HIV-1 Assembly, Release and Maturation

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The assembly and budding of retroviruses involves a series of regulated steps, driven primarily by the viral Gag polyprotein precursor, $Pr55^{Gag}$. In the case of HIV-1, assembly and budding occur predominantly at the plasma membrane. $Pr55^{Gag}$ is comprised of four major domains - matrix (MA), capsid (CA), nucleocapsid (NC) and p6 - and two spacer peptides, SP1 and SP2. Concomitant with particle release from the host cell plasma membrane, the viral protease cleaves $Pr55^{Gag}$ into its individual components, a process that triggers particle maturation.

The MA domain of $Pr55^{Gag}$ is the major viral determinant responsible for directing Gag to the plasma membrane. We showed a number of years ago that the host cell phospholipid $PI(4,5)P_2$ plays an important role in directing Gag to the inner leaflet of the plasma membrane. The MA domain also plays a central role in the incorporation of the viral envelope (Env) glycoproteins into virions. Although Env incorporation is not required for Gag assembly *per se*, it is required for the formation of infectious particles. Our recent work has defined the structural requirements for Env incorporation. Specifically, the formation of MA trimers in the assembling virions appears to play a central role in Env incorporation. We are also investigating the role of host cell factors in HIV-1 Env glycoprotein incorporation.

We demonstrated that a betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated $Pr55^{Gag}$ processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate to mature CA. BVM was shown in multiple clinical trials to be safe and effective in reducing viral loads in HIV-1-infected patients. However, single-amino-acid polymorphisms in the SP1 region of Gag reduced HIV-1 susceptibility to BVM in patients, leading to the discontinuation of BVM's clinical development. To overcome this problem, we carried out an extensive medicinal chemistry campaign to develop "second-generation" maturation inhibitors based on the BVM scaffold. We identified a set of BVM derivatives that demonstrate increased potency against consensus clade B strains of HIV-1 and are active against primary isolates with polymorphisms in SP1. The best of these analogs also retain significant activity against BVM-resistant mutants. Identification of resistance mutations has provided novel insights into the mechanism of action of these "second-generation" maturation inhibitors, and the role of the CA-SP1 region in HIV-1 assembly and maturation.