INDUCED MATURATION OF HIV-1 VIRIONS

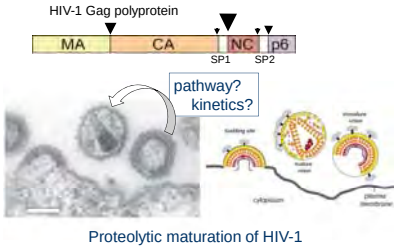
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Introduction

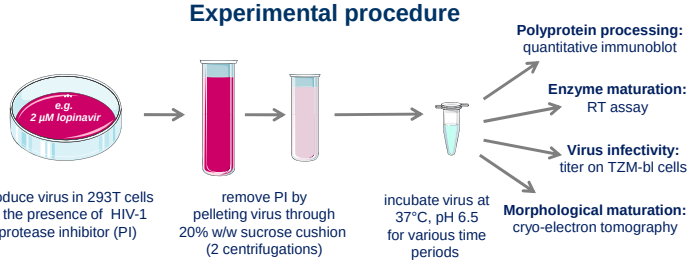
HIV-1 virions assemble at the plasma membrane of producing cells as immature, non-infectious particles. Processing of the Gag and Gag-Pol polyproteins catalyzed by the viral protease (PR) activates the viral enzymes and results in dramatic structural rearrangements within the particle. Formation of the mature viral core structure is a prerequisite for HIV-1 infectivity; Gag proteolytic processing and morphological maturation thus represent important targets for antiretroviral therapy.

When and how fast this maturation event occurs are two surprisingly basic questions from HIV-1 biology that still remain unanswered. Whereas the architectures of starting and end points of the process - immature and mature virions - are structurally well characterized, true structural intermediates elucidating the pathway of morphological maturation are lacking. Biochemical ensemble measurements and structural analyses are hampered by the fact that HIV-1 release and maturation in tissue culture occur in an asynchronous manner between individual cells and between individual particles released from the same cell. We have therefore developed a method to synchronize HIV-1 proteolytic maturation based on protease inhibitor wash-out, with the aim to allow a more detailed analysis of the maturation process.



Proteolytic maturation of HIV-1

Experimental procedure



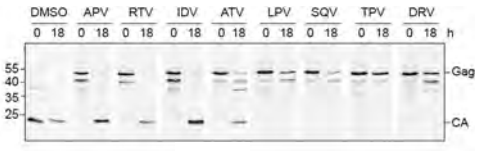
produce virus in 293T cells in the presence of HIV-1 protease inhibitor (PI)

remove PI by pelleting virus through 20% w/w sucrose cushion (2 centrifugations)

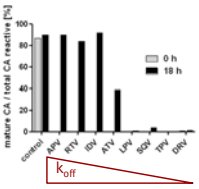
incubate virus at 37°C, pH 6.5 for various time periods

1. HIV-1 protease activation within assembled virions by protease inhibitor wash-out

Immunoblot of virus samples (αCA) produced in the presence of 5 µM of protease inhibitors directly after wash-out (0h) or after overnight incubation at 37°C (18 h)



Relative degree of Gag processing (integrated band intensities, LiCor)



Off-rates determined for PR:inhibitor complexes *in vitro* at 35°C* or 20°C**

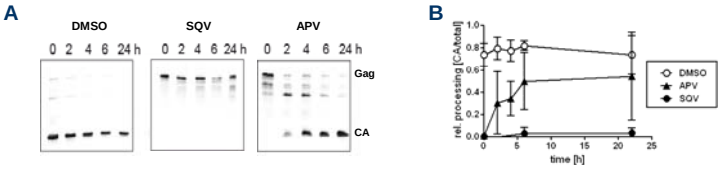
drug	k_{off} [s^{-1}]	k_{off} [s^{-1}]
Amprenavir (**APV**)	0.01690	0.00086
Ritonavir (**RTV**)	0.01100	0.00081
Indinavir (**IDV**)	0.00950	0.00078
Atazanavir (**ATV**)	0.00170	0.00014
Lopinavir (**LPV**)	0.00097	0.00016
Saquinavir (**SQV**)	0.00074	0.00015
Tipranavir (**TPV**)	n.d.	0.00011
Darunavir (**DRV**)	n.d.	0.0000008

* Shuman et al., J Mol Recognit 2004
** Dierynck et al., J Virol 2007

- complete protelytic maturation of Gag can be achieved
- efficiency of wash-out correlates with inhibitor off-rate

all further experiments were performed using APV (negative control: SQV)

2. Time course of induced Gag maturation

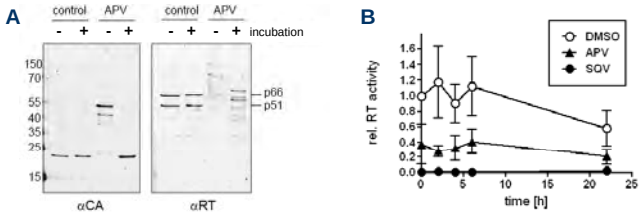


A. Samples taken at the indicated times of induced maturation were analyzed by quantitative immunoblot (LiCor) using antiserum against recombinant HIV-1 CA.

B. Relative Gag processing was determined by quantitation of integrated band intensities of CA versus total CA-reactive band intensities. The graph shows mean values and SD from four independent experiments.

3. Induced PR activation leads to aberrant Gag-Pol maturation

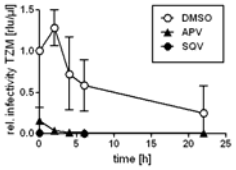




A. Pol processing was analyzed by quantitative immunoblot (LiCor) of start and end point samples using the indicated antisera.

B. Reverse transcriptase activity of samples taken after the indicated times of induced maturation was measured by Sybr Green PCR enhanced RT assay according to Pizzato *et al.* (2009).The graph shows mean values and SD from four independent experiments. Values were normalized to the start value of the respective DMSO control.

4. Induced Gag maturation does not restore particle infectivity

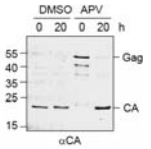


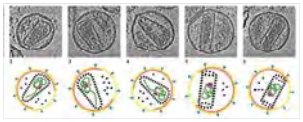
Samples were taken at the indicated time points after inhibitor wash-out and single-round infectivity was determined by titration on TZM-bl indicator cells. At 44 h.p.i., virus-induced luciferase was quantitated in the cell lysate using a commercially available assay (SteadyGlo, Promega).

The graph shows mean values and SD from four independent experiments. Values were normalized to the start value of the respective DMSO control sample.

5. Morphological analysis of ‚*in vitro* matured' virions

HIV-1_{NL4-3} was purified from tissue culture supernatant of 293T cells grown in the presence of DMSO or 2 µM APV, respectively, by centrifugation through an Iodixanol density gradient. Samples were analyzed by cryo-electron tomography either directly or after 20 h inucbation at 37°C, pH6.5.





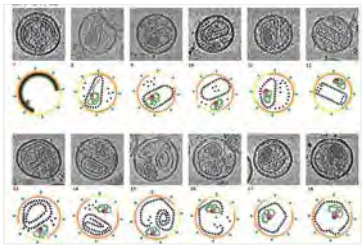
control t=0 h, t = 20 h

❖ particles from DMSO treated cells display mainly typical mature morphology

APV t = 0 h



37°C, pH 6.5

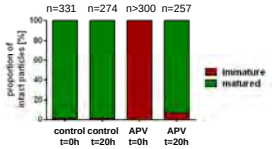


APV t = 20 h

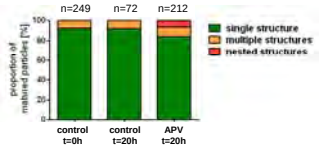
❖ particles from APV treated cells display immature morphology

❖ after induced maturation, particles display a range of morphologies

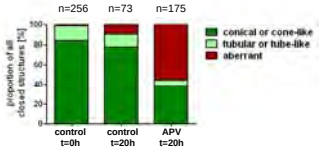
Statistical analysis of tomograms



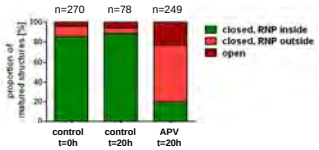
❖ induced maturation results in morphological rearrangements



❖ a single capsid derived structure is seen in most cases, but multiple or nested structures are also observed



❖ cone- or tube-like structures can be observed following induced maturation, but the majority of cores is aberrant



❖ in most cases, the RNP is detected outside of the core structure

- Induced maturation leads to structural rearrangements within the particle
- Particles with typical mature morphology are only exceptionally detected
- Core structures formed are mainly of aberrant shape and/or do not enclose the nucleocapsid

Summary and Conclusion

- ❖ Inhibitor wash-out can lead to complete HIV-1 Gag processing within assembled particles; however, this occurs with rather slow kinetics ($t_{1/2}$ ~ 4,5 h).
- ❖ Formation of mature RT, as well as RT enzymatic activity, are deficient.
- ❖ Gag processing is accompanied by morphological rearrangements, but the majority of particles appears structurally aberrant and infectivity is not restored.
- ➢ Induction of polyprotein processing within the assembled HIV-1 virion does not faithfully reproduce the events during HIV-1 assembly and maturation. Most likely, this reflects differences in the kinetics of individual steps.