

POSTER 12

MUTATIONS IN THE THUMB OF HIV-1 RT AFFECT RT STABILITY

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We show that a significant fraction (more than 50%) of the single mutations we generated in the thumb subdomain of reverse transcriptase (RT) affect the stability of RT in virions. The mutant virions contain fragments of RT that can be detected in western blots; however the degree of degradation varies from mutant to mutant. As expected, the titer of virions that contain unstable RTs is markedly reduced. There has been at least one previous report of a point mutation in the thumb subdomain that led to the complete loss of RT in virions, and there is another report that a triple mutation in the fingers subdomain resulted in an unstable RT, which gave the virus a temperature sensitive (TS) phenotype. Many (but not all) of the unstable RT thumb subdomain mutants we analyzed have a TS phenotype. We have done a preliminary survey of mutations in other subdomains to show that the instability phenotype can be seen with single point mutations in other subdomains. The stability of the RT mutants is enhanced by the addition of a protease inhibitor, suggesting that the viral protease plays an important role in the degradation of the mutant RTs. Taken together, these data suggest that the stability of a mutant RT in virions could be a major factor in determining viral titer, and by extension viral fitness, which could place a significant limit on the RT mutations that are acceptable to the virus.