

Figure 2 Mechanisms of PARP1 activation. (a) Recognition of a single-strand break by the DNA-binding domain of PARP1. ZnF2 (magenta) binds the minor groove of the DNA and caps the broken strand, whereas ZnF1 (blue) binds the major groove of the DNA. The tips of these zinc fingers also interact. The DNA single-strand break shown here was modeled on the DNA used in the crystal structure described by Ali *et al.*³. (b) *Cis* compared to *trans* automodification models for human PARP1. In the *cis* activation model proposed by Langelier *et al.*⁴, a single PARP1 protomer collapses on a DNA end, triggering intramolecular interactions and conformational changes that increase the flexibility of the catalytic domain to induce automodification. In the *trans* activation model proposed by Ali *et al.*³, two copies of PARP1 heterodimerize at the site of damage, enabling the modification of one protomer by the catalytic domain of its dimer partner.

activate *in-trans* automodification, wherein the catalytic domain of one protomer acts on the automodification domain of the other protomer (Fig. 2b). Although this is an attractive idea, further work will be required to determine the relative contributions of *cis* compared to *trans* models of automodification.

The discovery that the chemical inhibition of PARP1 can lead to dramatic synthetic lethality in cells deficient in the human breast cancer tumor suppressors, BRCA1 and BRCA2, has led to the idea that these inhibitors could provide powerful new therapies for cancer patients with mutations in either BRCA1 or BRCA2 (refs. 6,7). Indeed, the results of phase 2 clinical trials have generally provided support for the clinical efficacy of PARP1 inhibitors, even in patients with cancers that have become resistant to other therapies⁸. Understanding the structural mechanisms of PARP1 activation will be key to guide the development of new inhibitors for this crucial DNA-damage response enzyme.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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to the way in which ZnF2 recognizes DNA in the structure described by Ali *et al.*³; ZnF3 and the WGR domain also make limited contacts with the DNA backbone, as well as with ZnF1. Langelier *et al.*⁴ argue that these interactions drive a conformational change in the catalytic domain that leads to an enhancement of the flexibility and catalytic activity of this domain. Although the PARP1 automodification domain is not present in the structure described by

Langelier *et al.*⁴, the relative arrangement of the ZnF3, WGR and catalytic domains suggest that a flexible automodification domain might be able to access the catalytic active site in *cis* without the need to invoke a catalytic dimer.

Ali *et al.*³ clearly validate the key role of ZnF2 in DNA recognition and show that dimerization of the DNA-binding domain facilitates the recognition of strand discontinuities in DNA. The implication is that dimer formation could

Nef-arious goings-on at the Golgi

James H Hurley & Juan S Bonifacino

HIV-1 avoids the immune detection of infected cells by preventing class I molecules of the major histocompatibility complex (MHC-I) bound to viral peptides from reaching the cell surface. A new structure shows how Nef turns MHC-I from a noncargo into a cargo for the clathrin adaptor AP-1, thus directing MHC-I to the lysosome instead of the plasma membrane.

In the late 1980s, HIV-1 infection was effectively a death sentence. Since then, the three enzymes encoded by the HIV-1 genome, protease, reverse transcriptase and integrase,

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have become some of the most intensively scrutinized proteins ever studied because they were the clear first lines of attack against the virus. A structural analysis of these enzymes accelerated the discovery of the inhibitors that make up the highly effective antiviral cocktail treatment currently in use¹. Now that HIV-1 can be treated effectively, at least in the developed world, attention has turned to the issue of resistance to the currently used antiviral drugs. Ultimately, it is hoped that HIV-1 infection can be eradicated from patients. The challenges of combating drug resistance and eradicating infection will require new lines of attack.

With a genome coding for just 15 proteins, HIV-1 makes for itself a virtue of necessity by

adeptly turning myriad host cell processes to its own ends. The host cell machinery is involved in many steps of the viral life cycle, including cellular entry, genome integration, transcription, RNA export, translation and the assembly and release of viral particles. All of these viral processes are accomplished while dodging the restriction factors of the host cell, as well as evading detection and destruction by the immune system. The dependence of HIV-1 on so many host factors² is a vulnerability for the virus given that the host proteins are not subject to the same selective pressures that yield drug-resistant variants of viral proteins. Thus, in recent years, the field of HIV-related structural biology has shifted much of its focus to understanding

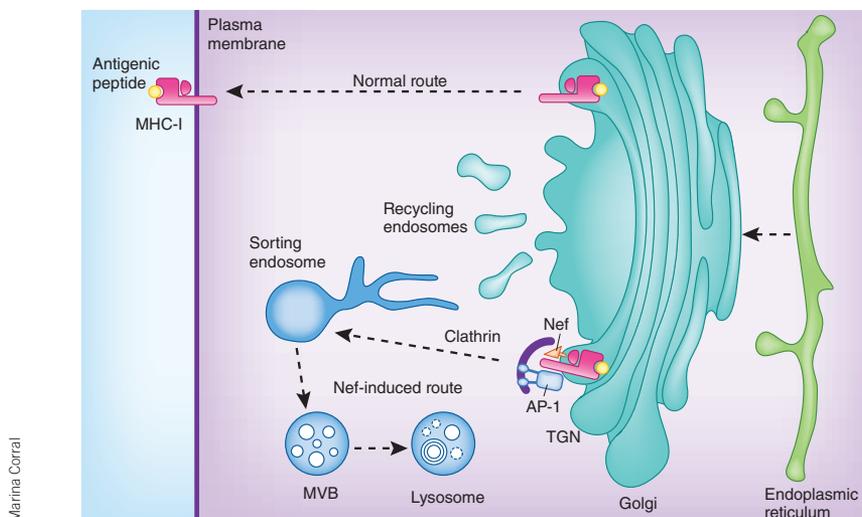


Figure 1 Schematic of the normal trafficking of MHC-I-peptide complexes and its redirection by HIV-1 Nef. MVB, multivesicular body. TGN, trans-Golgi network.

host-virus interactions. In this issue, Jia *et al.*³ provide a particularly insightful addition to this recent trend and reveal how the intracellular trafficking machinery is subverted to prevent detection of infected cells by the immune system.

Interactions of HIV-1 proteins with the trafficking machinery of the host occur at several stages². Some interactions involve viral structural proteins, such as the Gag polyprotein, which co-opts various adaptors, regulatory proteins and lipids for targeting to sites of budding at the plasma membrane. Once Gag arrives at the plasma membrane, it assembles into buds without the help of host factors, but the buds then require the membrane scission apparatus of the host's endosomal sorting complex required for transport (ESCRT) for their release as infectious particles. Other interactions involve the viral accessory proteins Vpu and Nef, whose only purpose is to interfere with various host-cell functions that limit the replication and release of the virus from the infected cells⁴. Vpu acts by preventing the delivery of the newly synthesized co-receptor CD4 from the

endoplasmic reticulum to the cell surface and by antagonizing the restriction factor tetherin at the plasma membrane. Nef also downregulates CD4, but by a different mechanism that involves the cooperative assembly of a tripartite complex with the clathrin-associated adaptor protein complex AP-2 at the plasma membrane, leading to accelerated CD4 endocytosis and lysosomal degradation. Another function of Nef is to downregulate MHC-I to block the deployment of antigen-loaded MHC-I molecules to the cell surface and thus prevent detection of the infected cells by cytotoxic T lymphocytes⁴. This function involves interactions with another clathrin-associated adaptor protein complex, AP-1, which is a component of clathrin coats that are associated with the trans-Golgi network (TGN) and endosomes. Newly synthesized MHC-I molecules normally do not interact with AP-1 on their way to the plasma membrane. The presence of Nef, however, induces the formation of a MHC-I-Nef-AP-1 complex, halting transport of MHC-I molecules to the plasma membrane and ultimately diverting them to lysosomes for degradation (Fig. 1).

Biochemical and functional analyses revealed that assembly of the MHC-I-Nef-AP-1 complex involved a large number of structural elements in the three proteins. This complexity was borne out in the crystal structure of the ternary complex, which shows the cytosolic tail of MHC-I nestled within a long, narrow groove at the interface of Nef with the $\mu 1$ subunit of AP-1 (ref. 3). A key element is Tyr320 in the cytosolic tail of MHC-I, which fits into a pocket on $\mu 1$ that is similar to that binding the tyrosine residue in the YXX Φ sorting signals on the $\mu 2$ subunit of AP-2 (ref. 5) (Y is considered position 0, X is any amino acid, and Φ is a bulky hydrophobic amino acid).

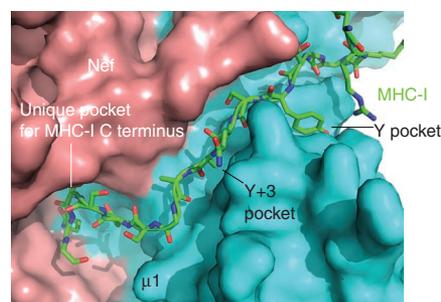


Figure 2 A unique binding pocket created in the AP-1-Nef complex that does not exist when AP-1 or Nef are alone.

Notably, the MHC-I tail has alanine in place of a large hydrophobic residue at the Y+3 position, a difference that prevents MHC-I from entering lysosomes under normal circumstances. This seemingly small change is so effective because the structure of $\mu 1$ is quite rigid in this region. The situation is analogous to what happens when large hydrophobic residues that make up the densely packed cores of proteins are mutated to alanines in protein stability studies: when the positions are rigid, the cavities created by these mutations are energetically costly. For example, a cavity created by mutation of leucine to alanine can affect stability by up to 5 kcal mol⁻¹ (ref. 6). In the context of a binding event, such an increase in free energy corresponds to a three order of magnitude decrease in affinity. The surroundings of the cavity are too tightly packed for Nef to fill it, so Nef indirectly compensates by forming entirely new interactions with the MHC-I tail. For example, the $\mu 1$ -Nef assembly forms a completely new, deep binding pocket for residues at the Y+7 to Y+11 positions (Fig. 2). The portion of the pocket contributed by $\mu 1$ has no known host function and would thus seem to be a potential target for pharmacologic inhibition. To validate this pocket as a potential drug target, it would be interesting to profile the effects of mutational disruptions on MHC-I downregulation compared to the sorting of endogenous cargo.

AP-1 localization to the TGN and endosomes is central to the ability of the Nef-AP-1 complex to intercept and redirect MHC-I to lysosomes. The small GTPase Arf1 is the key determinant for the recruitment of AP-1 to these compartments. Jia *et al.*³ present an attractive model for the engagement of AP-1 with MHC-I at TGN and endosomal membranes based on the crystal structure of AP-2 in the activated, YXX Φ -bound conformation⁷. The AP-2-based model is a useful starting point, but AP-2 functions at the plasma membrane and is principally activated by the lipid phosphatidylinositol-4,5-bisphosphate (PIP₂), not by Arf family GTPases. Full understanding of the mechanism by which AP-1 participates in Nef-induced MHC-I downregulation will ultimately require elucidation of the structure of the complete active AP-1 complex and the nature of its assembly with Arf1, cargo and lipids on TGN and endosomal membranes.

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