

ANALYSIS OF CLEAVAGE SITE DETERMINANTS WITHIN HIV-1 SUBTYPE A, B, AND C GAG POLYPROTEINS

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INTRODUCTION

The structural (MA, CA, NC), regulatory (p2, p1, p6^{GAG}) and enzymatic (PR, RT, IN, RNase H) proteins that comprise the virus core are initially translated as a part of the gag and gag/pol polyprotein precursors^{1,2,3}. Processing of these precursors is accomplished by the viral PR (Fig. 1) encoded within gag/pol. Both the order and the kinetics of cleavage appear to be critical steps in the generation of fully infectious viral particles^{1,4} (Fig. 2).

While a relatively large body of literature analyzes subtype B gag/pol processing^{1,5-8} there are no data about the order or the rate of gag/pol cleavages in subtypes A, C or any other subtypes. This study will analyze *in trans* processing events in subtype A and C gag polyproteins and will bring qualitative and quantitative information regarding the order and the rate of processing in subtype A and C gag polyproteins. By comparing the results obtained with those for subtype B, we will provide additional proofs that genetic variability in gag polyprotein has an impact on the rate and the order of the processing.

SUBTYPE B, A, AND C PROTEASE SEQUENCES

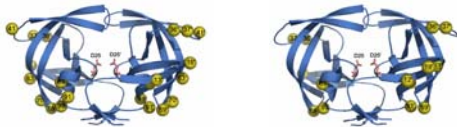


Fig. 1. Ribbon representation of HIV-1 A (left-side cartoon) and C (right-side cartoon) proteases (sequences retrieved from NIH AIDS Reagent Program). In blue is represented the back-bone of subtype B enzyme (PDB: 1MRW). Yellow spheres show the location of naturally-occurring polymorphisms in the subtype A and C proteases.

CLEAVAGE SITES IN GAG POLYPROTEIN

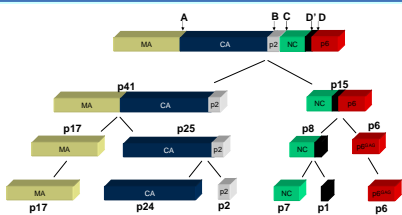


Fig. 2. HIV-1 gag polyprotein consists of three structural (matrix-MA, capsid-CA, nucleocapsid-NC) and three accessory/regulatory (p2, p1, p6^{GAG}) proteins. These polyproteins are cleaved by the viral protease. Processing, a highly regulated event, occurs *in trans* at five well-defined cleavage sites within gag.

ALIGNMENT OF HIV-1 SUBTYPE A, B, AND C GAG POLYPROTEINS CLEAVAGE SITES

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Subtype_B ---S-QV-SQNYPIVQNIQ---
Subtype_C ---G-KV-SQNYPIVQNLQ---
Subtype_A ---N-S-SQNYPIVQNAQ---
Subtype_B ---T-N-S-A-T-I-M-M-Q-R-G-N-F-R---
Subtype_C ---Q-S-H-N-I-M-M-Q-R-G-N-F-K---
Subtype_A ---S-T-I-N-T-I-M-M-Q-R-G-N-F-R---
Subtype_B ---K-G-R-P-G-N-F-L-Q-S-R-E-P---
Subtype_C ---K-G-R-P-G-N-F-L-Q-N-R-E-P---
Subtype_A ---K-G-R-P-G-N-F-P-Q-S-R-T-E-P---
    
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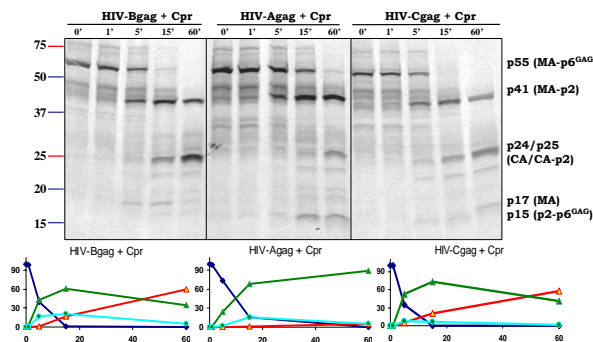
Fig. 3. Two cleavage sites (not shown) – CA/p2, NC/p1 – are well conserved, while the other three (shown above) – MA/CA, p2/NC, p1/p6^{GAG} – are polymorphic.

COMPARISON OF THE NUMBER OF LABELED METHIONINES IN EACH PROTEIN SPECIES

Protein	HIV-1 subtype B	HIV-1 subtype A	HIV-1 subtype C
Matrix (p17)	0	0	1
Capsid (p24)	11	11	10
Spacer peptide p2	2	2	2
Nucleocapsid (p7)	2	2	2
Spacer peptide p1	0	0	0
p6 ^{GAG}	0	1	0

Table 1. HIV-1 subtype A and B matrix (p17) protein, as well as p1 and subtype B and C p6^{GAG} proteins have no labeled methionine residues, hence cannot be visualized on the autoradiographs.

IN TRANS PROCESSING OF HIV-1 SUBTYPE A, B, AND C GAG POLYPROTEINS



IN TRANS PROCESSING OF HIV-1 SUBTYPE A, B, AND C GAG POLYPROTEINS

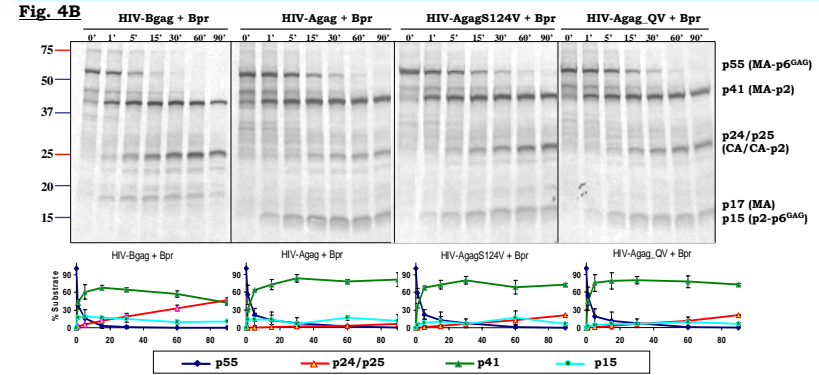


Fig. 4A, B. Time courses of HIV-1 subtype B, A, and C gag polyproteins processed *in trans* by addition of subtype C protease – Cpr (Fig. 4A), and subtype A protease – Apr (Fig. 4B). The positions of molecular mass markers are shown on the left. The composition and the calculated molecular weight of the products based on published sequence are shown on the right. Products are presented in abbreviated form by their N- and C-terminal domains by accepted nomenclature. Aliquots were removed at time points, in minutes ('), indicated on the top of each lane. Plots of the time course of cleavage of four precursors (p55 – navy, p24/p25 – red, p41 – green, p15 – blue) were derived from densitometric analysis of the gels of the processing reactions.

RESULTS AND DISCUSSIONS

The pattern of cleavage of subtype A, B, and C gag polyproteins was dependent on the gag sequence and seemed not to be influenced by the protease added *in trans*, indicating that there are determinants in gag that modulate the processing events.

We also noticed that subtype A gag is processed at a slower rate than the other gag polyproteins analyzed in this study. This is demonstrated by the slower build-up of p24/p25. When a S124V mutation is introduced at P5 or a Gln-Val dipeptide is inserted at the P6-P5 positions of the MA/CA cleavage site, we observed a 3-fold increase in p24/p25 yield during subtype A gag processing by *in trans* addition of subtype B protease. However, the amount of p24/p25 produced by subtype A gag cleavage is less than p24/p25 amount produced by subtype B gag cleavage, indicating that there are other regions within gag polyprotein involved in controlling cleavage at MA/CA site.

REFERENCES

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