Cotranslational Folding and "Constrained Monomers" in the Maturation of HIV-1 Protease

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HIV-1 particle formation and release occur with oligomerization of Gag polyprotein precursor and budding through the cellular plasma membrane. Maturation to an infectious virion depends on multiple proteolytic cleavages of the viral polyproteins by the viral protease, PR. PR is part of the Gag-Pro-Pol polyprotein, a minor frameshifted translational variant of the Gag protein that is incorporated in the budding virion with Gag. PR is active as a dimer and must exist both in an active form in the context of the Gag-Pro-Pol precursor and as the mature dimer. Here we study the cotranslational folding of the PR monomer within frameshifted transframe-protease-reverse transcriptase (TF-PR-RT) constructs by *in vitro* translation to explore early steps of PR folding and activation. We demonstrate cotranslational folding of ribosome-bound PR at its conserved α-helix near the C-terminus. The experimental design included constructs that were either released from the ribosome, or retained on the ribosome by a translational arrest peptide constraining the PR domain to a monomeric state. Unexpectedly, we find that released TF-PR-RT dimers are refractory to cleavage by PR, while ribosome-bound monomeric chains are efficiently cleaved. We suggest that the "constrained isolation" of PR monomers on the ribosome in this system is analogous to PR monomers entering the budding virion in the context of the Gag-Pro-Pol precursor. These observations suggest a model for virion maturation in which dimerization of a subset of Pro-Pol precursors initiates cleavage of PR monomers that then dimerize and carry out most of the proteolytic processing needed for virion maturation.