Role of Spacer Peptide 2 in HIV-1 Replication

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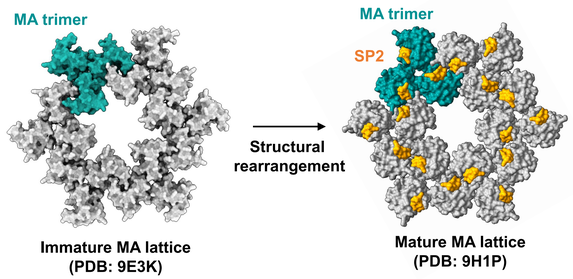
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Background

Structural studies have shown that the HIV-1 matrix (MA) protein assembles into hexamers of trimers beneath the viral membrane and undergoes a conformational rearrangement during virion maturation. A subsequent structural study suggested that this transition is triggered by binding of spacer peptide 2 (SP2), released during protease (PR)-mediated Gag processing, to MA. Although SP2-triggered MA maturation has been linked to Env-mediated membrane fusion, earlier studies reported only minor effects on infectivity when SP2 was deleted, or its cleavage blocked. We have also recently reported that the MA-L20K/E73K/A82T mutant replicates with WT kinetics in multiple T-cell lines despite lacking an organized mature MA lattice, leaving the physiological role of MA maturation unclear.

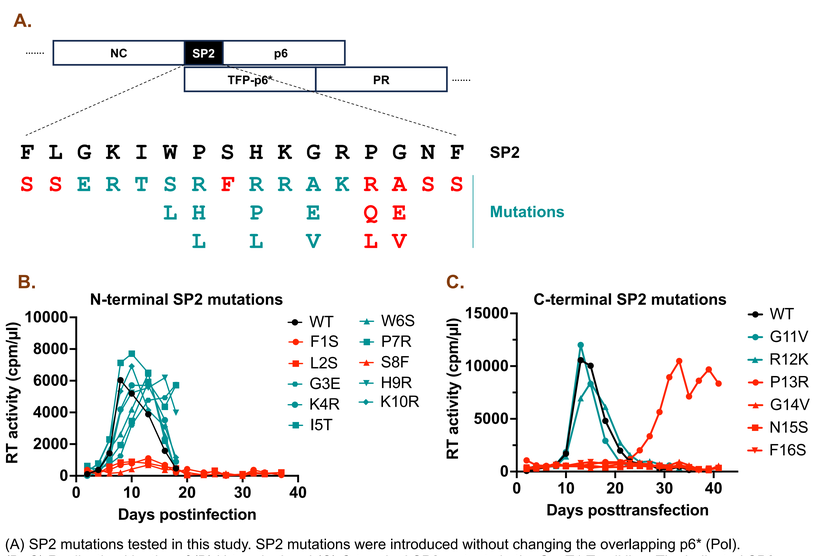


- **The N-terminus of SP2 is predicted to associate with the viral membrane**
- **The C-terminus of SP2 is proposed to interact with the MA lattice to trigger its maturation**

Methods

We developed a liposome-based assay showing that SP2 addition converts liposome-bound MA from the immature to the mature MA lattice. To investigate the role of SP2 in virus replication, we introduced SP2 mutations without altering the overlapping p6* region. We evaluated the impact of SP2 mutations on replication kinetics, infectivity, and fusion kinetics.

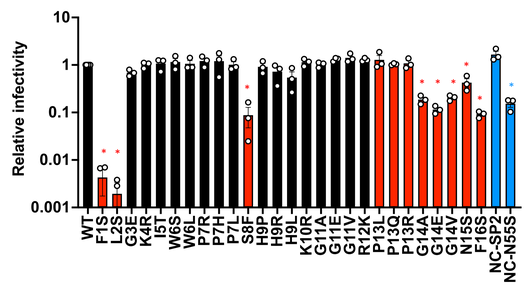
Figure 1. Some SP2 mutations impose defects in HIV-1 replication



(A) SP2 mutations tested in this study. SP2 mutations were introduced without changing the overlapping p6* (Pol).
(B- C) Replication kinetics of (B) N-terminal and (C) C-terminal SP2 mutants in the SupT1 T-cell line. The indicated SP2 mutants were transfected into the SupT1 T-cell line, followed by measuring RT activity in the culture supernatant at the indicated time points. Replication-defective mutants are highlighted in red.

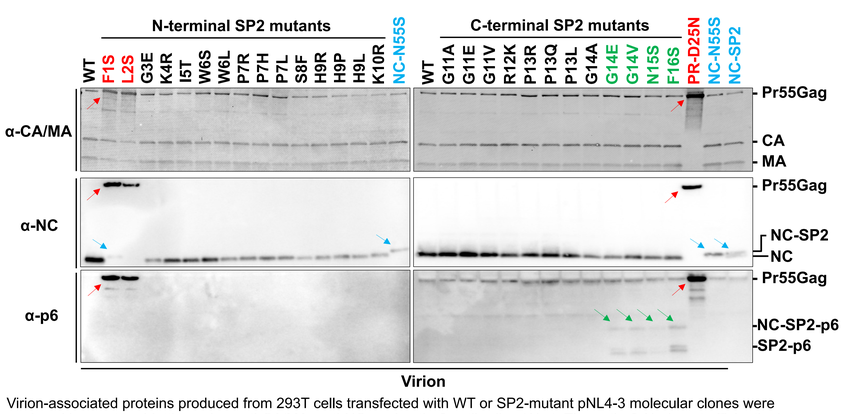
- **SP2 mutations affect virion maturation**
- **SP2-induced MA lattice maturation or dissolution of the immature MA lattice plays a regulatory role in membrane fusion kinetics**

Figure 2. Some SP2 mutations impose defects in HIV-1 infectivity



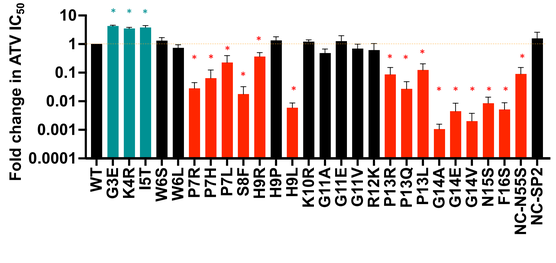
- RT-normalized virus stocks produced from 293T cells were used to infect TZM-bl cells.
- Luciferase activity was measured at 48 h postinfection. *, P < 0.05 by one-sample t-test.
- Defective mutants in SupT1 (Fig.1) are highlighted in red.
- NC-N55S: NC-SP2 cleavage site mutant
- NC-SP2: NC-N55S + SP2-I5T Previously described SP2-release-deficient mutant

Figure 3. C-terminal SP2 mutations impair processing at SP2-p6 junction



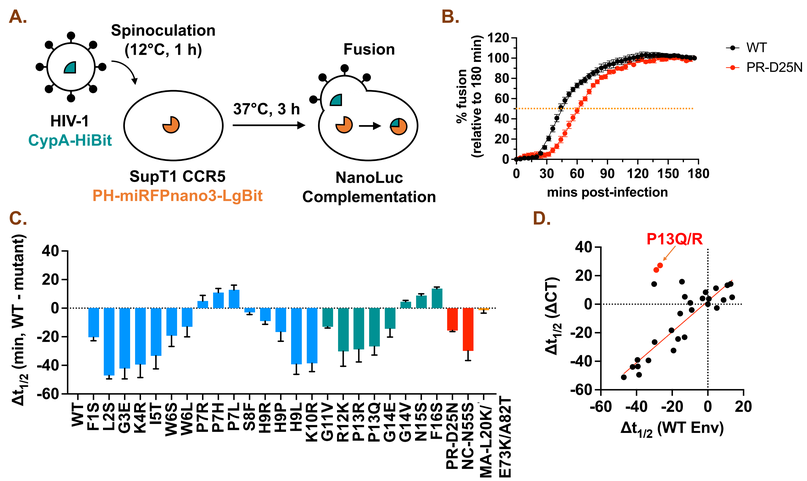
Virion-associated proteins produced from 293T cells transfected with WT or SP2-mutant pNL4-3 molecular clones were analyzed by western blotting using anti-CA/MA, anti-NC, and anti-p6 antibodies (Abs). The anti-p6 Ab do not efficiently detect mature p6 but recognize the Gag precursor Pr55Gag and several processing intermediates. PR-D25N is a protease-deficient mutant. NC-SP2 and NC-N55S are cleavage-site mutants at the NC–SP2 junction. Mutations affecting Gag, NC–SP2, or SP2–p6 processing are highlighted in red, blue, or green, respectively. Bands resulting from defective cleavage are indicated by arrows of the corresponding color.

Figure 4. Many SP2 mutations affect sensitivity to protease inhibitors despite no obvious defect in proteolytic Gag processing



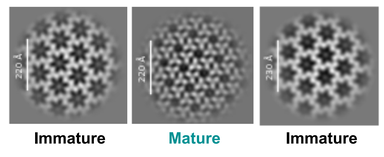
- 293T cells were transfected with the indicated proviral clones in the presence of atazanavir (ATV).
- At 48 h post-transfection, the supernatants were used to infect TZM-bl cells. Data from at least three independent experiments are shown as means ± SEs. *, P < 0.05 by one-sample t-test.
- PI-resistant and PI-hypersensitive mutants are highlighted in green and red, respectively

Figure 6: SP2 amino acid 13 regulates fusion kinetics through Env-CT



(A) Schematic representation of the experimental design. The target SupT1 CCR5 T-cell line expresses PH-miRFP670nano3-LgBiT, which is predominantly localized to the plasma membrane. CypA-HiBiT is incorporated into virions via the CA–CypA interaction. Following spinoculation at low temperature, cells were incubated at 37 °C and accumulation of NanoLuc luminescence was measured over time. The ΔEnv mutant produced no detectable NanoLuc signal (not shown).
(B) NanoLuc signal normalized to the value at 3 h post-incubation. Fusion kinetics were quantified as the time required to reach 50% of maximal fusion ($t_{1/2}$).
(C) Relative fusion kinetics of SP2 mutants pseudotyped with full-length WT Env. N-terminal and C-terminal SP2 mutants are indicated in blue and green, respectively. The SP2-release-deficient mutant and the mutant defective in MA lattice maturation (MA-L20K/E73K/A82T) are shown in red and orange, respectively.
(D) Correlation between WT Env– and ΔCT Env–mediated fusion kinetics for SP2 mutants. Each dot represents an individual SP2 mutant. Most SP2 mutations affected fusion kinetics independently of the Env cytoplasmic tail, except for **SP2-P13Q/R (highlighted in red).**

Figure 7: SP2-P13 mutations impair MA lattice formation on liposomes



- Purified MA protein was incubated with liposomes in the presence or absence of SP2 peptide, followed by negative-stain electron microscopy.
- Representative two-dimensional (2D) class averages of MA are shown. WT MA assembled into an ordered lattice on the surface of liposomes.
- In the absence of the SP2 peptide, the MA lattice resembled the immature MA lattice observed in virions.
- In the presence of WT SP2 peptide, the MA lattice closely resembled that of the mature MA lattice found in virions

Conclusion

- **Some SP2 mutations impose defects in HIV-1 infectivity and replication in multiple T-cell lines**
- **SP2 mutations alter susceptibility to protease inhibitors, which is likely correlated with changes in virion maturation**
- **Release of SP2 is required for particle infectivity**
- **SP2-triggered MA lattice maturation, or dissolution of the immature MA lattice, is not essential for HIV-1 infection, but appears to play a regulatory role in membrane fusion kinetics**
- **SP2 mutations alter fusion kinetics, which is likely correlated with SP2-triggered conformational rearrangement in the MA lattice**
- **These findings suggest that SP2 has functions both related to, and distinct from, MA lattice maturation**