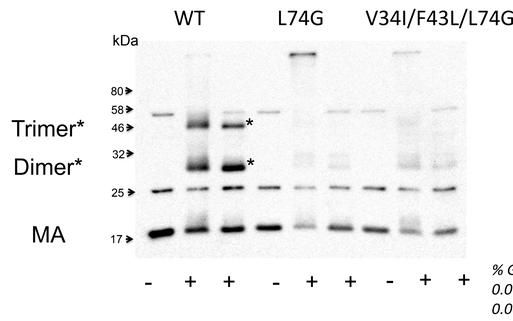


Jurkat cells were transfected with WT pNL4-3 and L74G mutants. Cells were split every other day. Supernatant was collected every day for RT analysis. Comparable results were obtained using L74E mutants.



HeLa cells were transfected with L74G mutant viruses. At 48 h posttransfection, virus particles were collected, analyzed by SDS/PAGE and western blotting with anti-gp41 Ab and HIV-1 Ig. Env incorporation was calculated as the ratio of virion gp41 to virion CA; all numbers were expressed relative to WT. For the single-cycle infectivity assay TZM-bl cells were infected with equivalent amounts of WT and mutant viruses. The luminescence values of WT were normalized to 100%. Comparable results were obtained using L74E mutants.

MA Trimerization Assay for L74G Viruses



293T cells were transfected with L74G viruses bearing Q62K and S66K mutations. At 48 h posttransfetion, viral samples were collected, treated with glutaraldehyde, separated by SDS/PAGE, and analyzed by western blotting with anti-MA Ab.

CONCLUSIONS

- MA trimers have been detected in HIV-1 virions using a developed cross-linking assay

- Disruption of MA trimers caused by mutations in the trimer interface results in a loss of Env incorporation and consequently low viral infectivity

- Truncation of the long CT restores the Env incorporation level probably eliminating steric hindrances between the long CT and MA domains and allows the mutant viruses to replicate (Tedbury et al., 2016)

- MA trimer-defective mutants L74E and L74G do not incorporate Env

- Compensatory mutations F43I and F43L at the trimer interface together with V34I mutation restore Env incorporation, infectivity and replication activity of mutant viruses

FUTURE WORK

- Cross-linking assay with glutaraldehyde and different reagents, other MA mutants and protease-deficient virions
- Characterization of mutant MA-membrane lattices
- Crystallization of 74LE/LG MA proteins