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Ancient and ongoing viral adaptation to antagonize primate SAMHD1

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Abstract

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Restriction factors are components of the innate immune defense against viral pathogens. They inhibit viral replication by operating as molecular barriers to steps of the viral life cycle, and viruses have evolved mechanisms to counteract these blocks. The restriction factor SAMHD1 prevents lentiviruses such as Human Immunodeficiency Virus and the related Simian Immunodeficiency Virus (SIV) from replicating in myeloid cells and resting T-cells. Many lineages of lentiviruses, including HIV-2 and other SIVs, encode accessory proteins Vpr or Vpx that serve to abrogate host SAMHD1 restriction by causing degradation of the antiviral factor. Selective pressure for the host to escape infection and for the virus to persist result in the rapid evolution of SAMHD1 to escape viral antagonism, characteristic of a molecular arms race between host and virus. In contrast to other well-studied restriction factors, only a subset of extant lentiviruses antagonize SAMHD1. This trait evolved in one ancient ancestor of a subset of modern viruses, but was lost in SIV that infects chimpanzees, the viral precedent of HIV-1. Thus, HIV-1 that founded the pandemic does not have a method of SAMHD1 antagonism. To address the questionable necessity of SAMHD1 antagonism,I examined viral adaptation to SAMHD1 polymorphism occurring in naturally infected primates. I show that SAMHD1

antagonism must be important for viral fitness because viruses have adapted to distinct SAMHD1 variants present in theirprimate host population.

I further examined the molecular basis of lentiviral adaptation to degrade SAMHD1 by mapping species-specificity in SAMHD1 antagonism by the viral proteins Vpr and Vpx. I show thatdespite high sequence diversity in *vpr* and *vpx* genes and divergent SAMHD1 targeting, Vpr and Vpx evolution is constrained by the need to bind the host ubiquitin ligase machinery used to cause SAMHD1 degradation. Capitalizing on this conservation and a structure of one crystallized Vpx protein, I designed chimeric Vpr and Vpx proteins and mapped specificity of Vpx of the SIVsmm/mac / HIV-2 lineages to two discrete regions of Vpx. I propose a model to explain how viral antagonists adapt to bind rapidly escaping targets while maintaining their function. Due to extensive and conserved interaction with a host ubiquitin ligase protein, the sequence available for Vpx and Vpr to bind target SAMHD1 is limited to dedicated regions which are evolutionary and structurally flexible, allowing sampling of great molecular diversity to reestablish target binding without compromising antagonist function and overall structure.

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Chapter 1: Introduction

Origins of Human Immunodeficiency virus

Since emergence, the Human Immunodeficiency Virus (HIV) pandemic has infected over 75 million people and as of 2013, an estimated 35 million people are currently living with HIV[1]. HIV is a type of retrovirus called a lentivirus, and without antiretroviral treatment, HIV infection causes Acquired Immunodeficiency Syndrome (AIDS)[2, 3]. HIV infects hostimmune cells, particularly CD4+ T-cells, though macrophages and dendritic cells are also infected. Chronic HIV infection causes progressive immune cell death and failure of immune defenses, leaving patients susceptible to opportunistic infections and cancers.

Human immunodeficiency viruses arose from cross-species transmissions of lentiviruses that infect African primates called Simian immunodeficiency viruses (SIVs)[4].The vast majority of HIV infections stem from a single event among many documented instances of a primate lentivirus crossing to humans[5]. There are two types of human lentivirus, HIV-1 and HIV-2, with the type denoting their primate species of origin. Both types are further divided into groups derived from independent transmission events. HIV-1 descends from four separate transmissions of SIV infections of great apes[6]. HIV-2 has crossed to humans on at least eight separate occasions from SIV infections of the sooty mangabey[7, 8]

The HIV pandemic is the worldwide spread of HIV emerging from one transmission of SIV that is hypothesized to be a chimpanzee hunter in Cameroon[9].This strain is called pandemic HIV-1 or group M (“major”). The other groups of HIV-1 are called group O, group N, and group P[6, 10]. Groups M and O are of a similar estimated age, with transmission to humans

occurring around the year 1900[9, 11]. However, groups O, N, and P either infect a very limited number of individuals or remain endemic to the geographical region of origin[10, 12, 13]. Group O originates from western lowland gorillas, and the strain is the second most prevalent group of HIV-1, infecting an estimated 100,000 individuals in west central Africa[14].Group NHIV infects less than 20 individuals and group P has been identified in 2 [10, 15, 16]. HIV-2 is also divided into groups denoting separate transmissions from sooty mangabeys, collectively infecting about 1-2 million people in west central Africa[4, 17-20].

Prior to 1960, the estimated population dynamics of HIV-1 group Mare indistinguishable from the second most prevalent group of HIV-1, group O[9]. However, the growthof group M accelerated around 1960. The sudden eruption of the established virus suggests that a precise mix of social factors are responsible for the pandemic, attributed to positioning of Kinshasa along key railways and iatrogenic effects of vaccination campaigns[9].However, biological factors likely support the past and present spread of group M. While all cross-species transmissions are contingent upon rare sets of circumstances allowing viral replication in a new species, HIV-1 group M seems better suited for spread in human populations than other human lentiviruses[21]. The virus is more transmissible and far more pathogenic than other HIVs[22]. Such differences in viral characteristics are determinedboth by thevirus transmitted from theantecedent primate host and by the early eventsoccurring in adaptation to humans.Particularmolecular adaptations can determinethe outcome ofviral transmissions between species or propagation new populations[23].

Simian immunodeficiency viruses that surfaced to endanger humans represent only a fraction of an extensive lentiviral reservoir. Over 40 genetically distinct SIVs circulate in modern primates, reflecting a minimum of 12 million years of evolution in simian hosts[24, 25].

Each virus is named,"SIV" followed by a three letter code describing the primates species infected. For instance, lentivirus that infects chimpanzee is called SIVcpz, while SIV that infects the red-capped mangabey is called SIVrcm. The convoluted history of primate lentiviruses encompasses not only the storiesofparallel, coevolvinghost-virus pairs but also frequent incidents of viral extinctions and reemergence[26]. Host switches and genetic recombination of lineages further expand viral diversity and complicate coevolutionary stories[27, 28]. Recombination of viral lineages is presumably rare as it requires coinfection of an individual, yet all eight of the major lentiviral clades show evidence of recombination, illustrating the colossalnumber of events sampled over millennia[29]. Lentiviral evolutionary history thus provides a rich source of examples of past and present viral adaptation to hosts. By studying these ancient and current adaptations to cognate and novel hosts, we can begin to understand thefactors that pressure viruses to adapt as well as the molecular mechanisms underlying these adaptations.

Virus host interactions: dependence and evasion

While lentiviral history is rife with examples of cross-species transmission, amultitude of barriers usually block potential transmissions. For example, the chimpanzee subspecies*Pan troglodytes verus* is heavily exposed to SIV of the preferred prey, the western red colobus, yet the apes remain free of SIV infection[30]. One explanation is that lentiviruses rely on an array of host factorsto complete their life cycle, and these factors may differ between divergent species.Virus-host protein interactions dictate nearly every intracellular step of the virus life cycle including entry via binding surface receptor, trafficking within the cell, and the usurpation of cellular machinery for virion[31]. Theinability of a viral protein to bind a necessary host factor, such as a cell surface receptor used for viral entry,can severely obstruct replication in

nonnative hosts[23]. Thus genetic variationbetween primate hosts has the capacity to halt cross-species transmission of viruses because past viral specialization on a one host's species-specific factorsmay hinder viral transmissibility to another species. In this way, host genetics create a formidable barrier to host switching.

Viral evolutionary pressures shape primate host immunity

The evolution of primate hosts is shaped by selective pressure to avoid or attenuate lentiviral infection. The Old World monkeys (OWM), or *Cercopithecidae,*appear to be the most ancient simian reservoir and are often referred to as the natural hosts, distinct from the comparatively recent infections of hominid hosts and the lack of SIV infections in New World monkeys[25, 32, 33]. Infection of natural hosts is characterized by a lack of pathogenesis attributed to protective measures driven by millions of years of selective pressure by ancient viral infections. Not unlike hosts with progressive disease, natural hosts experience robust acute viremia that proceeds to a state of chronic infection. However, infected individuals preserve mucosal immunity, are not subject to chronic inflammation and immune dysfunction, and do not progress to AIDS[34-36]. The mechanism of nonpathogenicity must rely in some part on host features, as viruses of natural hosts tend to be highly pathogenic after gaining a replicative foothold in novel hosts[37]. By restricting infection of key target CD4+ T cell subsets, particularly the CD4+ central memory T cell subset (Tcm), natural hosts seem to preserve immune function. For instance, CD4+ Tcm of sooty mangabeys isprotected from infection in comparison to other memory T cell subsets such as CD4+ effector memory T cells[38]. The mechanism of protection is thought to be altered expression of the CCR5 chemokine receptor which is a co-receptor used for viral entry[38, 39]. Similarly, African green monkey T memory

T cellsdownregulate the CD4receptor [40]. Preservation of the CD4+ Tcm correlates with survival following SIV challenge in pathogenic infection of rhesus macaques, which demonstrate no sparing of CD4+ Tcm infection in comparison to other subsets[41]. Conversely, decline of the Tcm predicts disease progression in SIV infections of macaques [42]. Thus it is thought that the programmed sparing of CD4+ Tcm by downregulation of receptors is a protective adaptation of the natural hosts driven by millions of years of co-evolution with lentiviruses.

Lentivirusеshave also driven the evolution of primate innate immune effector genes called restriction factors.Restriction factors are antiviral proteins that potently block various steps of the viral replication cycle within host cells. To thrive in primate populations, extant lentiviruses must circumvent this array of replicative blocks, thusthey encode accessory proteins dedicated to antagonism of restriction factors[43]. The opposing selective pressures for virus and host survival ignite an evolutionary arms race in which both antiviral protein and viral antagonist evolve rapidly at the protein-protein interface: the host to escape antagonist binding and the virus to reestablish it. This genetic conflict is exemplary of the “Red Queen” hypothesis in which there is continuous selection for change to maintain a *status quo*[44]. The successive rounds of selection generally occur in multiple,concurrently coevolving virus-host lineages, leading to species-specificity in restriction-antagonism relationships. Ancient arms races have both renderedrestriction factors among the most rapidly evolving genes in primate genomes and imparted primates differential susceptibility to modern viruses[45].

A side effect of recurrent selection for change is signatures of positive selection in restriction factor genes[46, 47]. Positive selection can be detected and appreciated by using computational comparison of a panel of primate gene sequences[45]. This type of analysis compares the number of amino acid altering mutations compared to an expected number of

changes based on silent mutations in the gene. Analysis of positive selection in restriction factors can powerfully illuminate timing of ancient viral pressures by determining primate lineages exhibiting the strongest signal of selection for change[48]. Positive selection analyses can also identify specific amino acid sites that are recurrently changing in multiple primate lineages, suggesting they lie at the surface targeted by a viral antagonist.

Lentiviral restriction factors

The study of restriction factors and their mechanisms of halting viral replication illuminatesthe selective pressures that drive viral adaptation. In addition, the repertoire of host immune defenses and the capacity of a virus to evade those immune defenses determines host susceptibly to infection[48]. Four restriction factors are known to potently inhibit lentiviral replication and drive the evolution of viral evasion:APOBEC3G, tetherin, TRIM5α, and SAMHD1. These antiviral host proteinsare extensively studied due to their ability to determine host susceptibility, potential therapeutic exploitation, and implications for studying lentiviral infection in animal models.

APOBEC3G is an antiviral protein that causeshypermutation of the retroviral genome during reverse transcription in a target cell[49, 50]. Primate genomes contain seven syntenic Apobec3 genes (A-H)[51, 52]. All of the APOBEC3 proteins restrict lentiviruses or endogenous retroviruses with varying degrees of efficacy, but APOBEC3G is the most potent anti-HIV effector of the APOBEC3s[53, 54]. An entire lentiviral accessory protein, Vif, is devoted to antagonizing APOBECs. Vif binds APOBEC and to a Cullin 5 E3 ligase to induce APOBEC3 degradation[55, 56]. All lentiviruses, with the exception of equine infectious anemia virus, encode Vif[57]. Other lentiviral antagonist genes are specific to primate lentiviruses, suggesting

that APBOBEC3s represent a most ancient pressure shaping lentiviral evolution. Hypermutated sequences can be amplified from HIV patients, indicating the restriction factor is a relevant pressure to extant lentiviruses.

Tetherin is a broad-acting restriction factor that anchors budding virions to the cell surface, preventing infection of new target cells [58, 59]. The anchoring of virions to the cell surface has also recently been shown to make infected cells more susceptible to killing by antibody dependent cellular cytotoxicity[60]. In most lentiviral lineages, the Nef accessory protein is responsible for tetherin antagonism[61-63]. Nef mediates the endocytosis of tetherin and causes it to accumulate in lysosomal compartments rather than restrict at the cell surface[64]. A subset of primate lentiviruses, including HIV-1,encode a Vpu protein and rely on this protein to antagonize tetherin by sequestering or degrading the restriction factor[58, 65].

TRIM5α is another restriction factor derived from a family of proteins, though it appears to be the most potent antiretroviral TRIM[66, 67]. TRIM5α is thought to cause premature uncoating of the viral caspid, halting infection[68]. The restriction factor also has a role in sensing incoming virus and intracellular signaling[69]. Lentiviruses do not encode an antagonist of TRIM5α. Instead, they acquire escape mutations in the capsid protein, and TRIM5α mutants that reestablish binding are subsequently selected[70].

Many other proteins affect lentiviral replication including putative restriction factors. For instance, Schlafen 11 is an interferon-stimulated gene that inhibits HIV-1 replication when overexpressed[71]. Schlafen 11 inhibits viral protein synthesis by interacting with tRNA to enable expression of translation of RNA with human codon usage but inhibit translation of RNA with viral codon bias[71]. Schlafen 11 is a member of a family of *SFLN* genes, many of which show signatures of positive selection and are likely involved in inhibition of a range of

viruses[72]. However, no lentiviral antagonist has been identified. Similarly, MX2 is known to inhibit HIV-1 at a post-entry step[73]. There is some species specificity in the interaction, and viral capsid dictates sensitivity or resistance to MX1. IFITM is another recently discovered HIV-1 inhibitor. IFITM incorporates into virions and inhibits fusion in new target cells[74]. These newly uncovered lentiviral restriction factors due show signs of positive selection, implying that there is direct protein interaction and pressure for hosts to escape or reestablish viral protein binding, but the nature of these relationships are not yet well understood. In summation, primate hosts express an array of antiviral proteins that block lentiviral replication with varying degrees of potency, and we may have only just begun to appreciate the myriad of innate immune defenses shaping lentiviral evolution.

SAMHD1 is a restriction factor antagonized by Vpx and Vpr

My thesis involves another restriction factor called SAMHD1. SAMHD1 was identified by mass spectrometry as the Vpx-binding factor that protects monocytes, macrophages and dendritic cells from infection by HIV-1, and additionally, the restriction factor was later shown to protect resting T-cells from infection[75-77]. Unlike most other lentiviral restriction factors, SAMHD1 is an essential human gene. Individuals born with mutations that abrogate enzyme functionhave Aicardi-Goutieres syndrome and exhibit symptoms of congenital infection, signifying that SAMHD1 prevents improper immune activation[78]. Interestingly, mice knockouts of SAMHD1 are not lethal and have no apparent phenotype[79, 80]. The antiviral mechanism of SAMHD1 was first proposed to be hydrolysis of dNTPs, creating such low dNTP conditions in noncycling cells that viral replication is halted atreverse transcription[81, 82]. Though this is one possibility, the mechanism of SAMHD1 restriction is still debated[83-86].

SAMHD1 is not antiviral in cycling cells, including the main lentiviral target activated CD4+ T cells. My thesis examines the importance of SAMHD1 antagonism in lentiviral fitness by using an evolutionary approach to study viral adaptation to host SAMHD1.

Only a subset of viruses encode an antagonist of SAMHD1. Two major clades of lentivirus (SIVrcm and SIVsmm) encode a Vpx gene that bridges SAMHD1 to a Cul4 ubiquitin ligase resulting in SAMHD1 tagged for proteasomal degradation[87-89]. In a subset of lineages that do not encode Vpx, the related viral protein Vpr mediates SAMHD1 degradation by the same mechanism[90].[1]All lineages encode *vpr*, and in all lentiviruses tested, the Vpr protein induces G2 arrest in host cells, though the significance of arrest is unclear[87, 91-95]. In an ancestor of a subset of modern lentiviruses, the Vpr protein gained the ability to degrade host SAMHD1 on top of ancestral Vpr functions[90]. The *vpr* gene was later duplicated in one lineage by a recombination event, resulting in viruses that encode both *vpx* and *vpr*[96, 97]. Thus extant lentiviruses either encode Vpr that does not degrade SAMHD1, Vpr that does degrade SAMHD1, or both Vpr and Vpx.

Types of adaptive mechanisms

Singular mutationsat an established interface

The simplest mechanism of viral adaptation envisioned in an arms race scenario is the mutation of a limited number of amino acids in the canonical antagonist. Adaptive changes are thus are those that accommodate or leverage thenovel binding surface presented by a host escape mutation. Host and viral evolution hence inches forward in successive rounds of minor advancements at a given set ofvirus-host protein interfaces. This model is repeatedlysupported

[1]For brevity, let "Vpx/r degradation of SAMHD1" imply "Vpx/r-mediated degradation of SAMHD1" throughout my thesis.

by experimental primate infection studies. For instance, when forced to replicate in the presence of Vif-resistant A3G restriction, SIVagm Vif incurred anadaptive mutation within one year of replication*in vivo*[98]. A single amino acid change in Vif (Y84C) dramatically improved antagonism of A3G in all clones isolated from a monkey homozygous for the resistant *A3G* allele[26]. A similar retrospective study of SIVmac and its predecessor SIVsmm found that restrictive rhesus macaque A3G drove a single amino acid change in Vif (G17E) which was instrumental in SIVsmm adaptation to macaques[99]. In both cases, the precedent resistance of host A3G was ascribed to a single polymorphism mapping to the proposed Vif binding face of A3G. That is, the H130 polymorphism in AGM A3G and R60 in rhesus macaque A3G appear on the same face of the N-terminal active domain when modeled onto the crystal structure of APOBEC3C[99]. Thus in two separate observations of A3G-Vif coevolution, host escape mutations and viral counter adaptations map to single amino acid changes on a single interface.

In a similar example, HIV-1 Nef regained the ability to antagonize chimpanzee tetherin in a single *in vivo* passage, and this gain maps to two amino acids in a single C-terminal loop[100]. As a descendent of SIVcpz, HIV-1 Nef certainly had the ability to antagonize chimp tetherin in the past. The study shows that after evolving under relaxed conditions in humans for one hundred years, Nef function is preserved and species-specific antagonism can be accomplished by altering a single surface of the antagonist. In a third example,a viral escape scenario was also mediated by limited amino acid changes. A study of SIVsmm replication in rhesus macaques expressing restrictive TRIM5 selected for viral escape within two years[101]. The different macaques expressed two separate, restrictive TRIM5 alleles, a TRIM5α variant and a TRIM5Cyp variant. Escape from the restrictive TRIM5α mapped to two amino acids in viral capsid. Escape from TRIMCyp mapped to mutations occurring within a known 5 amino acid

CypA binding loop. Thus both cases of escape and cases of gain in antagonism can be dictated by limited alteration of a recurrently changing interface.

Aiming off target: invention of a novel interface

While single amino acid substitutions in host or viral proteins can profoundly affect replication outcomes, there is a great deal of diversity in mechanisms of viral innovation in the arms race.For instance, an unusually formidable host maneuver at the protein interface may force selection for the antagonist to target a novel surface of the antiviral protein. In one example, an insertion in Colobinae A3G drove a lateral shift in the sequence targeted by SIVcol Vif[25]. The insertion maps to the face of APOBEC targeted by Vif and presumably causes steric hindrance of Vif binding atthe prior site, selecting for Vif that binds adjacently.

The lentiviral antagonists Vpr and Vpx present the most remarkable case of recurrent retargeting. The antiviral targetSAMHD1 is bound at a minimum of three independent locations depending on the viral lineage of Vpx/r. Vpx of the SIVrcm/mnd-2 targets the N terminus of SAMHD1, while Vpx of the SIVsmm lineage targets the far C-terminus[102]. SAMHD1 degrading Vpr target even additional surfaces. SIVagm Vpr of appear to have a level of dependence on both termini of the protein, while SIV infecting Debrazza’s monkey relies on yet a third interface in the middle of the protein[102]. The drivers of differential SAMHD1 targeting are unknown. The N-terminal targeting Vpx proteins exhibit species-specificity characteristic of participants in an evolutionary arms race[90]. Interestingly however, Vpr/x that target the middle or the far C-terminus of SAMHD1 act broadly, likely due to conservation of the motifs they target[90]. The conservation of protein motifs targeted by a lentiviral antagonist is unusual in the Old World monkeys, where the pressure of ancient infections should be visible in terms of

positively selected sites. The lack of positive selection in the OWM SAMHD1 C-terminus suggests that Vpx retargeting may be a recent event or that SAMHD1 evolution is constrained here.

Novel interfaces can also be generated when an atypical protein develops the ability to antagonize. HIV adaptation to human tetherin is repeatedly an example of this process. The SIV accessory protein Nef is usually responsible for tetherin antagonism in primate SIV infections, including the precursor viruses of HIV-1 and HIV-2[61, 62]. Human tetherin, however, bears a five amino acid deletion at the site of Nef binding, effectively eliminating antagonism by Nef. To replicate in human cells, HIVs have employed unconventional viral proteins to assume the task of tetherin antagonism. Amongst the independent transmissions of HIV and subsequent adaptations to humans, at least three distinct proteins mediate tetherin antagonism. HIV-1 group M and group N gained the ability to antagonize tetherin in the Vpu accessory protein, though group M tetherin antagonism is far more effective (Sauter, 2009 ; Sauter, 2012]. Though the deletion in human tetherin severely obstructs binding by Nef, a recent study found that HIV-1 group O Nef is used for tetherin antagonism but targets a region adjacent to the deletion[103]. Group O Nef is thus another example of canonical antagonist retargeting. HIV-2 viruses employ a third antagonist, the Env protein[104, 105]. Adaptations in *env* are fascinatingly repeatable *in vivo*, as a study of SIVΔ*nef* adaptation to rhesus macaques discovered that gains in antagonism mapped to mutations in the gp41 subunit of Env[106]. The differential proteins used to counteract human tetherin highlights the idea that the development of robust antagonism is problematic and effective solutions may be rare events, such as the occasional beneficial mutation in other viral genes.

Recombination supports large molecular leaps

Extreme barriers to viral emergencehave the power to drive extraordinary adaptations by restraining all but the rarest and most advantageous events sampled over millenia. In spite of known exposure risks, extant lentiviruses infect only a subset of hominids, including humans, two species of chimpanzee, and western lowland gorillas. Even after extensive sampling, no infections have been discovered in other great ape species[30, 107-109]. Interestingly, hominid restriction factor genesdisplay strong signals of positive selection, and even the genes of uninfected species, such as the orangutan, have undergone a curious amount of diversification[70, 90]. One explanation is that ancient, extinct lentiviruses may have shaped hominoid immunity and rendered them highly resistant to contemporary lentiviruses that infect OWM. All extant hominoid SIV and HIV-1 infections stem from common ancestor that is far younger than the viruses that infect OWM natural hosts. This ancestor virus was formed by two lentiviral lineages, SIVrcm and SIVmus, that recombined in a chimpanzee host[33]. The recombination event dramatically restructured the *vif* gene in such a way that it boosted antagonism of chimpanzee A3G, facilitating the emergence of lentiviruses in hominoids[110]. Gain of function due to striking protein reshuffling in a co-infected individual must be highly unusual. However, the diversity generated in such recombination events may be the only means to support the leap to new fitness peaks in combatinginsurmountable restriction.

Functional circumvention

In one example of viral gene loss, a separate viral gene acquired a compensatory gain of function rather than develop antagonism *de novo*. The recombination event underlying Vif

reconstruction was coupled with the complete loss of SAMHD1 degradation capability in the SIVcpz ancestor[110]. The virus retained neither the *vpx*gene of SIVrcm nor the *vpr*gene of SIVmus. The emergence of this virus is remarkable and speaks to the power of A3G selective pressure, especially given the severe attenuation of Vpx mutants in experimental infections of macaques[111]. However, a lack of Vpx does not prevent infection but rather hindered only dissemination and disease progression. Low-level replication of the SIVcpz ancestor likely provided a crucial window of time to develop compensatory adaptations to combat the SAMHD1 block. For instance, one hypothesis proposes that, rather than selecting for SAMHD1 antagonism via an atypical gene, adaptive mutations in reverse transcriptase served as a compensatory mechanism and allowed the virus to circumvent the need for SAMHD1 degradation[112].

Neofunctionalization

SAMHD1 degradation is an interesting viral adaptation because it is the only case of a protein neofunctionalization recent enough to analyze its genesis and detail its evolutionary history. The degradation function evolved on top of the existing functions of the Vpr protein[90]. The Vpr usurps the host cellular ubiquitin ligase machinery, specifically binding the substrate receptor DCAF1 of the Cullin 4 ubiquitin ligase[88]. The exact mechanism is unclear, but Vpr changes the host cell environment to allow more efficient viral replication. Vpr induces cell cycle arrest as well as markers of DNA damage in infected cells, and HIV-1 Vpr was recently shown to prematurely activate the SLX4 complex, which is involved in DNA repair[113]. However, the relationship between arrest, DNA damage, and SLX4 activation is unclear[114]. On top of some or all of these Vpr functions, Vpr began to mediate degradation of SAMHD1

using the same CUL4 DCAF1 ubiquitin ligase complex[90]. Modern day descendants of this ancestral virus include four of the eight major clades of lentivirus. SIV infections of AGM, De Brazza's monkey, and the Sykes monkey maintain a multifunctional Vpr protein[90]. In contrast, an ancestor of two major clades of SAMHD1 antagonizing viruses experienced a recombination event in which the *vpr* gene was duplicated, resulting in the genesis of viruses encoding both *vpr* and *vpx*genes[96, 97]. All SIV Vpx proteins maintain the ability to degrade their cognate SAMHD1, but do not have the ability to induce G2 arrest. Instead, Vpr performs its original functions in these viruses. Vpx proteins are more related to each other and to SAMHD1-degrading Vpr than to their own Vpr proteins, indicating that either the *vpr* or *vpx* gene originated from an external source[90]. Regardless of which gene was the incoming gene in the recombination event, the ability to cause G2-arrest was lost in the contemporary *vpx* gene. The duplication was perhaps adaptive because it allowed subfunctionalization of Vpr and Vpx. Hence, by relaxing the need to maintain multiple functions, Vpr and Vpx could hypothetically perform their respective functions more efficiently.

Constraints on viral evolution: viruses are not always winning

Despite the staggering diversity and power of lentiviral adaptive mechanisms, viruses are not perpetual victors in the arms race. A short life cycle, error-prone replication, and exponential growth in acute infection propagate immense viral diversity[115], and exploration of sequence space is far more rapid and extensive than that achieveda by primate host. However, many elements constrain lentiviral evolution.

The length of the lentiviral genome is one major limitation to adaptation. While large DNA viruses can package hundreds or thousands of kilobases, packaging constraints limit the

lentiviral genome to about 10kb[116]. Likely due to size constraints, lentiviral genomes encode overlapping genes. These multiple reading frames place additional pressures on viral evolution because adaptive mutations in one gene may be deleterious in another. Further, key elements of RNA structure must be preserved, such as the stem loop driving the gag-pol frameshift and the rev response elementthat is crucial for RNA export[117, 118].

Another constraint on lentiviral adaptation is the multifunctionality of accessory proteins. For instance, SIV Nef antagonizes host tetherin but also downregulates the CD4 receptor, presumably to deter superinfection[119, 120]. Even Vif, a protein seemingly devoted the singular pursuit of APOBEC3 antagonism, must overpower not one but a family of rapidly diverging antiviral APOBEC3 proteins. The functions of Vpr and Vpx are an especially intriguing example of multifunctionality pressures. Vpr from nearly all SIV lineages induces G2 arrest in Old World monkey cells[121-123]andthe extensively studied HIV-1 Vpr manipulates many additional aspects of the cellular environment with unclear importance[124, 125]. Further, somelineages of lentivirus encode Vpr proteins that degrade SAMHD1 in addition to maintaining the ability to induce G2 arrest, meaning one protein is responsible for at least two major functions[90]. In lineages that encode Vpx, these two activities are ascribed to separate proteins; Vpx mediates degradation of SAMHD1 while Vpr maintains ancestral Vpr activities. Such subfunctionalization is presumably adaptive because each protein can specialize on its cognate task, which may be especially advantageous when the multiple host targets are rapidly evolving. Yet, a number of extant lentiviruses including SIVagm, SIVdeb, and SIVmus did not experience a duplicating recombination and still encode a dual functional Vpr. The maintenance of a single multifunctional protein may indicate that useful recombinatory events are rare. However, with up to 70% of wild adult AGMs harboring SIV infections, the hypothetical

moleculartradeoff may not necessarily translate to measurable consequences[126]. The success of viruses that experienced subfunctionalization still suggests that relief of multifunctional constraint could be adaptive.

Similar to the problem of multifunctionally, the antagonism of restriction factors not only relies on binding of the targeted restriction factor but also binding of additional, conserved host proteins. For example, Vif binds conserved components of the Cullin5 ubiquitin ligase complex, and a conserved PTAP motif in lentiviral Gag is crucial for binding ESCRT proteins to allow for viral budding [55, 56, 127, 128].This type of host machinery is generally composed of essential proteins that are very conserved. Therefore, viral antagonists likely need to maintain binding of a conserved factor while simultaneously sampling mutations to improve binding of a rapidly evolving restriction factor target. The need to maintain binding of multiple host factors is another limitation on viral evolution.

Heterozygosity of restriction factors also limits the effectiveness of viral antagonism. By comparing restriction factor sequences across primates, the key amino acids that are rapidly diversifying are revealed, and they often differ even between closely related species. For many obscure or endangered species, only one genome is available for this type of analysis. However, when deeper sequence analysis of a single species is feasible, restriction factor polymorphisms within species are exposed[98, 129]. Infection of heterozygous hosts may hinder the speed of lentiviral adaptation. Indeed a study of SIVagm Vif evolution in aheterologous AGM species demonstrated that virus was able to evolve robust antagonism of a resistant APOBEC3G variant within two years of infection[98]. However, the gain of function was only observed in the host homozygous for the resistant allele. In a host expressing both a degradation-sensitive and resistant A3G, Vif did not acquire any gain in antagonism. Further, Vif that adapted to novel

A3G lost potency in antagonism of the previously sensitive A3G variant, indicating a clear tradeoff in the molecular ability to adapt to A3G variants[98]. Widespread within species polymorphism alongside evidence for molecular tradeoffs suggests that restriction factors may be subject to balancing selection, a phenomenon in which heterozygote advantage drives the maintenance of multiple alleles in a population[130]. Thus, to effectively spread in a population, virus antagonists must find molecular solutions that combat multiple, independent host escape mutations.

Is SAMHD1 antagonism an imperative for lentiviral fitness?

APOBEC3G, tetherin, and TRIM5alpha are lentiviral restriction factors that present robust blockades to infection, forcing viral adaptation in order to replicate successfully. Past host switches are marked by adaptations to some or all of these factors. In addition, *in vivo* adaptations to resistant forms of all three restriction factors have been observed in limited timespans or even single passages[98-100]. The evolutionary pressures presented by SAMHD1 are different, however. The lentiviruses that infect Colobinae and a subset of guenons diverged prior to Vpr neofunctionalization, and they never evolved the ability to antagonize SAMHD1 in their own lineages[90]. Further perplexing is the complete loss of the *vpx* gene in the ancestor of hominid lentiviruses, as Vpx is essential to the dissemination and pathogenesis of SIVs encodingthe gene[110, 111].

In the first part of my thesis, I show that SAMHD1 is an antiviral barrier driving the evolution of SIV antagonists in natural infections of modern primates. SIV infections of African Green monkeys provide a unique and powerful system to study virus-host coevolution. The AGMs comprise at least four geographically isolated species, and each population harbors a

genetically distinct lentivirus[131, 132]. The populations have been diverging for 1-3 million years, allowing a focused study of viral adaptation to host over short a relatively short evolutionary timespan[28, 133]. I show that AGM SAMHD1 is polymorphic, and Vpr of SIVagm virus subtypes have adapted to their autologous host SAMHD1. In many examples, polymorphisms render SAMHD1 resistant to antagonism by Vpr from other SIVagm subtypes, but even resistant SAMHD1 variants are antagonized by their autologous virus. Because the recently diverged SIVagm subtypes maintain antagonism of their own host species SAMHD1,the restriction factor must be an important element shaping lentiviral evolution in contemporary natural infections.

Viral adaptation to restriction factors in experimental infections studies yield clues to the interacting surface of a viral antagonists. This has been observed in cases of Vif adaptation to degrade APOBEC3G, Nef adaptation to antagonize tetherin, and viral capsid escape of TRIM5[98, 100, 101]. However, there are no observations of Vpr/x adaptation *in vivo* to restrictive SAMHD1. In fact, I found that SIVagm Vpr did not adapt to degrade resistant SAMHD1 after a year of replication in a novel host. For the second part of my thesis, I delve into the molecular determinants of specificity in Vpr and Vpx degradationof SAMHD1, as the amino acids determining specificity in different lineages point to the types of mutations the antagonist would need to adapt to resistant SAMHD1.I used an evolutionary and structural approach to map specificity using chimeric viral proteins. I concluded that the nature of ubiquitin ligase substrate receptor binding is conserved across Vpr and Vpx evolution. The conservation facilitated the design of robust breakpoints for chimeric proteins of different viral lineages. Depending on the lineage, the specificity of Vpx/r in degrading SAMHD1 maps to one or two regions in the viral protein. These regions are analogous across Vpr and Vpx proteins,

supporting a model in which viral antagonists maintain binding of host machinery by conserving overall structure, but they dedicate evolutionarily flexible regions to chasing rapidly evolving host targets.In summation, I demonstrate that SAMHD1 is a relevant pressure shaping the evolution of Vpr in natural SIV infectionsand that the mechanism of viral adaptation to SAMHD1 restriction is amino acid change occurring in structurally flexible regions of Vpr and Vpx which are designated to target SAMHD1.

Chapter 2: Materials and Methods

Amplification and sequencing of AGM SAMHD1

RNA and genomic DNA samples were derived from AGM PBMCs or cell lines, and sample origins and extractions were described in [98]. Additional samples of AGM fibroblasts were obtained from the Systems Bio Sample Repository at UCLA. RNA and genomic DNA were extracted using RNeasy mini kit (QIAGEN) and the DNeasy blood and tissue mini kit (QIAGEN). SAMHD1 was amplified from RNA using the One-Step Superscript III RT-PCR system. For each sample, bulk PCR product were sequenced from RT-PCR amplifications using OWM SAMHD1 specific primers. In the case of heterozygotes, cDNA was TA cloned using the pGEM T-Easy vector system (Promega) and individual clones were sequenced. The first and last exon of each haplotype were also amplified and sequenced from genomic DNA to ensure there were no polymorphisms at the ends of the gene.

Amplification of viral Vpr genes

Viral RNA isolation from plasma of the experimentally infected Sabaeus monkeys was described in [98]. Vpr sequences were amplified using the One-Step Superscript III RT-PCR system and the following primers: GCTATAAGGGGAGAGAGATTCGTCTT (F) and CAAAGCTGACAGTGATAGCAACACTT (R). Vpr cDNA was TA cloned using the pGEM T-Easy vector system (Promega), and ~10 individual clones were sequenced for each timepoint.

Accession numbers

The GenBank accession numbers for the AGM *SAMHD1* haplotypes and the SIVagm.Ver90 vpr sequences isolated from experimentally infected monkeys are KF741041-KF741096.

Plasmids

Human SAMHD1 and RCM SAMHD1 cDNA was reverse transcribed and amplified from RNA obtained from 293T cells (human) and an RCM cell line obtained from Coriell Cell Repositories using RT-PCR with Superscript III One-Step RT-PCR (Life technologies). SAMHD1 cDNA was cloned into the pLPCX expression plasmid and fused to a C-terminal hemagglutinin (HA) tag. ΔC RCM SAMHD1 was made from RCM SAMHD1 truncated at site 611 using overlapping PCR [102]. Each distinct AGM SAMHD1 haplotype was cloned from cDNA and ligated into the LPCX vector containing a C-terminal HA-tag. Cells were co-transfected with 200 ng LPCX-HA-SAMHD1 expression plasmid and between 30 and 200 ng of pCDNA3.1 3xFLAG-Vpr expression plasmidHIV-1 Q23-17 Vpr and SIVmac239 Vpx were amplified from proviral clones and cloned into the pcDNA3.1 expression vector fused to an N-terminal 3X-FLAG epitope tag. The Q23-17 HIV-1 provirus was a gift from Julie Overbaugh [134] and SIVmac239 provirus was obtained from the NIH AIDS Research and Reference Reagent Program [135]. SIVrcmNG411 Vpx was codon optimized and synthesized (Genscript). SIVagm.Gri677, SIVagm.Sab1 and SIVagm.Tan1 were amplified and cloned from provirus plasmids, and ligated into the pcDNA3.1 vector with an N-terminal 3xFLAG epitope tag. SIVagm.Tan1 Vpr contains a stop codon at position 34, which was changed to the tryptophan conserved in all other sequenced SIVagm Vprs by site directed mutagenesis (QuickChange II, Agilent Technologies). SIVagm.Sab92018 Vpr was amplified and cloned from viral RNA provided by Christian Apetrei (University of

Pittsburgh). The SIVagm.Ver90 Vpr was cloned from cDNA amplified from virus that was previously isolated from a vervet monkey [136].Vpx and Vpr chimeras were created using overlapping PCR and cloned into the pcDNA3.1 expression vector fused to a 3X-FLAG N-terminal tag. HIV-1 NL43ΔEnv with GFP reporter gene inserted into *env* with chimeric p6 (pHIV/SIV p6 17-26) was a gift from Nathaniel Landau [137]. Point mutations in SAMHD1, Vpx/r, and pHIV/SIV 17-26 were created using site directed mutagenesis (QuickChange II, Agilent Technologies).

Degradation assays

293T cells were plated in a 12 well dish at 1.6 x 10^5 cells per ml and transfected the following day using TransIT-LT1 (Mirus Bio). Cells were cotransfected with 200 ng of LPCX-HA-SAMHD1 expression plasmid and between 10 and 800 ng of pCDNA3.1 3xFLAG-Vpx/r expression plasmid. Different amounts of Vpr and Vpx plasmid were transfected to attempt to normalize for similar expression, and appropriate empty vector pcDNA3.1 was added to maintain constant total DNA transfected. Cells were harvested 48 hours post transfection for western blot analysis. Cells were lysed in RIPA buffer for 15 minutes on ice and spun at 13,000 rpm for 10 minutes to remove cell debris. 20 µg of protein were heated in sample buffer for 5 minutes and loaded onto NuPAGE Novex 4-12% Bis-Tris gradient gels (Invitrogen). Epitope-tagged proteins were detected using HA-specific antibody (Babco) and anti-FLAG M2 antibody (Sigma-Aldrich). Anti-tubulin (Sigma-Aldrich) antibody was used to ensure equal loading. Horseradish peroxidase conjugated secondary antibody (SantaCruz Biotech) was used to detect primary antibodies.

Phylogenic analysis

Viral amino acid sequences were gathered from the Los Alamos National Lab HIV sequence database (www.hiv.lanl.gov). Sequences were aligned using MUSCLE [138] and minor adjustments were made.

Virus production and virion packaging assay

239T cells were 6 well dish at 1.6 x 10^5 cells per ml and transfected to following day using TransIT-LT1 (Mirus Bio). Cells were transfected with 1.2ug proviral plasmid, 400ng L-VSV-G and 10-800ng Vpx. Viral supernatants and cells were collected 72 hours after transfection. Viral supernatants were filtered using a .2um sterile filter, aliquoted, and stored at -80C. Infectivity was normalized by infection of SupT1 cells, analyzed by subsequent flow cytometry analysis. Cells were lysed using RIPA buffer and used for western blot analyses. To analyze virions by western blot, 1mL of viral supernatant was centrifuged at 15,000xg for 1hr. Supernatant was aspirated and viral pellets were reconstituted in sample buffer with one fifth of volume loaded onto NuPAGE Novex 12% gel. Blots were probed withanti-p24 antibody (NIH AIDS reagent program) anti-FLAG M2 antibody (Sigma-Aldrich).

Cell culture and DC infections

Peripheral blood mononuclear cells (PBMCs) were purified from blood collected from Pall filters obtained from the Puget Sound Blood Bank. PBMCs were purified by Ficoll density gradient centrifugation, and monocytes were isolated using the Human Monocyte Isolation Kit II according to manufacture's instruction (Miltenyi Biotech). Monocytes were cultured for 6 days

in RPMI media with 10%FBS and antibiotics supplemented with 100 ng/mL IL4 and 50 ng/mL GMCSF (Gibco by Life Technologies) to differentiate to DCs, adding fresh cytokines every second day. After culture, MDDCs were positive for DC-SIGN and CD11. For infection, 2 x 10^5 MDDCs of donor 1 were infected with a multiplicity of infection (MOI) of 2 and analyzed by flow cytometry 48 hours post-infection. Multiplicity of infection was calculated on SupT1 cells. MDDCs from donor 2 were infected with three doses of virus at MOI .2, 2.5, and 5.

Chapter 3: Antagonism of SAMHD1 is actively maintained in natural infections of Simian Immunodeficiency Virus

INTRODUCTION

Simian Immunodeficiency Viruses (SIVs) naturally infect over 40 species of African primates and have given rise to HIV-1 and HIV-2 in humans [4, 24]. These primate lentiviruses have evolved to counteract host-specific, intracellular immune defenses called restriction factors, which can potently obstruct viral replication [47, 48]. Viral accessory proteins are largely responsible for the circumvention of host restriction, and a defining feature of restriction factors is their engagement in a molecular “arms race” to continually escape recognition by these rapidly adapting viral antagonist proteins. [46, 139].

SAMHD1 is deoxynucleoside triphosphate triphosphohydrolase that restricts lentiviral replication in myeloid and quiescent CD4+ T cells by suppressing cellular dNTP pools below the level required for reverse transcription and possibly by other mechanisms [75-77, 81, 83, 140]. The viral accessory proteins Vpr and Vpx relieve SAMHD1 inhibition by bridging the restriction factor to an ubiquitin ligase complex, targeting it for proteasomal degradation [88, 89]. The viral genes *vpr* and *vpx*are paralogous, but only two major lineages of lentivirus encode *vpx*, while all encode *vpr*. [87]. In lineages that encode both genes, the Vpx proteinis used for SAMHD1 antagonism while in lentiviral lineages that do not encode *vpx*, the Vpr protein sometimes functions to degrade SAMHD1 [90]. However, a subset of lentiviruses do not encode any SAMHD1 antagonist, including pandemic HIV-1 [90, 141]. Neither HIV-1 nor its precursor, SIVcpz, encode a *vpx* gene, and their respective Vpr proteins do not degrade SAMHD1 due to a deletion that occurred during the generation of SIVcpz [110].

SAMHD1 exhibits classic features of a gene entrenched in virus-host genetic conflict, including episodes of rapid evolution and species-specificity of the SAMHD1-Vpx/Vpr interaction [90, 141]. Additionally, extant Vpx/Vpr proteins bind SAMHD1 at strikingly different interfaces, which also hints at the strength of selective pressure to recover antagonism after host switching or emergence of SAMHD1 escape variants [102]. A lack of SAMHD1 degradation by HIV-1 is therefore perplexing. Additionally, *in vivo* studies show that the *vpx* gene, and thus presumably SAMHD1 antagonism, is critical for SIV dissemination and progression to AIDS in macaque models of infection [111, 142, 143]. Therefore, we sought to examine the importance of SAMHD1 antagonism for viral fitness by an independent method of studying viral adaptation to host SAMHD1 in natural infections of extant primates.

SIV infections of African Green Monkeys (AGMs) provide a unique opportunity to study the evolutionary forces governing virus-host interactions. AGMs comprise at least four related species of the genus *Chlorocebus*. These primates inhabit most of Sub-Saharan Africa, though the individual species, commonly known as the sabaeus, vervet, grivet, and tantalus monkeys, are mostly geographically distinct [131]. While they share a most recent common ancestor less than 3 million years ago, each population is infected with a distinct subtype of SIVagm, named SIVagm.Sab, SIVagm.Ver, SIVagm.Gri, and SIVagm.Tan [28, 132, 133]. Here we ask whether SIVagm subtypes adapt to variation in SAMHD1 in natural and experimental infections of AGMs. We find that SAMHD1 is polymorphic in AGMs, and variable sites alter sensitivity to degradation by the SIVagm SAMHD1 antagonist Vpr. Furthermore, we show that the specificity of Vpr for SAMHD1 involves both the N- and C-terminal regions of SAMHD1 and this specificity has evolved independently from adaptations of other Vpr proteins for their host

SAMHD1. Evidence of viral adaptation to host restriction in AGMs indicates that SAMHD1 antagonism is indeed a component of viral fitness in the context of natural infections.

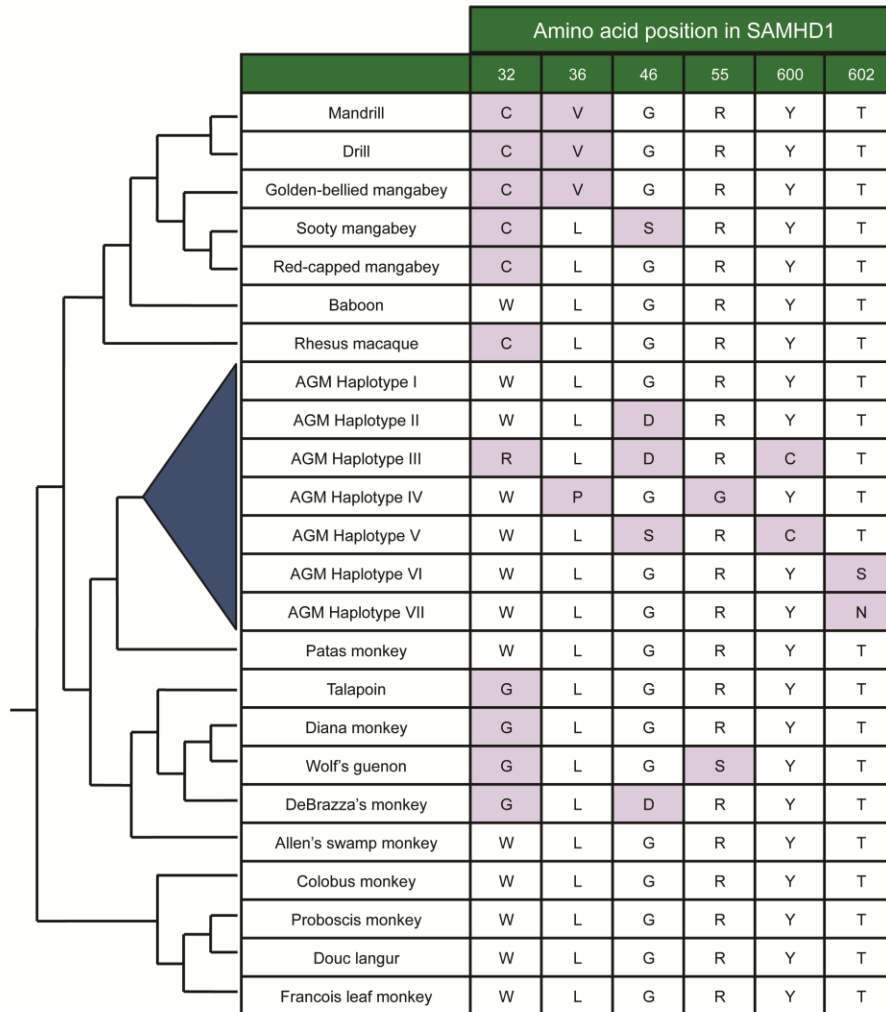
RESULTS

SAMHD1 is polymorphic in AGM species

A key feature of the virus-host molecular arms race is the selection for amino acid altering mutations in host antiviral proteins thatdisrupt binding by the viral antagonist. We sequenced *SAMHD1* from four different AGM species to search for polymorphism that could affect the interaction between SAMHD1 and SIVagm Vpr (Figure 1). Each of the species was represented by 9 to 11 samples, and two populations of sabaeus monkeys were included, one set taken from sabaeus monkeys in the original geographic range in West Africa and the other from a population introduced to the Caribbean islands [144]. Sequence analysis identified seven distinct haplotypes of AGM *SAMHD1* that yield variation at six amino acid positions (Figure 1). The seven haplotypes are unique sequences thatencompass all amino acid variation identified in AGM SAMHD1. Six of the 100 sequenced genes contain a silent SNP, but were counted as the haplotype of their amino acid sequence. Four of the variable amino acid sites are located at the N-terminus of the protein, and the other two are at the C-terminus.The middle of the gene is essentially devoid of both silent and amino acid altering SNPs. The locations of variation are consistent with previous studies of positive selection in primate SAMHD1, where sites exhibiting strong signals of positive selection were identified mostly in the N- and C-termini of the protein [90, 141]. The ancestral haplotype of AGM *SAMHD1* (the version that contains the ancestral version of each amino acid at each variable site) is maintained in AGMs and was

named Haplotype I, while the other haplotypes vary from this ancestral haplotype by one to threeamino acid changes. Sites 46 and 602 appear to have changed multiple times, as three different amino acids were observed at these sites. Variation occurs both at sites that are highly variable in other Old World monkey SAMHD1 as well as at sites that appear fixed in related primates (Figure 1). For example, SAMHD1 of other Old World monkeys also vary at all N-terminal sites, with extensive variation between primates occurring at sites 32 and 46. However, C-terminus altering polymorphisms have only been identified in AGM *SAMHD1* and appear invariant in other Old World monkeys.

The distribution of *SAMHD1* haplotypes among AGM species indicates that all populations harbor multiple versions of *SAMHD1*, thus in no population has a haplotype drifted to fixation (Figure 2A). However, there is a predominant haplotype in each species, which differ between populations. Several haplotypes were identified in at least three of the AGM species, including the reconstructed ancestral sequence, Haplotype I, as well as Haplotypes II and IV. The remaining haplotypes appear to have a more limited distribution. Haplotype III is found almost exclusively in sabaeus monkeys, though it was identified in one vervet sample. Haplotypes V, VI, and VII are unique to either grivet or vervet populations. Therefore, because each species exhibits a distinct composition of *SAMHD1* alleles, we were able to test whether autologous virus has adapted to antagonize the major and minor SAMHD1 variants in its host population.



	Amino acid position in SAMHD1					
	32	36	46	55	600	602
Mandrill	C	V	G	R	Y	T
Drill	C	V	G	R	Y	T
Golden-bellied mangabey	C	V	G	R	Y	T
Sooty mangabey	C	L	S	R	Y	T
Red-capped mangabey	C	L	G	R	Y	T
Baboon	W	L	G	R	Y	T
Rhesus macaque	C	L	G	R	Y	T
AGM Haplotype I	W	L	G	R	Y	T
AGM Haplotype II	W	L	D	R	Y	T
AGM Haplotype III	R	L	D	R	C	T
AGM Haplotype IV	W	P	G	G	Y	T
AGM Haplotype V	W	L	S	R	C	T
AGM Haplotype VI	W	L	G	R	Y	S
AGM Haplotype VII	W	L	G	R	Y	N
Patas monkey	W	L	G	R	Y	T
Talapoin	G	L	G	R	Y	T
Diana monkey	G	L	G	R	Y	T
Wolf's guenon	G	L	G	S	Y	T
DeBrazza's monkey	G	L	D	R	Y	T
Allen's swamp monkey	W	L	G	R	Y	T
Colobus monkey	W	L	G	R	Y	T
Proboscis monkey	W	L	G	R	Y	T
Douc langur	W	L	G	R	Y	T
Francois leaf monkey	W	L	G	R	Y	T

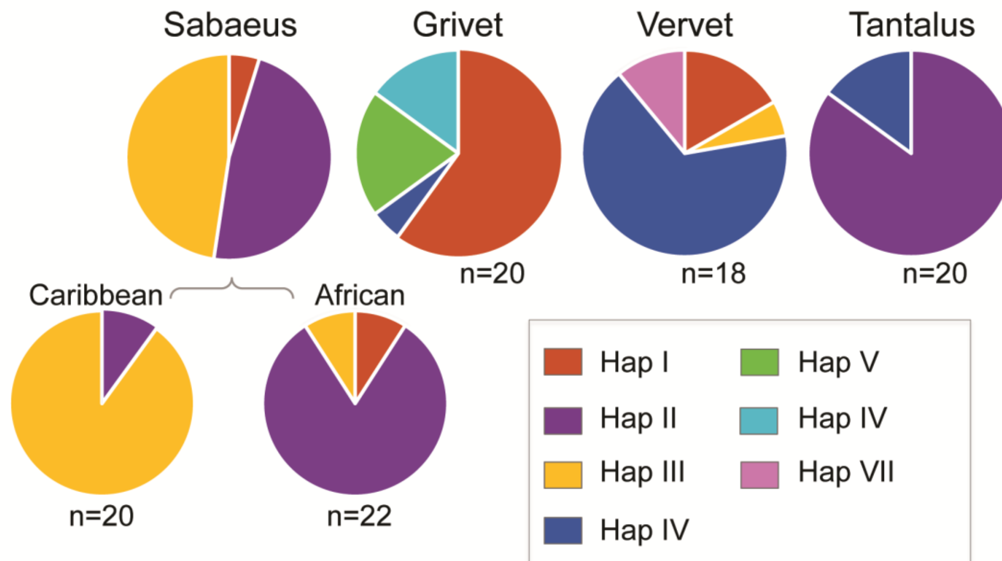
Figure 1AGM *SAMHD1* is polymorphic.The seven identified SAMHD1 haplotypes (blue triangle) differ at six amino acid positions across the gene. No other non-synonymous changes were found in any of the individuals outside of these sites, and only six of the 100 sequenced genes contain a synonymous SNP. Ancestral amino acids (white boxes), were inferred by maximum likelihood sequence reconstruction using ASR programs in Datamonkey [145, 146]. Purple boxes denote derived amino acid changes. For comparison, the amino acid present in Old World monkey SAMHD1 sequences is shown for each location of AGM SAMHD1 variation. The sources of these sequences were previously reported [90, 141]. The relationships of species according to Perelmon *et al*. 2011 are shown by cladogram [132].

AGM SAMHD1 polymorphisms have functional consequences for degradation by Vpr

To determine whether SIVagm Vprs demonstrate specific activity toward AGM SAMHD1, we cloned the seven *SAMHD1* haplotypes into a mammalian expression vector and assayed for sensitivity to Vpr-mediated degradation by viral proteins from each SIVagm subtype. Plasmids containing epitope-tagged SAMHD1 and Vpr were co-transfected into 293T cells and analyzed by immunoblotting. Levels of SAMHD1 in cellular lysates were compared to SAMHD1 expression in the absence of Vpr, and lower levels of SAMHD1 indicate that the tested Vpr is able to recognize and degrade the SAMHD1 variant.[2]

Western blot analyses show that some variants of AGM SAMHD1 are degraded by Vpr of all SIVagm subtypes. For instance, Vpr from all SIVagm subtypes have the ability to degrade ancestral SAMHD1 (Figure 2B). Additionally, Vpr from all SIVagm subtypes degraded the SAMHD1 variants encoded by Haplotype II and Haplotype VI, although one SIVagm.Sab Vpr appears slightly less efficient at degradation of the Haplotype VI variant than the other SIVagm Vprs. Thus the antagonism of ancestral SAMHD1 and two highly similar sequences is maintained by all SIVagm subtypes tested.

[2] While Vpr mediates degradation of SAMHD1, for brevity, let "Vpr degradation of SAMHD1" imply "Vpr-mediated degradation of SAMHD1" throughout my thesis.



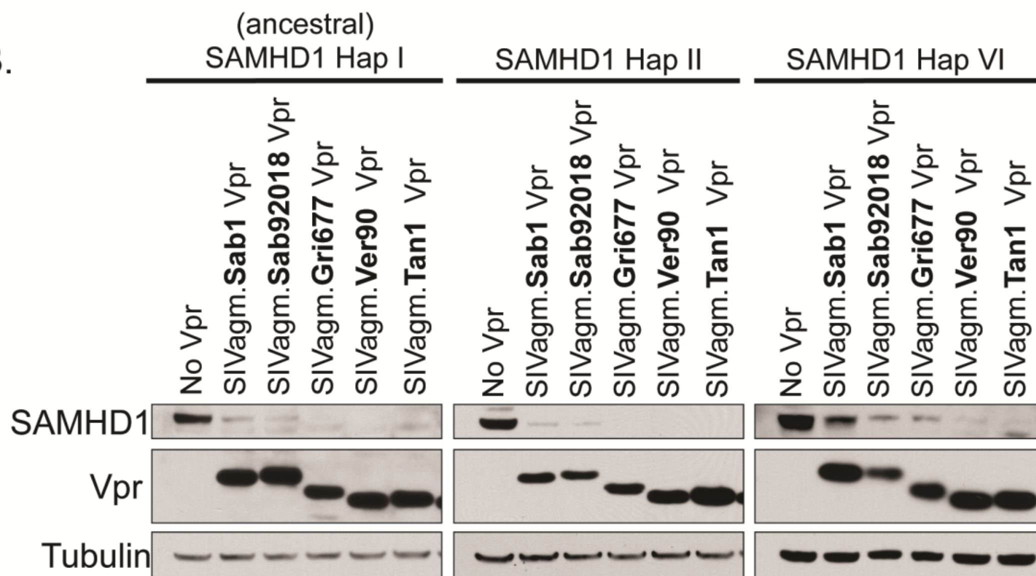


Figure 2All SIVagm Vprs have the ability to antagonize a subset of AGM SAMHD1 variantsA) Distribution of seven SAMHD1 haplotypes among AGM species. The grivet and tantalus pie charts represent 10 animals (20 *SAMHD1* genes sequenced) while the vervet pie chart represents 9 animals (18 *SAMHD1* genes). Sabaeus samples originate from 10 Caribbean (20 genes) and 11 African (22 genes) animals. **B)** Western blot analysis of HA-tagged AGM SAMHD1 expression in 293T cells with and without cotransfection of FLAG-tagged SIVagm Vprs. The Vpr of two SIVagm.Sab molecular clones are shown as they appear to have slightly different degradation abilities. Tubulin was probed as a loading control.

While three AGM SAMHD1 variants are sensitive to degradation by Vpr from all SIVagm subtypes, we identified viral lineage-specific differences in Vpr antagonism of the other four SAMHD1 variants, those encoded by Haplotypes III, IV, V, and VII (Figure 3A). The variants encoded by these haplotypes are each degraded by a different subset of SIVagm Vprs. Additionally, each viral Vpr is able to degrade some, but not all, of these SAMHD1 variants. For example, Haplotype III variant is degraded only by Vpr from viruses infecting sabaeus monkeys and is resistant to Vpr from all other SIVagm subtypes (Figure 3, left). This haplotype is found in both Caribbean and African sabaeus monkeys, suggesting SIVagm.Sab Vpr has adapted to antagonize this version of SAMHD1 due to its presence in the host population. Moreover, Haplotype IV is completely degraded by SIVagm.Ver and SIVagm.Tan Vprs with Vpr from the two SIVagm.Sab viruses exhibiting full and partial degradation activities. This haplotype, Haplotype IV, is found mostly in vervet and tantalus monkeys and is degraded by viruses naturally infecting these populations (Figure 3). Haplotype V variant is found only in the grivet population, and SAMHD1 is degraded by Vpr proteins from viruses infecting grivet and sabaeus monkeys but is resistant to the other Vprs (Figure 3). Finally, Haplotype VII, which is found only in vervets, has partial resistance to Vpr of the virus infecting sabaeus population (Figure 3). Thus, each lineage of SIVagm has evolved to recognize distinct SAMHD1 haplotypes.

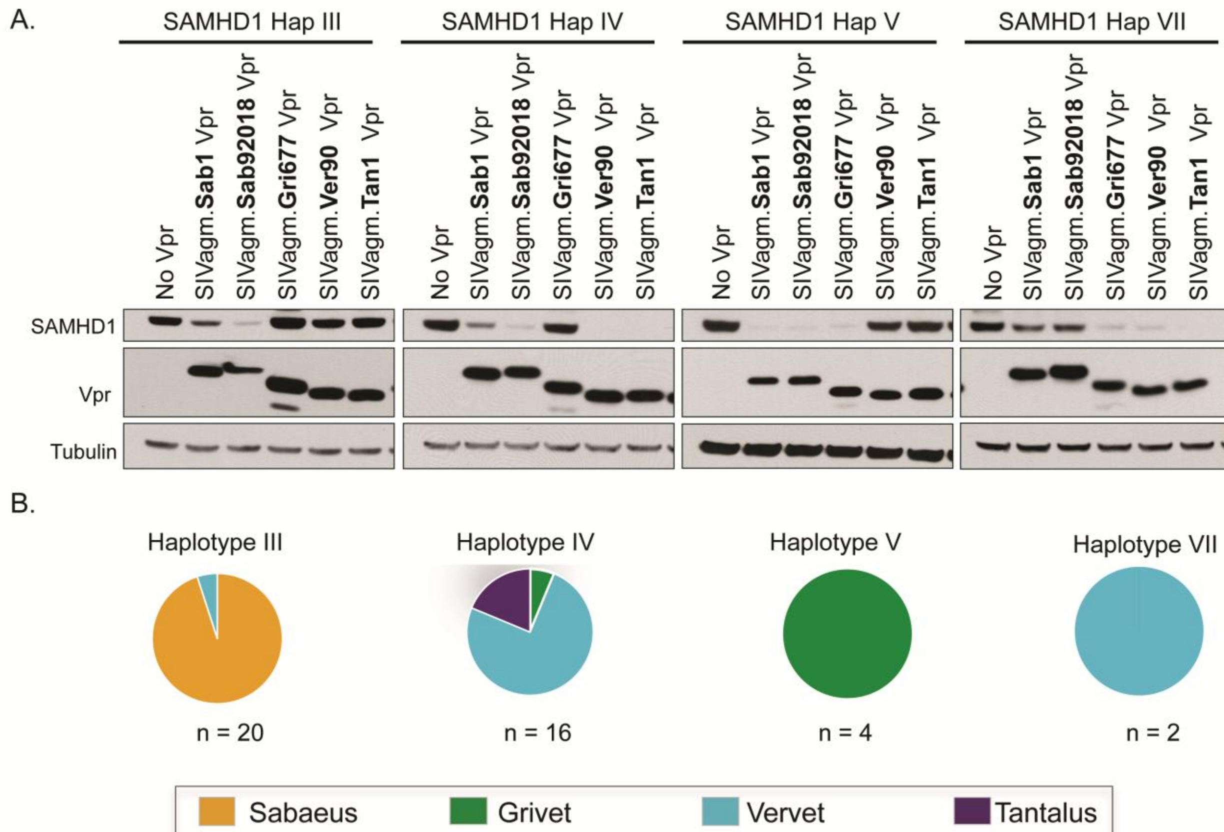


Figure 3 Vpr has adapted to autologous SAMHD1A) Western blot analysis of 293T cells cotransfected with HA-AGM SAMHD1 and FLAG-Vpr from virus infecting each population. Probing for tubulin serves as a loading control. **B)** Pie charts showing in which species each haplotype is most commonly found.

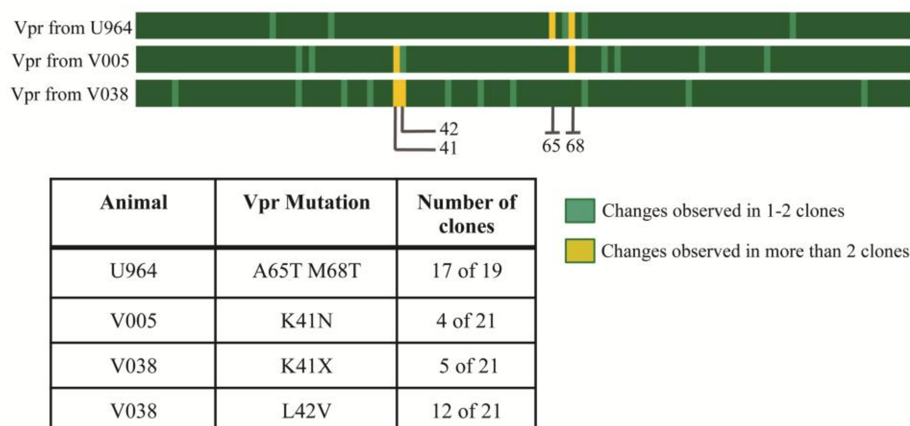
In summary, four AGM SAMHD1 variants demonstrate resistance to some but not all SIVagm Vprs, and this specificity is determined by only one to three amino acid changes in the SAMHD1 sequence. Two important points emerged from this analysis. First, SAMHD1 variants are always sensitive to Vpr from the population where the haplotype is most frequent (Figure 3B). Second, every virus is able to antagonize the predominant haplotype in its population (Figure 2A and 3A). In fact, despite the complex patterns of resistance and sensitivity observed for this interaction, each SIVagm Vpr is able to degrade all SAMHD1

variants found in the host population with two exceptions. These two exceptions are a single Haplotype III sequence found in one heterozygous vervet and a single Haplotype IV sequence found in one heterozygous grivet. The respective autologous SIVagm Vprs were not able to degrade the SAMHD1 proteins encoded by these haplotypes (Figure 3). It is possible that the low frequency of these alleles may not pose a significant selective pressure on the autologous virus, or that they represent rare instances of resistant hosts in a system experiencing ongoing genetic conflict between SAMHD1 and Vpr.

Interestingly, we did not find evidence that SIVagm.Ver Vpr adapted to improve degradation of resistant SAMHD1 up to one year after experimental infection of AGMs expressing the Haplotype III SAMHD1 (Figure 4). We studied Vpr evolution in three Sabaeus monkeys U964, V005, and V038. Two were homozygous for SAMHD1 haplotype III. The third (V005) is heterozygous for this resistant SAMHD1 and for the sensitive haplotype II. In a prior study, these three animals were experimentally infected with virus collected from a naturally infected vervet monkey, SIVagm.Ver90[136]. We amplified *vpr* sequences from the inoculating sera and from plasma samples collected from the experimentally infected animals at both 6 months and 1 year postinfection. A dominant *vpr* clone in the U964 animal contained two amino acid–altering mutations relative to *vpr* sequences of SIVagm.Ver90. These occur at amino acid positions 65 and 68, and most clones contained no additional amino acid alterations. The *vpr* sequences cloned from the other two animals contained numerous changes at amino acid positions 41 and 42.The majority of clones with these changes contained no other amino acid variations. Further, position 41 of *vpr* from virus in the V038 animal exhibited five separate amino acid alterations, but none were observed more than once. No identified clones demonstrated improvement in degradation of the resistant SAMHD1 variant (Figure 4)This

suggests that Vpr adaptation to SAMHD1 may require more than a single infection cycle to develop, or possibly that SIVagm.Ver Vpr is inherently ill-equipped to acquire the ability to tolerate particular mutations in Haplotype III SAMHD1. From a population level perspective, however, SIVagm Vpr is always active against the majority of SAMHD1 variants in its host population, despite mutations that render SAMHD1 resistant to the related viruses. Therefore, SIVagm has adapted to antagonize host SAMHD1 in evolutionarily shallow time, indicating that this function provides a selective advantage to the virus in infections of extant primates.

A.



Animal	Vpr Mutation	Number of clones
U964	A65T M68T	17 of 19
V005	K41N	4 of 21
V038	K41X	5 of 21
V038	L42V	12 of 21

B.

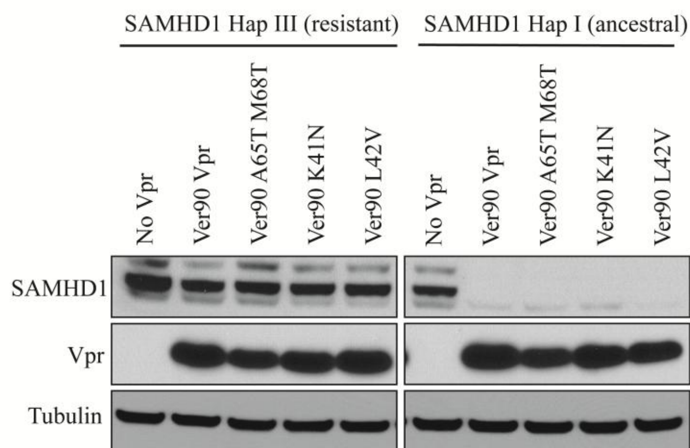


Figure 4Mutations acquired in SIVagm.Ver90 vpr after 6 months to 1 year of replication in three sabaeus monkeys expressing a resistant SAMHD1 variant. Approximately 10 *vpr* clones per time point were sequenced, and sequence alignments were prepared using MUSCLE (2). (A) A schematic of the *vpr* gene and the mutations acquired in each animal. Light green bars denote changes seen in only one or two clones. Yellow bars denote changes observed in over two clones out of ~20. Table shows specific changes observed in *vpr*. (B) SIVagm. Ver90 Vpr point mutants representing all commonly observed clones were tested both for a gain of ability to degrade the AGM SAMHD1 haplotype III. 293T cells were cotransfected with HA-tagged SAMHD1 and FLAG-tagged Vpr, and degradation was measured by Western blotting. Probing for tubulin serves as a loading control.

SIVagm Vprs are sensitive to independent escape mutations in AGM SAMHD1

Vpx and Vpr proteins bind SAMHD1 at strikingly different interfaces, but most appear to target either the N-or the C-terminus of the protein [102]. However, the region of SAMHD1 targeted by individual SIVagm Vpr proteins is not known, and variation at both the N- and the C-terminus appear affect the outcome of degradation (Figure 1 and 3A). We sought to map the individual amino acid changes responsible for resistance of a SAMHD1 variant to better understand how they affect interaction with Vpr. We chose the Haplotype III variant because it contains mutations at both N- and C- termini and is resistant degradation by multiple SIVagm Vpr proteins. We hypothesized that either the derived change at the N-terminus (site 32) or the derived change at the C-terminus (site 600) was responsible for the resistance of the Haplotype III SAMHD1 variant. As the third derived change in this haplotype is also present in Haplotype II, which is sensitive to all SIVagm Vprs, it should not influence degradation, and we thus excluded it from our analysis. To determine which mutation is responsible for SAMHD1 Haplotype III resistance, the residues were separately changed to the ancestral amino acid. We first asked whether Vprs that cannot degrade the Haplotype III SAMHD1 had activity against Haplotype III R32W (Figure 5A). Surprisingly, this change allowed for SAMHD1 to be completely degraded by SIVagm.Gri Vpr but had no effect on the activity of the other two Vprs. Thus change at site 32 has the ability to affect Vpr antagonism for one SIVagm subtype, but other features of Haplotype III must affect resistance to SIVagm.Ver and SIVagm.Tan Vpr. Indeed the cysteine at site 600 prevents SIVagm.Ver and SIVagm.Tan Vprs from degrading the Haplotype III variant. When this site is changed to the ancestral tyrosine, SIVagm.Ver and SIVagm.Tan Vprs are no longer inhibited (Figure 5A). While Haplotype III is resistant to all but

SIVagm.Sab Vpr, separate changes at the N- and C-termini are independently responsible for resistance to Vpr of different viral lineages (Figure 5B). Further, the mutations altering specificity occur at disparate regions of SAMHD1, indicating that Vpr from different subtypes SIVagm may bind SAMHD1 differently. Thus, while SIVagm *vpr* genes are related, there is remarkable diversity in their adaptation to SAMHD1, suggesting that these viruses have experienced selective pressure to counteract SAMHD1 restriction on an evolutionarily recent timescale.

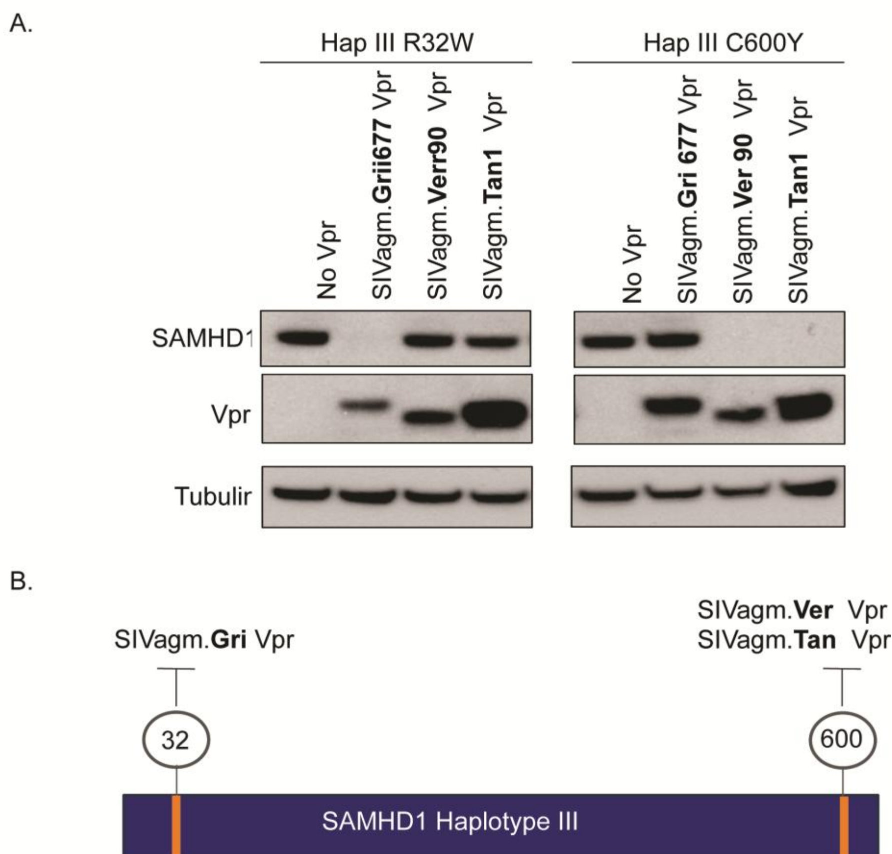


Figure 5 Multiple amino acids independently alter sensitivity to SIVagm Vprs (*A*) Western blot analysis of HA–SAMHD1 haplotype III point mutants R32W and C600Y. To assay for a gain of the ability to degrade SAMHD1, only SIVagm Vprs that lacked activity against wild-type SAMHD1 Haplotype III were included. Probing for tubulin serves as a loading control. (*B*) Schematic showing how amino acids in a single SAMHD1 variant independently affect interaction with SIVagm Vprs.

DISCUSSION

We show that SIVagm has adapted to polymorphism in the host restriction factor SAMHD1. Adaptation has occurred in the context of natural lentiviral infections and in an evolutionarily short timeframe of less than three million years. SIVagm Vpr adaptation to these SAMHD1 variants occurred even in the presence of mutations that render the restriction factor resistant to Vpr-mediated degradation by closely related viruses. Additionally, we found that separate residues in SAMHD1 independently confer resistance to Vpr from different SIVagm lineages, implying that Vpr may utilize multiple target surfaces to bind SAMHD1 despite the relatedness of SIVagm *vpr* genes. Maintained Vpr function due to viral adaptation requires host selective pressure and indicates that SAMHD1 antagonism is a component of viral fitness in natural infections.

Polymorphism in restriction factors which are targeted by viral antagonists is a classic feature of the virus-host arms race, and within-species variation in restriction factors may be indicative of balancing selection, in which multiple alleles are maintained due to heterozygote advantage [129]. AGM *SAMHD1* haplotypes contain amino acid changes in regions of the gene known to be evolving under positive selection and contain almost no silent mutations [90, 141]. The majority of nonsynonymous changes we identified in *SAMHD1* affect the protein's degradation by SIVagm Vpr, suggesting that amino acid variation occurs precisely at interfaces targeted by the viral antagonist. Such variation may have provided a historical selective advantage to hosts expressing resistant SAMHD1. The existence of minor alleles (1 of ~20 gene sequences per species sampled) that are resistant to autologous virus may indicate that this conflict is ongoing.

The recognition of SAMHD1 by Vpx and Vpr proteins from different SIV lineages has recently been shown to be evolutionarily dynamic. Some Vpx/Vpr proteins target the N-terminus of SAMHD1 while others target the C-terminus [102]. These seemingly extreme binding switches can be rationalized by the head to tail nature of the catalytically active SAMHD1 tetramer [147], as the far ends of the protein may be adjacent in physical space. Thus, antagonism could be reestablished after host escape by a Vpx/Vpr shift to target SAMHD1 at a new, but proximate, interface [102]. The previous study suggested SAMHDl antagonists depend on only one terminus of SAMHD1 for degradation, and that C-terminal binding versusN-terminal binding correlates with the two major viral lineages encoding *vpx* genes. However, within the evolutionarily short amount of time encompassing SIVagm strain divergence, SIVagm Vprs seem to have acquired distinct binding interfaces on SAMHD1. For example, SIVagm.Ver and SIVagm.Tan Vpr are highly sensitive to a single C-terminal mutation in SAMHD1, but tolerate extensive N-terminal variation. SIVagm.Gri Vpr is sensitive to N-terminal variation but not changes at the C-terminus, and further, SIVagm.Sab Vpr is broad-acting and tolerates both N and C-terminal variation (Figure 3A). The speed at which such complexity in the SAMHD1-Vpr interaction developed suggests that genetic conflict is ongoing and further supports the hypothesis that SAMHD1 antagonism is actively maintained and therefore advantageous to the virus.

The successful emergence of HIV-1 and its precursor SIVcpz is puzzling considering the evidence that SAMHD1 antagonism is a valuable component of viral fitness. We offer a possible explanation by suggesting that SAMHD1 antagonism is advantageous, but the cell-type specific nature of SAMHD1 restriction may mean selective pressure on the virus is limited to particular stages of infection, namely early stages thatrely on productive infection of myeloid

cells. The lack of restriction in cycling CD4+ T cells, the primary target cells, creates a unique scenario that may provide a window of opportunity for the virus to acquire compensatory mutations in cases where SAMHD1 is not effectively antagonized. We observed that SIVagmVer could replicate in AGMs, albeit at modest levels, even when at least the majority of Vpr clones lacked ability to cause the degradation of host SAMHD1 up to one year post-infection (Figure 4). However, we did not test minor variants or the possibilitythat this function was acquired by another viral protein. Additionally, if SIVagmVer Vpr is inherently poorlyequipped to acquire activity against the particular SAMHD1 variant tested, a failure to witness adaptation could be a consequence of the relatively short time frame of the study. However, because the samples used for tracking Vpr evolution *in vivo* were taken from animals infected by IV inoculation [136], we speculate that it may have bypassed SAMHD1 restriction. In contrast, restriction by the antiviral protein APOBEC3G is constant, and antagonism evolved in the same experimental animals tested here [98]. Thus despite our demonstration that SAMHD1 antagonism must be an important component of viral fitness, the successful emergence of SIVcpz is not implausible. Initial transmission to chimpanzees via blood borne exposure from hunting may similarly bypass the necessity of myeloid cell infection; subsequent low-level replication could allow time for compensatory mutations to arise. Nonetheless, we show that, in naturally infected populations, there is adaptation of SIVagm to actively maintain SAMHD1 antagonism, which underscores the importance of this viral function.

Chapter 4: Molecular basis of Vpr and Vpx adaptation to antagonize primate SAMHD1

INTRODUCTION

The innate immune response of primate hosts poses a significant barrier to lentiviral infection because an array of antiviral immune effectors, also called restriction factors, potently block viral replication [47]. Lentiviruses such as HIV-1 and HIV-2 express accessory proteins dedicated to the evasion of host immunity, which often operate by direct antagonism of restriction factors [43]. The conflicting selective pressures for host survival and viral replication drive rapid evolution at the interface between host and viral proteins [46]. However, molecular changes underlying lentiviral antagonist adaptation are poorly understood because the high mutation rate makes adaptive amino acid changes in the viral genome difficult to discern from a vast number of neutral mutations acquired by evolutionary drift, and others selected by the adaptive immune system such as CTL escape mutations.

The lentiviral accessory protein Vpx enhances infectivity of macrophages, dendritic cells, and resting T-cells by hijacking host degradation machinery to degrade the restriction factor SAMHD1 [75-77, 88, 89]. SAMHD1 blocks the virus at the reverse transcription step by a contested mechanism. Hydrolysis of dNTPs to maintain levels below those required for reverse transcription was the first hypothesized mechanism of SAMHD1 restriction, and later the importance of nuclease activity was proposed [81, 83, 85, 86, 140, 148, 149]. Vpx bridges

SAMHD1 to the ubiquitin ligase substrate-recognition receptor DCAF1, resulting in polyubiquitination of SAMHD1 and degradation by the proteasome [150, 151]. DCAF1 is the DDB1- and Cullin4-associated factor 1, a component of the CUL4-RBX1-DDB1-DCAF1 ubiquitin ligase complex [152]. Vpx causes the rapid degradation of SAMHD1 [153], relieving inhibition.

The *vpx* gene is encoded only by two major clades of lentivirus. Simian immunodeficiency virus of sooty mangabeys (SIVsmm) and its derivatives HIV-2 and SIV of macaques (SIVmac) comprise one clade of *vpx*-encoding lentiviruses. The other Vpx-containing clade is composed of SIVs of red-capped mangabeys (SIVrcm) and mandrills (SIVmnd-2) [154]. While only these two major clades of lentivrus encode *vpx*, the paralogous *vpr* gene is conserved across all extant primate lentiviral lineages [87, 96, 97]. Both Vpx and Vpr are small proteins expressed in the late stages of cellular infection. They are both packaged into nascent virions by association the p6 protein of the structural polyprotein Gag [155-157]. The function of Vpr is unclear, but Vpr has long been known to cause cell cycle arrest at the G2/M stage in a DCAF1-dependent manner [87, 91-95]. The induction of G2 arrest by Vpr is species-specific, and specificity maps to the C-terminal tail of Vpr [121, 158, 159]. The G2 arrest activity of Vpr is linked to the premature activation of the SLX4 complex, which is involved in DNA damage repair, and arrest has been proposed to benefit viral replication by protecting viral DNA from detection by immune sensors [113].

In a subset of lentiviruses that do not encode *vpx*, the Vpr protein is also able to degrade SAMHD1 [90]. The ability to degrade SAMHD1 first appeared in the Vpr protein in the ancestor of modern lentiviruses that degrade SAMHD1. This was a neofunctionalization event that was acquired on top of ancestral Vpr functions. These *vpr* genes are more closely related to

vpx genes than other *vpr* genes [90], and the genesis of the *vpx* gene itself is the result of a recombination event involving the gene for a SAMHD1-degrading Vpr in the ancestor of viruses that encode both *vpx* and *vpr*.

Vpx and Vpr exhibit about 25-40% amino acid sequence identity in pairwise comparison of sequence from different major clades. The species-specificity in SAMHD1 antagonism generates the expectation that some of the sequence diversity in Vpx and Vpr proteins should explain differential degradation of SAMHD1 variants. Vpx proteins of the two *vpx*-encoding lentiviral lineages not only target divergent SAMHD1, but opposite termini of SAMHD1 [90, 102, 141]. Vpx of the SIVsmm/mac and HIV-2 lineage is dependent on the C-terminus of SAMHD1 to ubiquintinate and degrade the protein, while SIVrcm and SIVmnd-2 are dependent on the N-terminus of SAMHD1 and have no reliance on the SAMHD1 C-terminus for degradation. [102]. Even additional surfaces of SAMHD1 are targeted by SAMHD1-degrading Vpr such as Vpr of SIV infecting the Debrazza's monkey [102].

HIV-1 Vpr is known to form three helices and to interact with host DCAF1 [160-166]. An especially of high degree of homology in the third helix of Vpx and Vpr proteins is likely a result of an essential DCAF1 binding interface [151, 166], and this presumption is now confirmed by crystal structure of complex of SIVmac Vpx bound to DCAF1 and a C-terminal portion of SAMHD1 [167]. Schwefel *et al* suggest that structural elements of DCAF1 binding may be maintained, as several DCAF1-interacting residues in Vpx_{smm} are conserved in the distantly related HIV-1 Vpr protein. Interestingly, a group has recently suggested that Vpx and Vpr do not interact with an identical subset of DCAF1 residues [168].

If Vpx and Vpr are under selective pressure to maintain extensive DCAF1 binding surfaces, the evolution of Vpx/r may be limited in the residues available to adapt toward

SAMHD1 targets. We hypothesized that one Vpx backbone could carry the specificity of a divergent Vpx. We identified conserved sequence flanking the hypothesized regions responsible for Vpx specificity and used these motifs as breakpoints to create chimeric Vpx proteins. We tested the chimeric viral proteins for gain of ability to mediate degradation of resistant SAMHD1. We successfully retargeted SIVrcm Vpx to exhibit the specificity of SIVmac Vpx, demonstrating a switch from N-terminal targeting to C-terminal targeting of SAMHD1. This study shows that overall DCAF1 binding can be functionally identical between the Vpx proteins and confirms that residues binding the human SAMHD1 degron in the structure of SIVsmm Vpx are sufficient to recreate SIVsmm specificity. However, the same amino acid swaps are not sufficient to create SAMHD1 degradation capability in a divergent HIV-1 Vpr background, suggesting there are unrecognized but important differences between viral proteins that can and cannot degrade SAMHD1.

RESULTS

Residues of Vpr and Vpx that bind the ubiquitin ligase are conserved across lentiviruses

The crystal structure of one Vpx protein, SIVsmm PBj Vpx, has been solved with the Vpx_{smm} bound to both the C-terminal WD40 domain of DCAF1 and to a C-terminal degron of human SAMHD1 [167]. The Vpx protein consists of a three helical bundle that exhibits extensive interaction with DCAF1, spanning the three helices and composing four separate interfaces. Two linear elements of SIVsmm Vpx compose one interface binding the SAMHD1 C-terminus (Figure 6A and 6B). These two linear elements involve the amino termini of helices 1 and 3 of Vpx thatcreate a hydrophobic pocket which hold a group of hydrophobic residues in

the C-terminal SAMHD1 degron [167]. Also likely important are two residues preceding helix 1 of Vpx,E15 and E16, which form polar interactions with two basic residues in the SAMHD1 C-terminal degron [167]. In addition, three residues in Vpx_{smm}mediate the bridging of all three proteins (Figure 6A) by either participating in dual interactions with both SAMHD1 and DCAF1 (W24, Y69) or by forming a hydrogen bond with the sole DCAF1 residue (D1092) directly binding SAMHD1 (K622) [167]. Thus SIVsmm Vpx participates in extensive interaction with DCAF1 and bridges the SAMHD1 C-terminal target to the host ubiquitin ligase by multiple binding interactions.

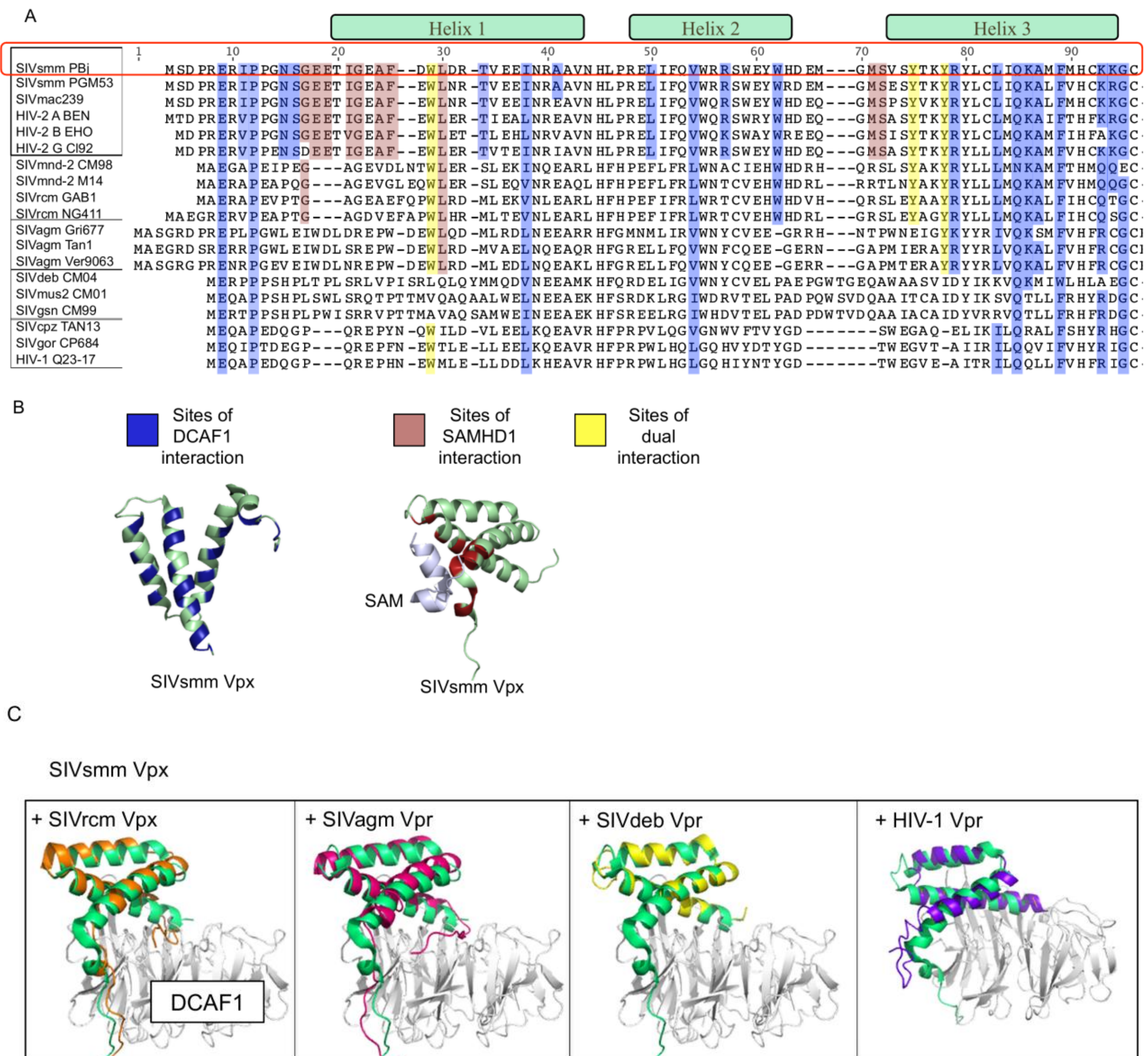


Figure 6 Ubiquitin ligase binding is conserved across Vpx and Vpr A) Full-length amino acid alignment of Vpr and Vpx from various HIVs and SIVs. Sequences were retrieved from www.hiv.lanl.gov and aligned using MUSCLE [138] Blue highlights indicate type-conserved amino acids that interact with DCAF1 based on crystal structure of SIVsmm Vpx, the first sequence, circled in red. Maroon highlights indicate type conserved amino acids that interact with the SAMHD1 C-terminal degron. Yellow highlighting indicates amino acids involved in both DCAF1 and SAMHD1 interactions .B) Locations of DCAF- interacting sites (blue) and SAMHD1-interacting sites (brown) of SIVsmm Vpx (green). C) SIVrcm Vpx (orange), SIVagm Vpr (pink), and SIVdeb Vpr (yellow) modeled onto structure of SIVsmm Vpx (green) using SWISS-MODEL[169].

Despite extensive diversity between Vpx and Vpr sequences from divergent lentiviral lineages, we hypothesized that all Vpx and Vpr proteins may form similar interactions with DCAF1 for several reasons. First, there is a high degree of sequence similarity in the third helix of HIV-1 Vpr and SIVmac and HIV-2 Vpx, and mutations falling in this helix, such as Q76A in Vpx and Q65R in Vpr, are known to disrupt DCAF1 binding [151, 166]. Second, the ability to degrade SAMHD1 evolved on top of ancestral DCAF1-dependent Vpr function, hinting that SAMHD1 ubiquitination may occur within the same Vpr-DCAF1 framework as the induction of G2 arrest [90]. To support the hypothesis that the conformation of DCAF1 binding is conserved throughout Vpx and Vpr evolution, we aligned diverse Vpr andVpx amino acid sequences using MUSCLE and assessed the conservation of the DCAF1-interacting sites (Figure 6A). In agreement with Schwefel et al, the majority of DCAF1-interacting amino acids are conserved in all Vpx proteins, regardless of the SAMHD1 terminus they targeted. In addition, there is extensive conservation of DCAF1-interacting residues in SAMHD1-degrading Vpr (Figure 6A). These include SIVagm Vpr from the viruses that infect African Green monkeys as well as Vpr of SIVsyk, SIVdeb, and SIVmus/mon/gsn lineages thatinfect various groups of guenons. A subset of these residues isconserved in the divergent HIV-1 and SIVcpz Vpr proteins thatdo not degrade SAMHD1. Further, modeled the amino acid sequence of various Vpx and Vpr onto the known SIVsmm crystal structure shows that overall structure is likely to be very similar in regard to the formation of 3 helices and DCAF1 interaction (6C). All together, evolutionary conservation suggests that Vpx and Vpr experience strong selective pressure to preserve precise interactions with DCAF1.

C-terminal SAMHD1 targeting maps to two regions of SIVmac Vpx

Extensive and conserved DCAF1-binding interfaces could limit the size and location of SAMHD1 interaction sites in Vpx and Vpr from different viral lineages. We hypothesized that the species-specificity of Vpx/r for degrading SAMHD1 would map to two regions corresponding to the stretches of SAMHD1-interacting sites observed in SIVsmm Vpx. In accordance, the sequence in these regions is very divergent acrossVpx and Vpr and could be responsible for species-specificity in SAMHD1 binding and degradation. If the amino acids controlling Vpx specificity are constrained by the need to conserve DCAF1 binding, one Vpx backbone could hypothetically carry the specificity of a divergent Vpx. Here, we sought to transfer the specificity of a C-terminally targeting Vpx to an N-terminally targeting Vpx in order to demonstrate the functional conservation of DCAF1 binding and map determinants of Vpx specificity for SAMHD1 degradation.

We made chimeric Vpx proteins by incorporating residues of SIVmac239 Vpx into SIVrcm411 Vpx. SIVmac Vpx is closely related the crystallized SIVsmm Vpx as well as HIV-2 Vpx. The proteins share the same specificity, for they target the C-terminus of human and a broad range of Old World monkey SAMHD1. SIVrcm Vpx does not rely on the C-terminus but rather on the N-terminus of SAMHD1 to target the protein. SIVrcm Vpx and SIVmac Vpr share only about 40% amino acid identity, posing a challenge to the creation of functional chimeric proteins. We combined structural information of SIVsmm Vpx with our evolutionary analysis of Vpx and Vpr in order to design of breakpoints (Figure 7A). We chose breakpoints by identifying sites involved in DCAF1 interaction that were very conserved across Vpr and Vpx and flanked the regions of predicted SAMHD1 interactions. From the alignment, it was clear that highly divergent sequence of varying length existed between the chosen sites in natural Vpr and Vpx

proteins, so we reasoned that the sequence of interest could likely be swapped with minimum effect on surrounding residues. We also took the structure of SIVsmm Vpx into account, confirming that the chosen swapped regions do not appear to be involved in interactions with other residues in Vpx_{smm}.

The crystal structure of SIVsmm Vpx shows two regions of Vpx form a pocket between the amino termini of helices 1 and 3 that binds the 19 AA human SAMHD1 C-terminal degron [167]. The amino acids interacting with SAMHD1 correspond to two linear regions in the Vpx amino acid sequence: G14 to L25 and M62 to Y69. The last two residues of this first SAMHD1-interacting region, W24 and L25, are also highly conserved with W24 interacting with both DCAF1 and SAMHD1. The amino terminus of SIVsmm Vpx prior to W24 appears to be free from intramolecular interactions, and thus its conformation should not depend on the context of Vpx. We hypothesized that the entire amino terminus of Vpx prior to W24 could be swapped with protein integrity maintained. The first region of SAMHD1 interaction is also flanked by the extremely conserved P9 thatforms a hydrogen bond between its main chain and Y1131 in the DCAF1 WD40 domain. An additional breakpoint following this residue served to further refine the sequence encoding specificity in this first SAMHD1 interaction region.

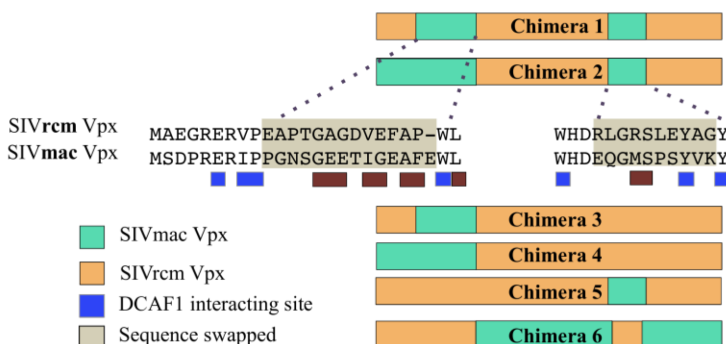
The second part of SIVsmm Vpx that interacts with SAMHD1 begins at residue M62 in a loop between helix 2 and helix 3 and extends to Y69 in helix 3. This stretch of sequence is flanked by two motifs conserved across Vpx, D58 E59 and R70 Y71, between which we replaced SIVrcm Vpx (peach) sequence with SIVmac Vpx (light green) sequence (Figure 7A). In all we created five chimeric *vpx* constructs to test the two regions of SIVmac Vpx for their individual and combined ability to mediate C-terminal targeting of SAMHD1 in the context of SIVrcm Vpx (Figure 7A).

To determine the ability of a Vpx to cause degradation of SAMHD1, we cotransfected 293T cells with plasmids containing epitope-tagged SAMHD1 and Vpx. Levels of SAMHD1 in cells lysates were analyzed by western blot, and a decrease in SAMHD1 expression compared to levels in the absence of Vpx indicates the viral protein is able to cause the degradation of SAMHD1. SIVmac Vpx is able to cause the degradation of human SAMHD1 while SIVrcm Vpx is not. Each region alone is unable to recapitulate human SAMHD1 degradation (Figure 7C). However, two chimeric Vpx proteins, Chimera 1 and Chimera 2, incorporate both regions of SIVmac in the SIVrcm context and both demonstrate a clear gain of ability to degrade human SAMHD1 (Figure 7B). In addition, Vpx Chimeras 1 and 2 lose the capacity to degrade C-terminally truncated SAMHD1, meaning they rely on the C-terminus for binding and degradation of SAMHD1 in contrast to parental SIVrcm Vpx, which mediates robust degradation of the ΔC RCM SAMHD1. Thus chimeric Vpx with SIVrcm backbone and two regions of SIVmac Vpx gains the specificity of SIVmac Vpx. Because each region alone was unable to recapitulate human SAMHD1 degradation, both regions of SIVmac Vpx are necessary to recapitulate SIVmac specificity.

To determine if the specificity of other Vpx proteins is governed by the same two regions mapped in SIVmac Vpx, we created a Vpx chimera in the SIVmac background with regions of SIVrcm sequence. This Chimera 6 Vpx shows a partial but not full gain of function in degrading ΔC RCM SAMHD1, which is degraded by SIVrcm Vpx but not SIVmac Vpx (Figure 7D). This indicates that this chimeric Vpx is either less efficient due to perturbation in structure in a nonnative context or that all determinants of N-terminally targeting SIVrcm Vpx are not included in the two regions controlling specificity of C-terminally targeting SIVmac Vpx. Thus, the DCAF1 binding by Vpx of divergent clades is functionally identical, and C-terminal

targeting specificity of SIVmac Vpx maps to two discrete regions of the protein that are sufficient to cause degradation of full length SAMHD1. These regions are also involved in determining specificity of N-terminal targeting Vpx, but may not encompass all determinants.

A



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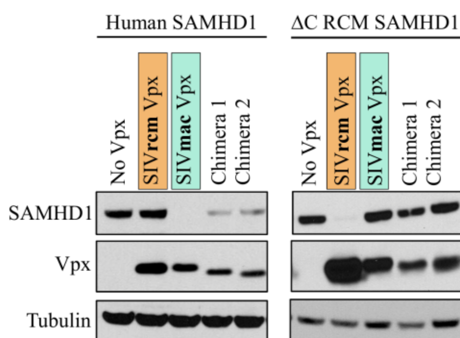

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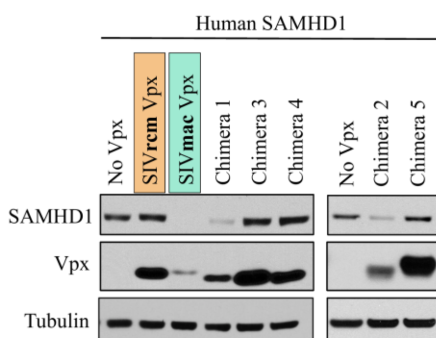

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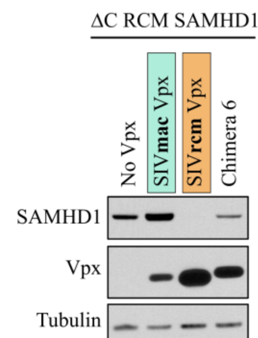


Figure 7 Human SAMHD1 degradation maps to two regions of SIVmac VpxA)Diagrams shows design of Vpx chimeras with SIVrcm Vpx sequence in peach and SIVmac sequence in light green. Blue squares denote sites of SIVsmm Vpx that interact with DCAF1. Maroon squares denotes sites if SIVsmm Vpx that interact with SAMHD1. Grey highlighting shows the amino acid sequence that was swapped to make chimeric *vpx* constructs. B-D) Western blot on cell lysates showing HA-tagged human SAMHD1 or HA-tagged ΔCRCM SAMHD1 expression in 293T cells with and without cotransfection of 3X-FLAG-tagged SIVrcm Vpx, SIVmac Vpx and Vpx chimeras. Tubulin was probed as a loading control.

SIVrcm Vpx chimeras will package into virions with SIVrcm packaging signal

HIV-2 and SIVmac Vpx dramatically boost the ability of HIV-1 to infect monocyte derived dendritic cells (MDDCs) by causing the degradation of SAMHD1 [75, 76, 170]. After mapping the determinants of human SAMHD1 degradation, we sought to test whether the chimeric SIVrcm/mac Vpx proteins would also enhance the ability of HIV-1 to infect human dendritic cells. Vpx is packaged into nascent virions by interaction with a packaging signal contained in p6 of the Gag polyprotein, but HIV-1 lacks both Vpx and the Vpx packaging signal [137, 171]. To infect DCs, Vpx is typically provided *in trans* by pretreatment of cells with SIV VLPs carrying Vpx. Infection of MDDCs has also been accomplished by an HIV-1 provirus containing a chimeric p6 sequence with the 10 amino acid minimum Vpx packaging signal of SIVmac239 [137].

Both of the methods to provide Vpx rely on the packaging signal of HIV-2/SIVmac Vpx. However, chimeric Vpx proteins are poorly packaged by both HIV-2 and the chimeric p6 HIV-1 (Figure 8B). An analysis of SIV p6 sequences revealed that the Vpx packaging signal in SIVrcm Vpx differs from the HIV-2/SIVmac sequence by 3 amino acids (Figure 8A). We reasoned that chimeric Vpx built in the SIVrcm Vpx backbone mightpackage more efficiently via the SIVrcm packaging signal. After changing the signal sequence from 17DPAVDLLKNY26 to the SIVrcm p6 version, 17DPAEAMLKNY26, the HIV-1 chimeric p6 construct was able to package chimeric SIVrcm/mac Vpx and SIVrcm Vpx to comparable levels as SIVmac Vpx (Figure 8B).

While the ability to tailor packaging of Vpx proteins is an exciting possibility, the mere 3 amino acid alteration to p6 caused a defect in virion infectivity. The defect is presumably a defect in maturation, as the Gag polyprotein p55 but not the cleaved p24 capsid protein is visible

by western blot of concentrated viral supernatant (Figure 8C). By creating a panel of virions varying in ratio of cleavage-capable and cleavage-defective Gag, we concluded that the infectivity defect is limited to virion maturation and not an effect of lower virion production, as levels of p55 steadily decrease and infectivity increases with increasing proportions of cleavage-capable gag (Figure 8C). When the Gag polyprotein is not cleaved into respective structural proteins, the virions remain immature and are not infectious due to impaired fusion [172]. However, by cotransfecting a 2:3 ratio of provirus encoding cleavage-capable Gag and and provirus encoding cleavage-defective, Vpx_{rcm}-packaging Gag, virions that both package chimeric Vpx and maintain infectivity can be produced (Figure 8D).

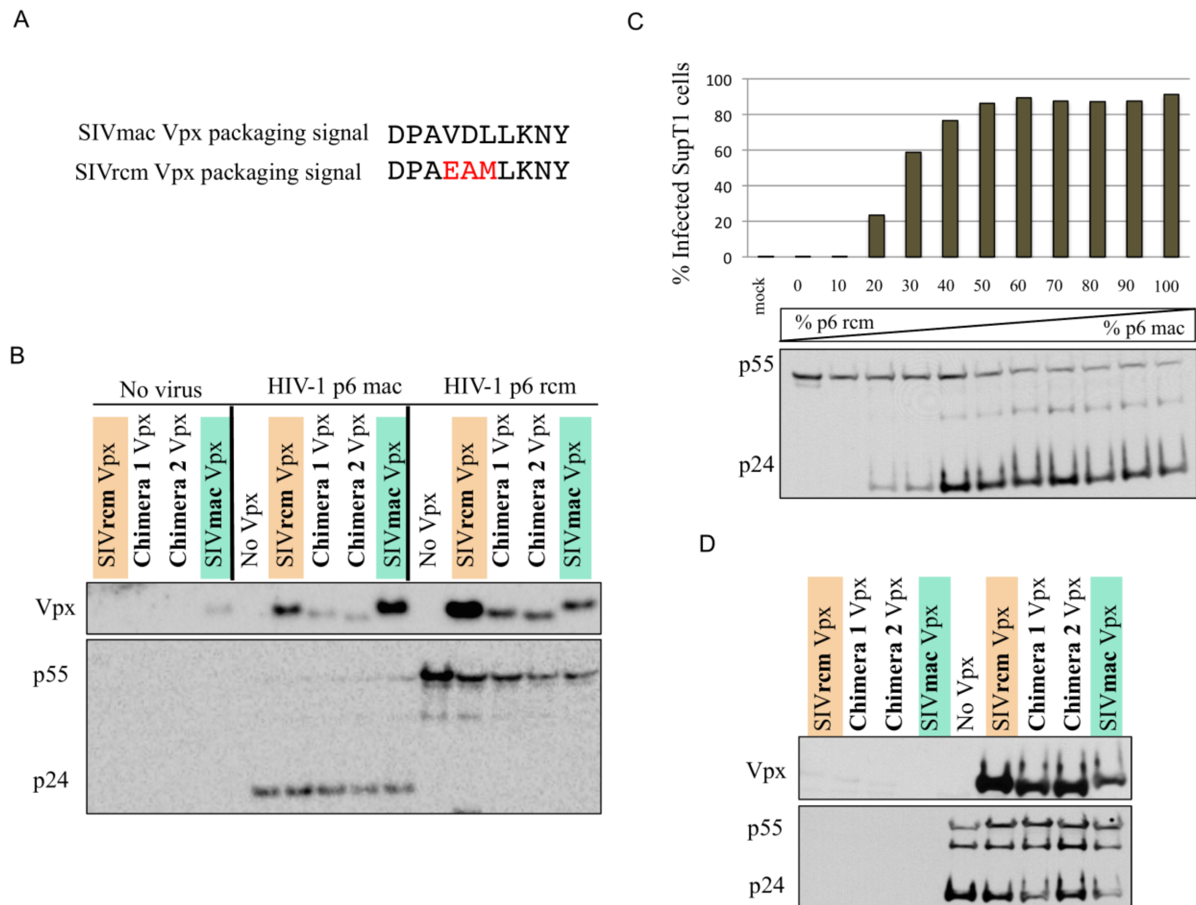
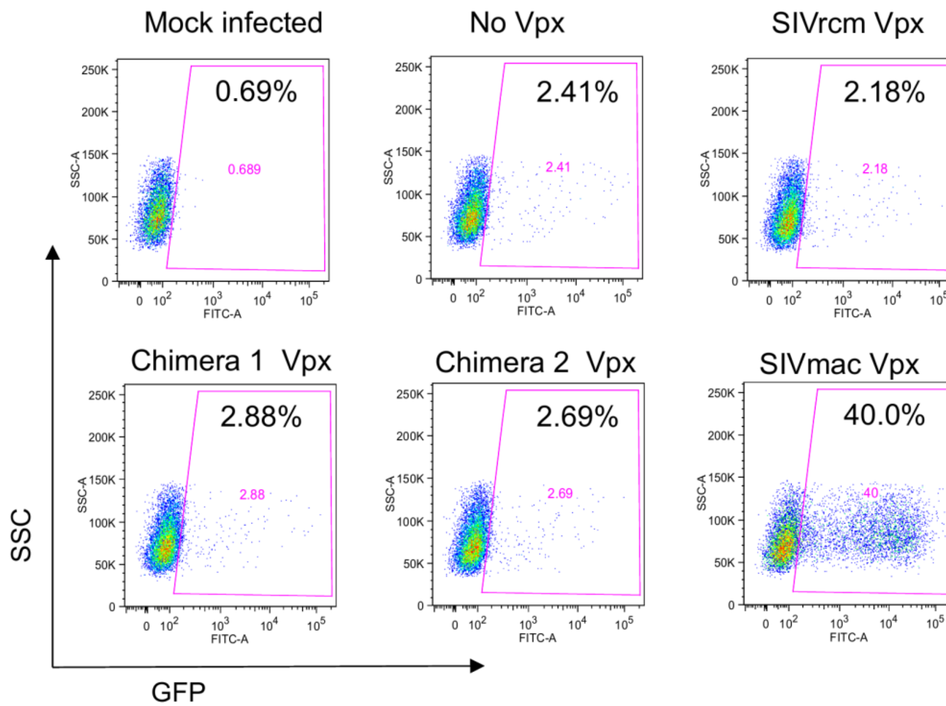


Figure 8 A system to package Vpx chimerasA) SIVmac and SIVrcm Vpx packaging signals B) Western blot on virions collected from concentrated supernatant collected from 293T cells cotransfected with either empty vector or proviral plasmid and either empty vector, SIVrcm Vpx (peach), SIVmac Vpx (light green), or Vpx chimeras 1 or 2. Virions were probed for 3X-FLAG tagged Vpx and p24 capsid protein to show Vpx packaging and Gag processing. 3X-FLAG tagged-Vpx were cotranfected with empty vector as negative packaging controls. HIV-1 p6 mac and HIV-1 p6rcm are an NL4-3 HIV-1 reporter proviral construct with an insertion of either the SIVmac or SIVrcm Vpx packaging signal in p6 of HIV-1 *gag*. C) Percentage of GFP-positive SupT1 cells infected with virus collected from supernatant of 293T cells transfected with different proportions of HIV-1 p6mac and HIV-1 p6rcm proviral constructs. Western blot shows probing for p24 capsid protein in concentrated virions. D) Western blot on concentrated virions in supernatant collected 293T cells cotransfected with a 2:3 ratio of HIV-1 p6mac and HIV-1 p6rcm proviral plasmid and 3X-FLAG-tagged Vpx.

SIVrcm Vpx chimeras with SIVmac Vpx specificity do not improve infectivity of human dendritic cells

After developing a system to deliver chimeric Vpx to target cells, we tested whether SIVrcm/mac chimeric Vpx could improve HIV-1 infectivity of MDDCs. We generated VSV-G pseudotyped HIV-1 GFP reporter viruses by cotransfecting 293T cells with VSV-G, Vpx, and a 2:3 ratio of provirus encoding cleavable Gag to provirus encoding cleavage-defective, Vpx_{rcm}-packaging Gag. Monocytes isolated from PBMCs from two donors were cultured with IL4 and GMCSF to induce differentiation into MDDCs, and confirmed by expression of DC-SIGN. After 6 days of culture with cytokines, MDDCs were infected with pseudotyped reporter HIV-1 containing either no Vpx, chimeric Vpx, SIVrcm Vpx, or SIVmac Vpx. SIVrcm Vpx does not degrade SAMHD1 and serves as a negative control. SIVmac Vpx readily degrades human SAMHD1 and serves as a positive control. Infection of DCs was profoundly inhibited in all conditions except the virus containing SIVmac Vpx (Figure 9A and B). In donor 1, approximately 2% of cells transduced with HIV-1 containing no Vpx, SIVrcm Vpx or chimeric Vpx were infected, while 40% of cells transduced with SIVmac Vpx were infected. MDDCs from donor two were exposed to three different MOIs of reporter virus and exhibited the same pattern of infection as donor 1, though with a lesser magnitude of infectivity by HIV-1 containing SIVmac Vpx (Figure 9B). Therefore, HIV-1 complemented with chimeric Vpx that degrades human SAMHD1 in cell lines is still blocked in MDDC infection.



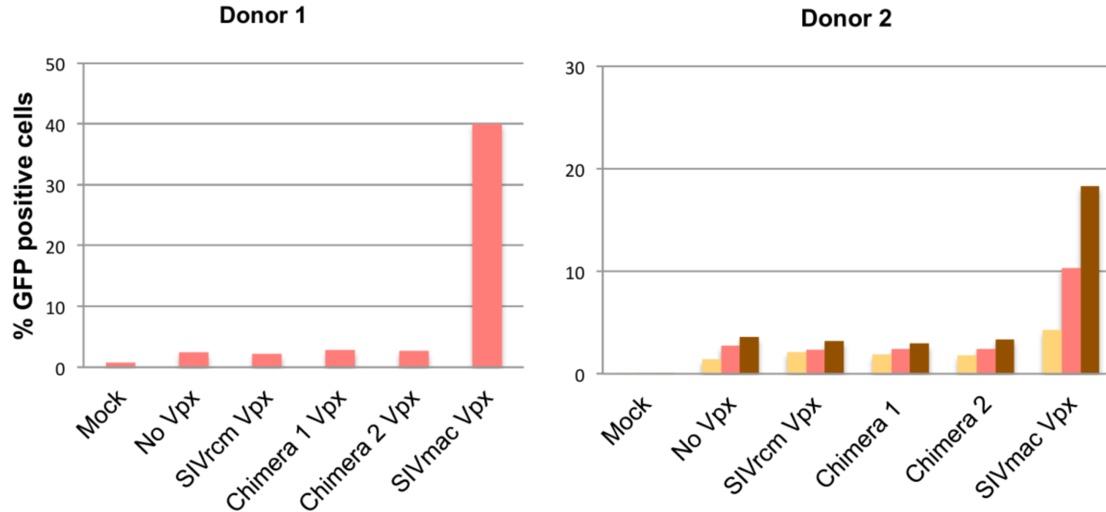


Figure 9 HIV-1 carrying chimeric Vpx still infects MDDCs poorly A) Flow graphs showing percentage of GFP positive MDDCs from Donor 1 infected with HIV-1 packaging no Vpx, SIVrcm Vpx, SIVmac Vpx, or Vpx chimera 1 or 2. B) Graphs of percentage of infected cells from donor 1 shown in A and donor 2. MDDCs from donor were infected with 3 MOIs of HIV-1 packaging no Vpx, SIVrcm Vpx, SIVmac Vpx, or Vpx chimera 1 or 2.

Determinants of specificity for SIVmac Vpx are not sufficient to develop SAMHD1 degradation *de novo*

SAMHD1 degradation arose as a neofunctionalization of Vpr, and even Vpr that do not degrade SAMHD1 maintain conservation of many amino acids mediating DCAF1 binding. An NMR structure of HIV-1 Vpr overlaid on the SIVsmm Vpx structure shows striking similarity between the three helices of the protein, with major differences seen in between the N-termini, the loops linking helix 2 and 3, and the unstructured C-terminal tails of Vpr and Vpx (Figure 6C). To determine whether the regions of SIVmac Vpx conferring specificity are sufficient to mediate degradation of SAMHD1 in a divergent, non-SAMHD1-degrading Vpr protein context, we replaced sequence elements of the primary isolate Q23-17 HIV-1 Vpr with sequence from SIVmac239 Vpx. Some of the amino acid sites previous used as breakpoints between Vpx chimeras are conserved in HIV-1 Vpr. These include W24 of SIVmac, allowing for a replacement of the HIV-1 Vpr N-terminus analogous to the strategy previously used in generating chimeric Vpx. Alignment of SIVmac Vpx and HIV-1 Vpr is poor in the area surrounding the second region needed for Vpx specificity, so we chose a panel of breakpoints based on structural overlay of SIVsmm Vpx and HIV-1 Vpr (Figure 10A). We tested a panel of Vpr/Vpx chimeric proteins for a gain in ability to degrade human SAMHD1.

Most of the panel of HIV-1 Vpr chimeras do not exhibit any gain in ability to degrade human SAMHD1 (Figure 10B). They are poorly expressed compared to Q23-17 HIV-1 Vpr, indicating some level of instability. One polar interaction existing in SIVmac Vpx, but not HIV-1 Vpr, is broken in some of the Vpr/Vpx chimeras. This is a polar interaction involving K68 in

helix 3 of SIVmac Vpx thatis without a corresponding polar contact in helix 1 when in the context of the chimera. A significant improvement in expression is seen with K68A mutation in multiple chimera backgrounds, but the stabilization does not improve SAMHD1 degradation capability (Figure 10C). The regions conferring the degradation specificity of SIVmac Vpx are not sufficient to generate SAMHD1 degradation capability in a non-SAMHD1-degrading Vpr background, indicating additional elements specific to Vpx and SAMHD1 degrading Vpr may be required for the ability to mediate degradation of SAMHD1.

Though the two hypothesized regions conferring specificity of Vpx were not sufficient to create SAMHD1 degradation de novo in an HIV-1 Vpr background, we did find that the C-terminus of SIVmac Vpx was not necessary for SAMHD1 degradation. A SIVmac Vpx with the C-terminus of HIV-1 Vpr, Vpr chimera 4, is able to robustly degrade human SAMHD1, despite rather poor expression of this chimera (Figure 10B, Vpr chimera 4). Therefore, the C-terminus of Vpx is not needed for SAMHD1 degradation.

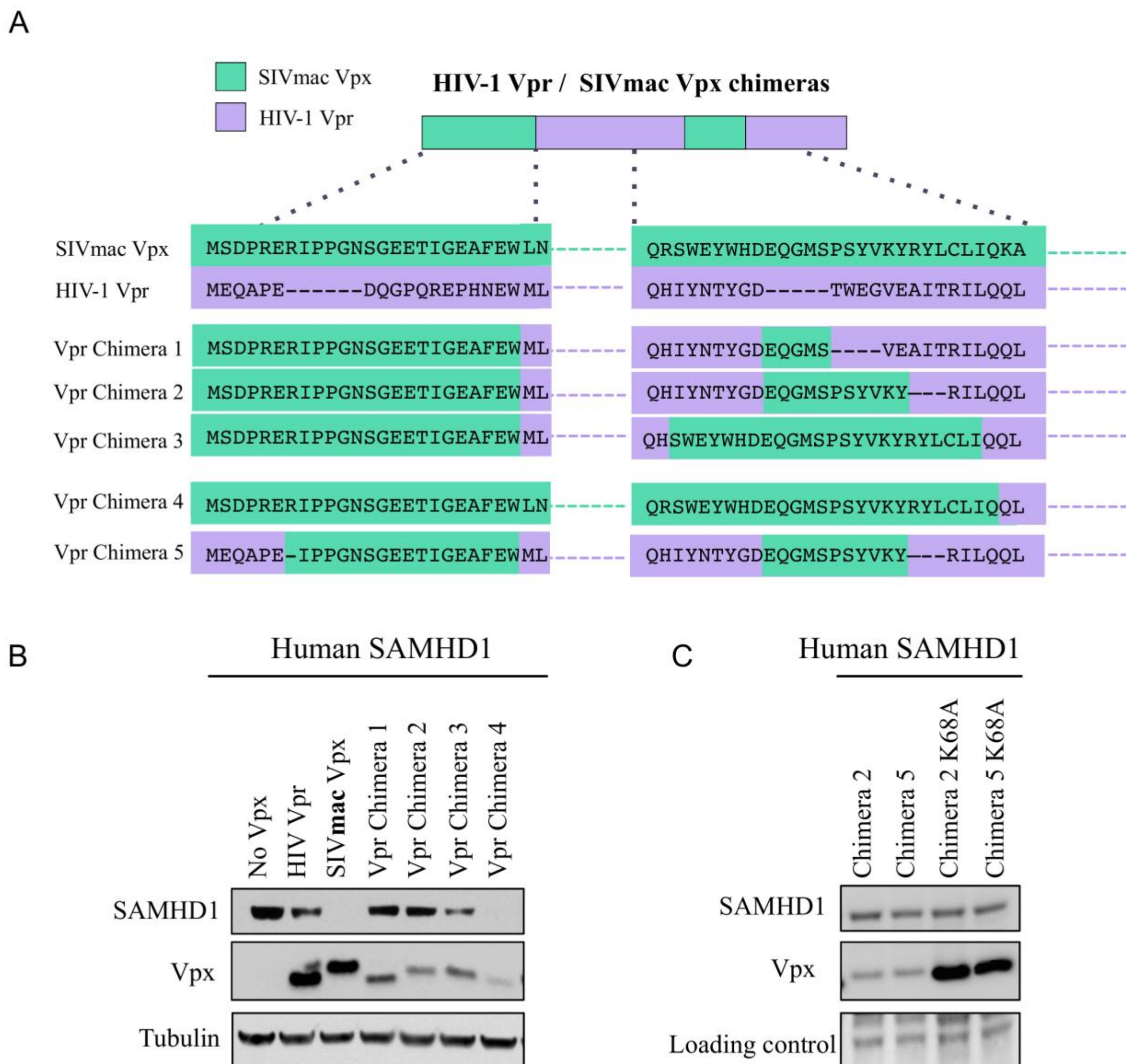


Figure 10 Regions conferring SAMHD1 degradation specificity are not sufficient to generate SAMHD1 degradation *de novo* in HIV-1 Vpr background. Diagram shows breakpoint design for Vpr/Vpx chimeras made in HIV-1 Vpr Q23-17 background (shown in purple) with regions containing specificity of SIVmac Vpx (shown in light green). B-C) Western blot of cell lysates showing HA-tagged human SAMHD1 expression in 293T cells with and without cotransfection of 3X-FLAG-tagged HIV-1 Vpr, SIVmac Vpx, Vpr/Vpx chimeras and chimera mutants. For B, tubulin was probed as a loading control. For C, a nonspecific band is shown as a loading control.

DISCUSSION

In this study, we sought to understand better how viral antagonists adapt to bind rapidly escaping host targets while maintaining overall antagonism function by studying the structure function relationships between SIV Vpx and SAMHD1 degradation. Amino acid sites corresponding to SIVsmm Vpx interactions with DCAF1 are widely type conserved across Vpx and Vpr protein evolution despite high levels of sequence variation between lineages. SIVrcm Vpx and SIVmac Vpx target opposite termini of SAMHD1, but DCAF1 binding is functionally conserved between these divergent Vpx proteins. The crystal structure of SIVsmm Vpx shows two linear regions of Vpx forming a SAMHD1 interacting interface with a C-terminal SAMHD1 degron [167], and we show these two regions are sufficient to mediate C-terminal SAMHD1 targeting of full length SAMHD1 in a divergent, N-terminally targeting Vpx background. While SIVmac Vpx specificity maps to two linear regions in Vpx, these sequence motifs are not sufficient to generate SAMHD1 degradation de novo in a nondegrading Vpr background, suggesting crucial unidentified factors are needed for SAMHD1 degradation capability independent of determinants of Vpx specificity.

SAMHD1 degradation specificity maps to two regions of SIVmac Vpx

An N-terminal requirement for Vpx to overcome SAMHD1 restriction has been previously established [150, 173]. Additionally, a recent study found that the N-terminus of SIVagm Vpr is necessary but not sufficient for SIVagm Vpr to degrade AGM SAMHD1, suggesting a second determinant is necessary [159]. In the case of divergent Vpx proteins, there is sufficient homology to map this second determinant to site beginning in the loop between

helix 2 and helix 3 and ending in the amino terminus of helix 3. Four amino acid residues in this regions interact with C-terminal SAMHD1 according to the SIVsmm crystal structure, but only one, site 62, differs between SIVmac Vpx and SIVrcm Vpx. The first region (residues 14 to 25) forming the Vpx_{smm} interface with SAMHD1 is extensive compared to the second region (62,63,66,69), likely leading to its rapid identification as a necessity for SAMHD1 degradation. However, in agreement with others, we found this region of sequence was not sufficient to cause degradation of human SAMHD1. A second regionwas necessary for a gain of function in SAMHD1 degradation, though it differed in only one SAMHD1-interacting residue, site 62. Of note though, other amino acid differences surrounding site 62 may be important for context.

We found that the regions responsible for specificity of Vpx that targets the C-terminus of SAMHD1 were partially responsible for the SIVrcm Vpx specificity, which targets the N-terminus of SAMHD1. We saw a modest increase in ΔC RCM SAMHD1 degradation by Vpx containing the hypothesized sequence conferring SIVrcm Vpx specificity in the SIVmac Vpx background, but the effect was not robust. This could indicate that additional sequence determinants are required to encompass all determinants of N-terminal targeting Vpx specificity. A recent study shows the solved crystal structure of SIVmnd-2 Vpx, which targets the N-terminus of SAMHD1, similar to SIVrcm Vpx [174]. Interestingly, as we observed in our study, this structure shows that the N-terminally targeting Vpx uses the same two regions as C-terminally targeting Vpx: an N-terminal region and the loop between helix 2 and 3. However, a separate interface is formed between the exposed residues of the amino terminus of helix 2 and the SAM domain of SAMHD1, strikingly involving a region of SAMHD1 (AA 32-69) containing multiple sites that display signatures of rapid evolution in Old World monkeys[90, 141, 174]. These residues were not included in our Vpx chimera made in the SIVmac Vpx with

regions conferring SIVrcm specificity, providing an explanation for our observation of partial but not full swap of specificity in this Vpx chimera.

A separate recent study used alanine mutagenesis of SIVrcm Vpx and identified the amino acids 23-WLHR-26 as key to SIVrcm degradation of RCM SAMHD1 [14]. This mutant lost binding and the ability to degrade SAMHD1 but maintained binding of DCAF1. However, the proposed 23-WLHR-26 motif contains DCAF1-interacting amino acids that are highly conserved across Vpx and Vpr evolution. In SIVsmm Vpx, the corresponding W24 is involved in binding both DCAF1 and SAMHD1 [167]. This residue may be important for SAMHD1 interaction in SIVsmm Vpx and possibly in SIVrcm Vpx, but given its extreme conservation, even in Vpr that do not degrade SAMHD1, this tryptophan is likely primarily of importance to proper DCAF1 binding. In fact, prior to the publication of the SIVsmm Vpx structure, a longer version of the exact motif was previously described as a crucial DCAF1 binding motif necessary for both Vpx degradation of SAMHD1 and Vpr induced G2-arrest [71]. Because the SIVsmm Vpx–DCAF1 interaction interface is extensive and conserved in other lineages, with especially strong conservation in helix 3 of Vpx/r, we believe that using DCAF1 binding as a control for proper folding of a motif falling in helix 1 is uninformative, though this control is widely used. Further, the 23-WLHR-26 motif cannot be responsible for SIVrcm Vpx specificity because this motif does not differ between Vpx sequences in a pattern corresponding to Vpx specificity in SAMHD1 degradation.

A possible additional role of Vpx

We did not see an increase in infectivity in dendritic cells the presence of chimeric Vpx that degrade human SAMHD1 293T cells. Several explanations for a lack of increase are

possible. First, Vpx produced by transfection is likely to be at present at significantly higher levels than when delivered by virion. Second, the maturation defect caused by alteration of p6, which isnecessary to package chimeric Vpx, may have interfered with the proper delivery of Vpx. In fact, a recent study suggests that proteolytic cleavage of the Gag polyprotein is likely required for Vpx release. However, this study didnot distinguish between a lack of Vpx release and a lack of virion fusion [175]. Because virions composed of unprocessed Gag are poorly fusogenic[172], we believed that circumventing this obstacle by mixing proportions of cleavage capable and cleavage resistant Gag would be sufficient to properly deliver Vpx. If cleavage of the p6 protein thatbinds Vpx is required for Vpx release, our packaging system is ineffective. Alternatively, chimeric Vpx is likely less efficient than wildtype SIVmac Vpx (Figure 7B), and SAMHD1 levels may need to fall below a certain threshold to result in relief of inhibition. There is also an interesting possibility that Vpx is important for an additional role early in infection that exhibits species-specificity ameliorated by SIVmac Vpx but not SIVrcm Vpx or chimeric Vpx. For example, Vpx could possibly antagonize additional restriction factors beyond SAMHD1 in a species-specific manner.

Molecular separation of function in multifunctional Vpr

The development of SAMHD1 degradation occurred once in lentiviral evolutionary history [90]. The *vpx* gene was lost in a recombination event restructuring the *vif* gene, leading to increased antagonism of chimpanzee A3G and lentiviral infections of hominids [110]. The ability to degrade SAMHD1 was not reinstated in another viral protein and did not reevolve in hominid lentiviral Vpr. Additionally, there seems to be no examples of convergent evolution in which a virus basal to the neofunctionalization of Vpr also gained the ability to degrade

SAMHD1 *de novo*. Combined with our difficulty in creating SAMHD1 degradation in a Vpr protein that does not degrade host SAMHD1, we conclude that the ability to degrade SAMHD1 was a likely a large molecular leap and the result of multiple fortuitous factors arising in an ancestral *vpr* gene.

Cassiday *et al* has recently suggested that Vpx and Vpr do not interact with an identical subset of DCAF1 residues, as they found differential binding of DCAF1 mutants by SIVmac Vpx and HIV-1 Vpr and further foundthat Vpr outcompetes Vpx in competitive binding assays [168]. We show that the majority of DCAF1-binding residues are conserved, and would predict that if not identical, DCAF1 binding is likely strikingly similar across Vpx and Vpr. However, despite conservation of DCAF1-interacting sites in HIV-1 Vpr, there are distinct differences between Vpr/x proteins that do and do not degrade SAMHD1. In general, the N-terminal region, C-terminal region, and an area stretching from the C-terminus of helix 2 to the N-terminus of helix 3 are very divergent while other features are highly conserved such as the three helical secondary structure and sites involved in DCAF1 interaction. An additional difference is a YRY motif present at the amino terminus of helix 3 present in every SAMHD1 degrading Vpr/x but absent in sequences of Vpr that do not degrade SAMHD1. However, HIV-1 Vpr/SIVmac Vpx chimeras containing this motif were still unable to degrade SAMHD1, indicating that additional factors crucial for SAMHD1 degradation are not yet recognized.

We determined that SIVmac Vpx does not require its native C-terminal region to degrade SAMHD1. Others have shown that the C-terminal tail of SIVagm Vpr is also dispensable for SAMHD1 degradation, while this C-terminal region is sufficient to change specificity of the G2 arrest activity of Vpr [159]. This observation sheds light on the conundrum of dual-functional Vpr proteins which both degrade SAMHD1 and induce G2 arrest. These functions are split

between Vpr and Vpx in lineages that encode both proteins, presumably to escape from the inherent constraint in adapting to multiple targets simultaneously. One explanation for how dual functional Vpr can adapt to multiple rapidly evolving targets is that these functions may be mostly molecularly separate, with discrete regions devoted to adaptation to SAMHD1 and the C-terminal region responsible for determining G2 arrest specificity.

In this study, we show that DCAF1 binding sites are conserved across diverse Vpr and Vpx proteins, allowing us to map determinants of specificity in SAMHD1 degradation to two linear regions of SIVmac Vpx. This functionally confirms that the interactions between SIVsmm Vpx and a C-terminal degron of human SAMHD1 shown by crystal structure are sufficient to cause degradation of full length SAMHD1. We also show that DCAF1 binding of Vpx is functionally conserved between Vpx of different lentiviral lineages. We propose a model where the evolution of Vpr and Vpx is constrained by the need to maintain extensive binding interfaces with DCAF1, resulting in the limitation of sequence available to bind rapidly diverging SAMHD1.

Chapter 5: Perspectives and Future Directions

In mythesis, I show that antagonism of SAMHD1 is maintained in natural SIV infections of modern primates. *SAMHD1* is polymorphic in geographically separated populations of African Green monkeys. SIV that infects each population has adapted to SAMHD1 variants present there, despite the majority of polymorphism conferring resistance to degradation by SIVagm Vpr of heterologous populations. Because each lentivirus adapted to antagonize resistant SAMHD1, SAMHD1 antagonism must be important for viral fitness. I have also shown that across lentiviruses, Vpr and Vpx maintain conserved binding with the ubiquitin ligase substrate receptor DCAF1, and Ihave mapped determinants of specificity of SAMHD1 degradation in at least one Vpx protein. However, several questions remain. Whysome viruses do not require SAMHD1 antagonism, how a loss of *vpx* could beadaptive, and why SAMHD1 antagonism did not reevolve after being lost are all prominent questions that need to be resolved.Additionally, the role of Vpx in transmission and pathogenesis is also not well understood. In this discussion, I will address these topics and demonstrate the utility of an evolutionary perspective in weighing these questions.

Is SAMHD1 important or not?

The maintenance of SAMHD1 antagonism in lentiviruses that naturally infect modern primates shows that SAMHD1 has the power to shape lentiviral evolution. The reasons why some but not all lentiviruses evade SAMHD1 is still unsettled. Different lines of evidence

suggest that SAMHD1 antagonism is somehow both essential and dispensable in lentiviral infection. Arguing against the importance of SAMHD1 antagonism, many lentiviral lineages do not encode a SAMHD1 antagonist. These include viruses basal to the evolution of SAMHD1 antagonism as well as those related to SIVcpz in which the *vpx* gene was seemingly lost without consequence [90, 110]. The lentiviruses basal to the neofunctionalization of Vpr are SIVs that infect *Colobinae*, a subset of guenons, and one of multiple lentiviruses that infect mandrills. These viruses are successful in their hosts despite a lack of SAMHD1 antagonism, infecting between 3 and 80 percent of individuals depending on the species [176]. This shows that, in particular contexts, SAMHD1 antagonism is not a necessity for lentiviral persistence in primate populations.

Moreover, the ability to antagonize SAMHD1 did not reevolve in during the entire evolution of SIVcpz or any lineage of HIV-1, suggesting the property is unnecessary in these viruses in the chimpanzee, gorilla, or human hosts. Finally, in my study of mutations arising during *in vivo* replication in AGMs, the virus did not evolve to ability to degrade a resistant SAMHD1, while it did evolve the ability to degrade another restriction factor, APOBEC3G [98]. However, in these experiments, peak viremia was lower than in the natural host of the virus, indicating possible restriction by SAMHD1 or other species-specific factors [136].

Despite the evidence that SAMHD1 antagonism is not necessary, *SAMHD1* gene has experienced bursts of rapid evolution in primate history [90, 141]. This means that resistant *SAMHD1* alleles were selected because they protect their hosts by hindering lentiviral replication. Additionally, Δ*vpx* versions of SIVsmm are attenuated in experimental infections of macaques [111]. The mutant virus is marked by a failure to disseminate to tissues and to cause pathogenesis. There is one caveat to concluding SAMHD1 antagonism is important based on

experimental infections with Δ*vpx* virus. The Vpx protein may be multifunctional, and a function apart from SAMHD1 degradation may be crucial for acute infection. In fact, at least one other function of Vpx is already proposed, as Vpx rescues HIV-1 from a post nuclear entry block in interferon-treated MDDCs [69]. Intriguingly, the block is independent of DCAF1 [177]. However, my studies show that SAMHD1 antagonism itself, rather thana different function of Vpr/x, is important for viral fitness in modern day viruses because viral Vpr has adapted to maintain antagonism of resistant SAMHD1 variants within an evolutionarily short timeframe. Thus, the selective advantage for the virus to maintain antagonism necessitates that SAMHD1 antagonism is a significant factor in viral success. Experimental infection using SIV that expressed Vpx that could not degrade macaque SAMHD1, but could potentially perform other Vpx functions may be a way to ask whether SAMHD1 degradation is needed for viral dissemination and progression. For example, a carefully chosen point mutant could potentially abrogate SAMDH1 degradation without disrupting other Vpx features. The amino acids E15 or E16, for instance, are crucial for SAMHD1 binding, but mutating either of them does not diminish DCAF1 binding and would be unlikely to affect the structure of Vpx because these sites lie in a flexible N-terminal region of Vpx [150]. Testing whether a mutant Vpx still rescues HIV-1 infection of IFN treated MDDCs could confirm that alternative functions of Vpx are unaffected. If SIV expressing mutant Vpx is still exhibits impairment in experimental infection, SAMHD1 antagonism and not another function of Vpx is likely crucial for viral dissemination and pathogenesis.

What is Vpx for?

Assuming that Vpx degradation of SAMHD1 is the primary function of Vpx, the properties of Δ*vpx* lentivirus can shed light upon early events in lentiviral transmission. SIVsmm Pbj is normally pathogenic in pigtail macaques, but without Vpx the virus is severely attenuated [111, 178]. The impairment is only observed in the spread of virus; there seems to be no impairment in initial infection [111]. Plasma viremia is detected early in infection, but it is delayed and of lesser magnitude than wildtype viremia. Additionally, inoculation by musocal or intravenous routes exhibits the same degree of impairment, which also indicates that Vpx is important for early viral amplification after and independent of transmission.

Until recently, the first events occurring in lentiviral transmission have been unclear. A longstanding hypothesis that proposed that dendritic cells capture the virus in tissues, mature and migrate to secondary lymphoid tissues, and then spread the virus to T-cells by trans-infection, where virions pass directly from one cell to another via a synapse formed between them [179, 180]. Variations on this hypothesis incorporated the idea that DCs may recruit CD4+ T cells to foci where local amplification occurs at the site of infection prior to migration. For instance, one study suggests exposure of epithelial cells to viral inocula causes the recruitment of plasmacytoid DCs, and that infection is then fueled by subsequent recruitment of CD4+ T cells [78]. Other hypotheses proposed that CD4+ T cells were the first targets [181], or initial cell infection was proportional to the availability of target cells [182]. However, the understanding of transmission an early events in infection islimited by the inability to visualize or detect signal from the very few initially infected cells in animal models.

A recent study using a dual-reporter lentiviral system capable of only a single round of replication has clarified the order of events in a macaque model [183]. A firefly luciferase

reporter aids the identification of foci of transduction in the female reproductive tract, and after creating cryosection of foci, a fluorescent reporter allows identification of particular transduced cells. This study suggests the earliest amplification occurs at local foci of CD4+ T-cells near the site of transmission. The kinetics of acute infection suggest that infection of draining lymph nodes occurs so rapidly that trans-infection by DCs cannot be the mechanism of the earliest viral amplification. The first infected cells must migrate directly to lymph nodes to fit the observed timing, though DC trans-infection could still play an important role later in acute infection. CD4+ cells already present at preexisting sites of inflammation are then thought to be the initial viral targets, as foci of densely transduced cells are present after infection of virus limited to a single round of infection.

The ease of transmission by Δvpx virus supports the idea that dendritic cells and resting T cells are not the first infected cells, as SAMHD1 would inhibit infection and likely inhibit transmission. Thus SAMHD1 is likely not antiviral in the very first cells CD4+ T cells infected during transmission, but some step of subsequent spreading is dependent on Vpr/x in viruses that encode a SAMHD1 antagonist. An inability to productively infect DCs might result in dampened cell to cell spread, which could explain the general impairment in amplification of Δ*vpx* lentivirus. Cell to cell spread of virus by virological synapse is more efficient than spread of cell free virus [184]. It would be interesting to compare the spread of WT SIVmac and SIV Δ*vpx* using the dual reporter system where early events can be visualized. If the earliest events in transmission are identical, this would definitively show that Vpx has no role in transmission. However, if the initial transduction is inhibited, a role of either resting T-cells or myeloid cells could be inferred, and more extensive cell marker staining could differentiate between the possibilities. Staining for levels of SAMHD1 in transduced and untransduced cells may also be

informative. If initial transduction of WT SIVmac and SIVmacΔ*vpx* are identical, by using SIV capable of more than one round of infection, a comparison of the kinetics of spread between wildtype and Δ*vpx* SIV is possible, as well as the identification of the specific step where SIVΔ*vpx* begins to lag.

If SAMHD1 antagonism is important, how could Vpx be lost?

Still mysterious is the glaring loss of the SAMHD1 antagonist gene in the genesis of SIVcpz. This loss can only be understood by viewing the scenario from an evolutionary perspective. The loss of *vpx* was inherently linked to the restructuring of *vif,* suggesting that antagonism of A3G is an absolute imperative for viral replication, while antagonism of SAMHD1 may be expendable under certain conditions [110]. Thus the improvement in antagonizing chimpanzee A3G outweighed the detriment brought about by loss of SAMHD1 degradation. A3G is a strong selective pressure in every target cell, in contrast to SAMHD1. Importantly, SIVs lacking the capacity to degrade SAMHD1 can still infect animals in experimental infections. In two cases, SIVsmm Δ*vpx* infection of macaques and SIVagm.Ver infection of Sabaeus monkeys, the virus is attenuated but not cleared [111, 185]. Natural infections may also sustain low-level infection despite a lack of SAMHD1 degradation, hypothetically allowing a window of time for secondary compensations to emerge.

SAMHD1 degradation may have been adaptive in such a way that it relaxed selection for a function crucial to viruses that do not degrade SAMHD1. This would explain why SAMHD1 antagonism is only essential to viruses that typically rely on Vpr/x. Properties unique to viruses that never degraded SAMHD1 could hint at what kinds of compensatory adaptations may have arisen in the SIVcpz/HIV-1 lineage. Indeed, rather than develop a novel method of SAMHD1

degradation, a current hypothesis proposes that the virus likely acquired mutations in reverse transcriptase that nullify SAMHD1 restriction. SAMHD1 operates at least in part by hydrolyzing cellular dNTPs to inhibit efficient reverse transcription. Recently, the reverse transcriptase enzymes of several HIV-1 isolates were shown to have significantly lower Km values for dNTP substrates compared to viruses that encode *vpx*, meaning HIV-1 RT is more efficient than other RT enzymes in conditions of low dNTP concentration [112]. It would be interesting to see if viral lineages basal to the neofunctionalization of Vpr have similarly low Km values. This would indicate that Vpx allowed for the relaxation of selection for supremely efficient RT enzymes. However, RT adaptations may represent an independent path around SAMHD1 restriction that may not be mirrored in viral lineages that never evolved a SAMHD1 antagonist.

Why hasn't Vpx reevolved?

The Vpr protein of neither SIVcpz nor any group of HIV-1 appears to degrade SAMHD1 of its host, meaning SAMHD1 antagonism has not reevolved since loss in the SIVcpz ancestor [90]. In fact, the one neofunctionalization of Vpr is the only known instance of a gain of function to degrade SAMHD1 in lentiviral evolutionary history. Given enough time, evolution of the *vpr* gene should sample all possible minor mutations, suggesting that the neofunctionalization involved substantial molecular change. Indeed, several obvious characteristics distinguish SAMHD1-degrading Vpx/r sequence amino acid sequence from sequence of Vpr that do not degrade SAMHD1. For instance, the N-terminus, particularly the region mapped to bind SAMHD1, is longer in Vpx/r that degrade SAMHD1. Additionally, the second binding interface, the loop between helix 2 and helix 3, is also longer in SAMHD1

degrading Vpx/r. Thus extending sequence in flexible regions while maintaining a rigid core structure may allow for association with new targets and may be a mechanism of novel function genesis.

Another example of Vpx-specific characteristic is a motif in helix 3. Two tyrosines in helix 3 of SIVsmm Vpx interact with both SAMHD1 and DCAF1 [167]. One of these tyrosines is conserved in all SAMHD1 degrading Vpx/r, while the other is conserved in nearly all. There is no equivalent motif in Vpr proteins that do not degrade SAMHD1. However, after the addition of the tyrosine motif to chimeric HIV-1 Vpr containing SIVmac specificity, chimeric Vpr/x is still unable to recapitulate human SAMHD1 degradation. Several possibilities can explain the failure to gain function. The HIV-1 and SIVmac Vpx/r proteins may be too divergent to create functional chimeric proteins due to unfavorable intramolecular interactions. The chimeric Vpr/x proteins appear to be expressed, but they should be tested for binding of the ubiquitin ligase subunit DCAF1 to ensure they are folding properly. Also possible is that other determinants may have been missed, and a crucial aspect of Vpx is not represented in the chimeric Vpr/x proteins. Regardless, the lack of reevolution of SAMHD1 antagonism in nature in combination withthe difficulty of recapitulating this function by logical designof Vpx chimeras hint that SAMHD1 antagonism may have been a molecularly complex gain of function. The original event generating the ability of Vpr to degrade SAMHD1 may be the result of a rare and fortuitous restructuring of Vpr that allowed for binding of a new target but maintained binding of DCAF1.

A recently published co-crystal structure of SIVmnd-2 Vpx bound to DCAF1 and the N-terminal region of mandrill SAMHD1 reveals a third and unexpected interface likely important for Vpx interaction with SAMHD1 [174]. In Vpx proteins that target the N-terminus of

SAMHD1, several amino acids along the exposed upper side of helix 2 interact with the SAM domain of SAMHD1. This domain is not included in the structure of SIVsmm Vpx, as only a very short SAMHD1 C-terminal degron was co-crystalized [167]. Interestingly, most amino acids composing the newly discovered interface are conserved in all Vpx proteins whether they target the N or C-terminus. The conservation of these sites between Vpx proteins implies that both N and C-terminal targeting Vpx proteins may bind the SAM domain. This supports the hypothesis that Vpx simultaneously binds multiple regions of SAMHD1 that are close together when the active SAMHD1 tetramer forms [102]. If true, Vpx/r did not radically change to bind opposite ends of a targeted protein, but simply shifted the degree of reliance on one interface relative to another in diverging viral lineages. While a structure of the SAMHD1 tetramer exists, it unfortunately does not include either the N or C-termini, so whether the termini assemble into a single surface is unknown [186]. A structure of the entire SAMHD1 tetramer as bound to Vpx and the WD subunit of DCAF1 would greatly inform the understanding of seemingly disparate SAMHD1 targeting.

Because the recently identified third interface may also exist between C-terminally targeting Vpx and their SAMHD1 targets, this motif should be included in the design of chimeric HIV-1 Vpr. The sites do not control specificity as they are conserved between divergent Vpx proteins, but they may still be crucial for degrading SAMHD1. The motif is absent in HIV-1 Vpr and is not included in the HIV-1 Vpr chimeras I tested. Including this interface could be the key to creating a gain of SAMHD1 degradation ability in the non- degrading HIV-1 Vpr. An HIV-1 Vpr chimeric protein that degraded SAMHD1 would not only delineate the components necessary in gain of function to degrade SAMHD1, it would also be useful in developing a more complete understanding of other Vpr functions. For instance, the evolutionary constraints on

SAMHD1-degrading Vprs are not well understood. A presumably adaptive duplication and subfunctionalization of Vpr occurred in one ancestral lentiviral lineage, but the costs and tradeoffs affecting contemporary multifunctional Vpr proteins are unclear. Interestingly, a chimera composed of SIVmac Vpx with the C-terminal tail of HIV-1 Vpr is able to degrade human SAMHD1, so the C-terminus of Vpx is not necessary for SAMHD1 antagonism. A swap of C-termini between SIVagm Vpr and HIV-1 Vpr also swaps specificity for causing G2 arrest in human and AGM cells, meaning specificity for G2 arrest is contained in the C terminal tail [159]. Therefore, these functions could be molecularly separate. There may be less constraint on the evolution of SAMHD1-degrading Vpr than expected if discrete parts of the antagonist protein are designated to separate roles, especially if flexible protein regions are designated to bind rapidly evolving targets. More studies of Vpr and Vpx mutants and chimeras could assist mapping of the exact parts of the proteins necessary for the multiple functions of Vpx and Vpr. For instance, mapping the determinants of Vpr interaction with the SLX4 complex and the determinants of Vpx rescue from the antiviral state created by IFN-treated MDDCs could both be informative. Additionally, crystallography studies would definitively show how Vpr and Vpx interact with various targets, greatly enhancing our understanding of the molecular basis of antagonist activities and the molecular mechanisms of antagonist adaptation.

Is a lack of pathogenesis caused by Vpx?

It is tempting to speculate that Vpx contributes to dampened pathogenesis in lentiviral infections. There are two examples of primate species harboring multiple lentiviral infections that differ in pathogenicity correlating with presence or absence of *vpx*. Lentiviral infections of humans include HIV-1 and HIV-2, but only HIV-2 encodes a *vpx* gene. Similarly, two

lentiviruses, SIVmnd-1 and SIVmnd-2, infect mandrills. SIVmnd-2 encodes Vpx while SIVmnd-1 does not. HIV-2 is less pathogenic than HIV-1. The two lentiviruses that infect mandrills both seem to be nonprogressive in their natural host. However, SIVmnd-1 and SIVmnd-2 exhibit different properties in experimental infections of novel hosts, rhesus macaques. SIVmnd-1 results in severe T-cell depletion and immune activation, while SIVmnd-2 shows little difference from an infection of the natural host two years following infection [187].

Another hypothesis pertaining to pathogenesis suggests that the loss of *vpx* in SIVcpz may have been adaptive, as it may have allowed the virus to hide from sensing via productive infection of dendritic cells[188]. Studies have suggested that when Vpx is present and reverse transcription is allowed to complete, monocyte derived dendritic cells mature and release interferon[137, 189]. The hypothesis proposes that completion of reverse transcription and sensing by DCs results in the mounting of a robust adaptive immune response. In contrast, by halting viral replication in DCs, the host immune response is impaired. Antigen presenting cells would present viral components, but the appropriate co-stimulatory signals to activate a robust T-cell response would be absent without sensing of virus in the same cell. With Vpx, the same antigen-presenting cell both senses the virus and presents antigen.In support of this hypothesis, HIV-2 infections result in polyfunctional T cell responses more often than HIV-1 infection [190]. A functional adaptive immune response to viral infection would thus account for the milder pathogenicity of HIV-2 in comparison to HIV-1. However, two points counter this hypothesis. The discovery that the loss of *vpx* was linked to a gain of activity in Vif antagonism of chimpanzee A3G convincingly demonstrates that the loss of *vpx* was a detrimental but necessary cost associated with the lentiviral jump from Old World monkeys to hominids[110]. Second, if loss of SAMHD1 antagonism was beneficial due to impaired viral sensing, the maintenance of

SAMHD1 antagonism would not be observed, as the virus would not experience selective pressure to regain antagonism of SAMHD1 each time a host escape mutation emerged in the virus-host arms race. Because my studies show that SAMHD1 antagonism is maintained, the loss of *vpx* cannot be adaptive.

Recent studies postulate that when viral nucleic acid is detected in resting CD4+ T-cells as a result of restriction by SAMHD1, the cells die by a highly inflammatory programed cell death called pyroptosis[191]. During pyroptosis, cells swell and burst, releasing contents including proinflammatory cytokines to promote further inflammation[192, 193]. SAMHD1 does indeed potently restrict virus in resting CD4+ T-cells, and they are the type majority of cells encountered by lentivirus during infection[77, 194]. This hypothesis links CD4+ T cell depletion with chronic inflammation, but several points are still unexplained. Notably, the same *vpx*-encoding virus results in profoundly different courses of infection in different hosts. SIVsmm infections of sooty mangabey are typical of nonpathogenic infections in a natural host. However, the same *vpx*-encoding virus is highly pathogenic when adapted to macaques, resulting in irrecoverable T cell depletion, chronic inflammation, and progression to AIDS. The SIVsmm Vpx degrades macaque SAMHD1, and the Vpx protein sequence is identical to SIVmac at sites involved in SAMHD1 binding, indicating it was likely preadapted to macaque SAMHD1[90]. Thus, a lack of Vpx degradation of SAMHD1 cannot be the reason for pathogenesis in macaques, and the presence or absence of *vpx* cannot be the sole factor contributing to viral pathogenesis.

An evolutionary perspective is a powerful tool to resolve contradictory findings. Because SAMHD1 has experienced recurrent episodes of rapid evolution due to lentiviral pressure, we can conclude that SAMHD1 escape from degradation by a viral antagonist is beneficial for the

host. If escape mutations in SAMHD1 resulted in chronic inflammation and progression to AIDS as a result of pyroptosis of nonproductively infected resting T-cells, the escape mutations would never have given the host an evolutionary advantage. One could argue that SAMHD1 escape mutations act at the level of preventing infection but are detrimental to hosts once infected, except that SAMHD1 antagonism does not seem to be a barrier to initial viral infection. Similarly, the loss of *vpx* in one ancestral lentiviral lineage was not directly adaptive but rather the consequence of a stronger force shaping lentiviral evolution, A3G. While the loss of vpx was likely detrimental initially, SAMHD1 antagonism was not regained, likely because the molecular change necessary to gain antagonism *de novo* is too extensive to have been sampled during recent hominoid lentiviral evolution and an alternative compensative was selected instead.

In mythesis I have employed an evolutionary perspective in studying the adaptation of lentiviruses to antagonize the restriction factor SAMHD1. I have shown that despite contradictory findings on SAMHD1 antagonism significance, lentiviruses adapt to gain antagonism of resistant SAMHD1 variants in population of modern primates, meaning that SAMHD1 antagonism is actively selected and therefore adaptive. I have also shown that lentiviral Vpr and Vpx adaptation to rapidly evolving SAMHD1 is constrained by maintaining binding of the ubiquitin ligase substrate receptor DCAF1, limiting sequence available for SAMHD1 interaction. However, why Vpx is crucial to success of a subset of lentiviruses, how Vpr gained the ability to degrade SAMHD1, and the role of SAMHD1 antagonism in viral pathogenesis are questions that remain unresolved.

Bibliography

1. UNAIDS, *Global Reports - UNAIDS report on the global AIDS epidemic*, 2013: Geneva.
2. Barre-Sinoussi, F., et al., *Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 868-71.
3. Gallo, R.C., et al., *Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 865-7.
4. Hahn, B.H., et al., *AIDS as a zoonosis: scientific and public health implications.* Science, 2000. **287**(5453): p. 607-14.
5. Keele, B.F., et al., *Chimpanzee reservoirs of pandemic and nonpandemic HIV-1.* Science, 2006. **313**(5786): p. 523-6.
6. Sharp, P.M. and B.H. Hahn, *Origins of HIV and the AIDS pandemic.* Cold Spring Harb Perspect Med, 2011. **1**(1): p. a006841.
7. Clavel, F., et al., *Isolation of a new human retrovirus from West African patients with AIDS.* Science, 1986. **233**(4761): p. 343-6.
8. Hirsch, V.M., et al., *An African primate lentivirus (SIVsm) closely related to HIV-2.* Nature, 1989. **339**(6223): p. 389-92.
9. Faria, N.R., et al., *HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations.* Science, 2014. **346**(6205): p. 56-61.
10. Vallari, A., et al., *Confirmation of putative HIV-1 group P in Cameroon.* J Virol, 2011. **85**(3): p. 1403-7.
11. Korber, B., et al., *Timing the ancestor of the HIV-1 pandemic strains.* Science, 2000. **288**(5472): p. 1789-96.
12. Ayouba, A., et al., *HIV-1 group N among HIV-1-seropositive individuals in Cameroon.* AIDS, 2000. **14**(16): p. 2623-5.
13. Peeters, M., et al., *Geographical distribution of HIV-1 group O viruses in Africa.* AIDS, 1997. **11**(4): p. 493-8.
14. D'Arc, M., et al., *Origin of the HIV-1 group O epidemic in western lowland gorillas.* Proc Natl Acad Sci U S A, 2015. **112**(11): p. E1343-52.
15. Delaugerre, C., et al., *HIV-1 group N: travelling beyond Cameroon.* Lancet, 2011. **378**(9806): p. 1894.
16. Plantier, J.C., et al., *Census and analysis of persistent false-negative results in serological diagnosis of human immunodeficiency virus type 1 group O infections.* J Clin Microbiol, 2009. **47**(9): p. 2906-11.
17. UNAIDS, *AIDS Epidemic Update.* 2006.
18. Gao, F., et al., *Human infection by genetically diverse SIVSM-related HIV-2 in west Africa.* Nature, 1992. **358**(6386): p. 495-9.
19. Damond, F., et al., *Identification of a highly divergent HIV type 2 and proposal for a change in HIV type 2 classification.* AIDS Res Hum Retroviruses, 2004. **20**(6): p. 666-72.
20. da Silva, Z.J., et al., *Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing?* AIDS, 2008. **22**(10): p. 1195-202.

21. Sauter, D., et al., *Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains.* Cell Host Microbe, 2009. **6**(5): p. 409-21.
22. Hodges-Mameletzis, I., G.J. De Bree, and S.L. Rowland-Jones, *An underestimated lentivirus model: what can HIV-2 research contribute to the development of an effective HIV-1 vaccine?* Expert Rev Anti Infect Ther, 2011. **9**(2): p. 195-206.
23. Parrish, C.R., et al., *Cross-species virus transmission and the emergence of new epidemic diseases.* Microbiol Mol Biol Rev, 2008. **72**(3): p. 457-70.
24. VandeWoude, S. and C. Apetrei, *Going wild: lessons from naturally occurring T-lymphotropic lentiviruses.* Clin Microbiol Rev, 2006. **19**(4): p. 728-62.
25. Compton, A.A. and M. Emerman, *Convergence and divergence in the evolution of the APOBEC3G-Vif interaction reveal ancient origins of simian immunodeficiency viruses.* PLoS Pathog, 2013. **9**(1): p. e1003135.
26. Compton, A.A., H.S. Malik, and M. Emerman, *Host gene evolution traces the evolutionary history of ancient primate lentiviruses.* Philos Trans R Soc Lond B Biol Sci, 2013. **368**(1626): p. 20120496.
27. Charleston, M.A. and D.L. Robertson, *Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny.* Syst Biol, 2002. **51**(3): p. 528-35.
28. Wertheim, J.O. and M. Worobey, *A challenge to the ancient origin of SIVagm based on African green monkey mitochondrial genomes.* PLoS Pathog, 2007. **3**(7): p. e95.
29. Salemi, M., et al., *Mosaic genomes of the six major primate lentivirus lineages revealed by phylogenetic analyses.* J Virol, 2003. **77**(13): p. 7202-13.
30. Leendertz, S.A., et al., *No evidence for transmission of SIVwrc from western red colobus monkeys (Piliocolobus badius badius) to wild West African chimpanzees (Pan troglodytes verus) despite high exposure through hunting.* BMC Microbiol, 2011. **11**(1): p. 24.
31. Bushman, F.D., et al., *Host cell factors in HIV replication: meta-analysis of genome-wide studies.* PLoS Pathog, 2009. **5**(5): p. e1000437.
32. Chahroudi, A., et al., *Natural SIV hosts: showing AIDS the door.* Science, 2012. **335**(6073): p. 1188-93.
33. Bailes, E., et al., *Hybrid origin of SIV in chimpanzees.* Science, 2003. **300**(5626): p. 1713.
34. Rey-Cuille, M.A., et al., *Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease.* J Virol, 1998. **72**(5): p. 3872-86.
35. Broussard, S.R., et al., *Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease.* J Virol, 2001. **75**(5): p. 2262-75.
36. Cichutek, K. and S. Norley, *Lack of immune suppression in SIV-infected natural hosts.* AIDS, 1993. **7 Suppl 1**: p. S25-35.
37. Letvin, N.L., et al., *Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III.* Science, 1985. **230**(4721): p. 71-3.
38. Paiardini, M., et al., *Low levels of SIV infection in sooty mangabey central memory CD(4)(+) T cells are associated with limited CCR5 expression.* Nat Med, 2011. **17**(7): p. 830-6.

39. Pandrea, I., et al., *Paucity of CD4+CCR5+ T cells is a typical feature of natural SIV hosts.* Blood, 2007. **109**(3): p. 1069-76.
40. Beaumier, C.M., et al., *CD4 downregulation by memory CD4+ T cells in vivo renders African green monkeys resistant to progressive SIVagm infection.* Nat Med, 2009. **15**(8): p. 879-85.
41. Letvin, N.L., et al., *Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys.* Science, 2006. **312**(5779): p. 1530-3.
42. Okoye, A., et al., *Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection.* J Exp Med, 2007. **204**(9): p. 2171-85.
43. Malim, M.H. and M. Emerman, *HIV-1 accessory proteins--ensuring viral survival in a hostile environment.* Cell Host Microbe, 2008. **3**(6): p. 388-98.
44. Van Valen, L., *A new evolutionary law.* Evolutionary Theory 1973. **1**: p. 1-30.
45. Nielsen, M.H., F.S. Pedersen, and J. Kjems, *Molecular strategies to inhibit HIV-1 replication.* Retrovirology, 2005. **2**: p. 10.
46. Daugherty, M.D. and H.S. Malik, *Rules of engagement: molecular insights from host-virus arms races.* Annu Rev Genet, 2012. **46**: p. 677-700.
47. Duggal, N.K. and M. Emerman, *Evolutionary conflicts between viruses and restriction factors shape immunity.* Nat Rev Immunol, 2012. **12**(10): p. 687-95.
48. Emerman, M. and H.S. Malik, *Paleovirology--modern consequences of ancient viruses.* PLoS Biol, 2010. **8**(2): p. e1000301.
49. Harris, R.S., et al., *DNA deamination mediates innate immunity to retroviral infection.* Cell, 2003. **113**(6): p. 803-9.
50. Mangeat, B., et al., *Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts.* Nature, 2003. **424**(6944): p. 99-103.
51. Jarmuz, A., et al., *An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22.* Genomics, 2002. **79**(3): p. 285-96.
52. OhAinle, M., et al., *Adaptive evolution and antiviral activity of the conserved mammalian cytidine deaminase APOBEC3H.* J Virol, 2006. **80**(8): p. 3853-62.
53. Bishop, K.N., et al., *Cytidine deamination of retroviral DNA by diverse APOBEC proteins.* Curr Biol, 2004. **14**(15): p. 1392-6.
54. Zheng, Y.H., et al., *Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication.* J Virol, 2004. **78**(11): p. 6073-6.
55. Mehle, A., et al., *A zinc-binding region in Vif binds Cul5 and determines cullin selection.* J Biol Chem, 2006. **281**(25): p. 17259-65.
56. Yu, X., et al., *Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex.* Science, 2003. **302**(5647): p. 1056-60.
57. Gifford, R.J., *Viral evolution in deep time: lentiviruses and mammals.* Trends Genet, 2012. **28**(2): p. 89-100.
58. Neil, S.J., T. Zang, and P.D. Bieniasz, *Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu.* Nature, 2008. **451**(7177): p. 425-30.
59. Van Damme, N., et al., *The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein.* Cell Host Microbe, 2008. **3**(4): p. 245-52.

60. Alvarez, R.A., et al., *HIV-1 Vpu antagonism of tetherin inhibits antibody-dependent cellular cytotoxic responses by natural killer cells.* J Virol, 2014. **88**(11): p. 6031-46.
61. Jia, B., et al., *Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2.* PLoS Pathog, 2009. **5**(5): p. e1000429.
62. Zhang, F., et al., *Nef proteins from simian immunodeficiency viruses are tetherin antagonists.* Cell Host Microbe, 2009. **6**(1): p. 54-67.
63. Schmokel, J., et al., *The presence of a vpu gene and the lack of Nef-mediated downmodulation of T cell receptor-CD3 are not always linked in primate lentiviruses.* J Virol, 2011. **85**(2): p. 742-52.
64. Serra-Moreno, R., et al., *Tetherin/BST-2 antagonism by Nef depends on a direct physical interaction between Nef and tetherin, and on clathrin-mediated endocytosis.* PLoS Pathog, 2013. **9**(7): p. e1003487.
65. Yang, H., et al., *Structural insight into the mechanisms of enveloped virus tethering by tetherin.* Proc Natl Acad Sci U S A, 2010. **107**(43): p. 18428-32.
66. Reymond, A., et al., *The tripartite motif family identifies cell compartments.* EMBO J, 2001. **20**(9): p. 2140-51.
67. Stremlau, M., et al., *The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys.* Nature, 2004. **427**(6977): p. 848-53.
68. Stremlau, M., et al., *Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor.* Proc Natl Acad Sci U S A, 2006. **103**(14): p. 5514-9.
69. Pertel, T., et al., *TRIM5 is an innate immune sensor for the retrovirus capsid lattice.* Nature, 2011. **472**(7343): p. 361-5.
70. Sawyer, S.L., et al., *Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain.* Proc Natl Acad Sci U S A, 2005. **102**(8): p. 2832-7.
71. Li, M., et al., *Codon-usage-based inhibition of HIV protein synthesis by human schlafen 11.* Nature, 2012. **491**(7422): p. 125-8.
72. Bustos, O., et al., *Evolution of the Schlafen genes, a gene family associated with embryonic lethality, meiotic drive, immune processes and orthopoxvirus virulence.* Gene, 2009. **447**(1): p. 1-11.
73. Goujon, C., et al., *Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection.* Nature, 2013. **502**(7472): p. 559-62.
74. Compton, A.A., et al., *IFITM proteins incorporated into HIV-1 virions impair viral fusion and spread.* Cell Host Microbe, 2014. **16**(6): p. 736-47.
75. Laguette, N., et al., *SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx.* Nature, 2011. **474**(7353): p. 654-7.
76. Hrecka, K., et al., *Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein.* Nature, 2011. **474**(7353): p. 658-61.
77. Baldauf, H.M., et al., *SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells.* Nat Med, 2012. **18**(11): p. 1682-7.
78. Rice, G.I., et al., *Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response.* Nat Genet, 2009. **41**(7): p. 829-32.
79. Rehwinkel, J., et al., *SAMHD1-dependent retroviral control and escape in mice.* EMBO J, 2013. **32**(18): p. 2454-62.

80. Behrendt, R., et al., *Mouse SAMHD1 has antiretroviral activity and suppresses a spontaneous cell-intrinsic antiviral response.* Cell Rep, 2013. **4**(4): p. 689-96.
81. Goldstone, D.C., et al., *HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase.* Nature, 2011. **480**(7377): p. 379-82.
82. Powell, R.D., et al., *Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase.* J Biol Chem, 2011. **286**(51): p. 43596-600.
83. White, T.E., et al., *The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation.* Cell Host Microbe, 2013. **13**(4): p. 441-51.
84. Welbourn, S., et al., *Restriction of virus infection but not catalytic dNTPase activity is regulated by phosphorylation of SAMHD1.* J Virol, 2013. **87**(21): p. 11516-24.
85. Hofmann, H., et al., *Inhibition of CUL4A Neddylation causes a reversible block to SAMHD1-mediated restriction of HIV-1.* J Virol, 2013. **87**(21): p. 11741-50.
86. Ryoo, J., et al., *The ribonuclease activity of SAMHD1 is required for HIV-1 restriction.* Nat Med, 2014. **20**(8): p. 936-41.
87. Tristem, M., A. Purvis, and D.L. Quicke, *Complex evolutionary history of primate lentiviral vpr genes.* Virology, 1998. **240**(2): p. 232-7.
88. Sharova, N., et al., *Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction.* PLoS Pathog, 2008. **4**(5): p. e1000057.
89. Schule, S., et al., *Restriction of HIV-1 replication in monocytes is abolished by Vpx of SIVsmmPBj.* PLoS One, 2009. **4**(9): p. e7098.
90. Lim, E.S., et al., *The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx.* Cell Host Microbe, 2012. **11**(2): p. 194-204.
91. Bartz, S.R., M.E. Rogel, and M. Emerman, *Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control.* J Virol, 1996. **70**(4): p. 2324-31.
92. He, J., et al., *Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity.* J Virol, 1995. **69**(11): p. 6705-11.
93. Jowett, J.B., et al., *The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle.* J Virol, 1995. **69**(10): p. 6304-13.
94. Re, F., et al., *Human immunodeficiency virus type 1 Vpr arrests the cell cycle in G2 by inhibiting the activation of p34cdc2-cyclin B.* J Virol, 1995. **69**(11): p. 6859-64.
95. Rogel, M.E., L.I. Wu, and M. Emerman, *The human immunodeficiency virus type 1 vpr gene prevents cell proliferation during chronic infection.* J Virol, 1995. **69**(2): p. 882-8.
96. Tristem, M., et al., *Origin of vpx in lentiviruses.* Nature, 1990. **347**(6291): p. 341-2.
97. Sharp, P.M., et al., *Gene acquisition in HIV and SIV.* Nature, 1996. **383**(6601): p. 586-7.
98. Compton, A.A., V.M. Hirsch, and M. Emerman, *The host restriction factor APOBEC3G and retroviral Vif protein coevolve due to ongoing genetic conflict.* Cell Host Microbe, 2012. **11**(1): p. 91-8.

99. Krupp, A., et al., *APOBEC3G polymorphism as a selective barrier to cross-species transmission and emergence of pathogenic SIV and AIDS in a primate host.* PLoS Pathog, 2013. **9**(10): p. e1003641.
100. Gotz, N., et al., *Reacquisition of Nef-mediated tetherin antagonism in a single in vivo passage of HIV-1 through its original chimpanzee host.* Cell Host Microbe, 2012. **12**(3): p. 373-80.
101. Wu, F., et al., *TRIM5 alpha drives SIVsmm evolution in rhesus macaques.* PLoS Pathog, 2013. **9**(8): p. e1003577.
102. Fregoso, O.I., et al., *Evolutionary Toggling of Vpx/Vpr Specificity Results in Divergent Recognition of the Restriction Factor SAMHD1.* PLoS Pathog, 2013. **9**(7): p. e1003496.
103. Kluge, S.F., et al., *Nef proteins of epidemic HIV-1 group O strains antagonize human tetherin.* Cell Host Microbe, 2014. **16**(5): p. 639-50.
104. Bour, S., et al., *The envelope glycoprotein of human immunodeficiency virus type 2 enhances viral particle release: a Vpu-like factor?* J Virol, 1996. **70**(2): p. 820-9.
105. Hauser, H., et al., *HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment.* Retrovirology, 2010. **7**: p. 51.
106. Serra-Moreno, R., et al., *Compensatory changes in the cytoplasmic tail of gp41 confer resistance to tetherin/BST-2 in a pathogenic nef-deleted SIV.* Cell Host Microbe, 2011. **9**(1): p. 46-57.
107. Prince, A.M., et al., *Lack of evidence for HIV type 1-related SIVcpz infection in captive and wild chimpanzees (Pan troglodytes verus) in West Africa.* AIDS Res Hum Retroviruses, 2002. **18**(9): p. 657-60.
108. Van Heuverswyn, F., et al., *Genetic diversity and phylogeographic clustering of SIVcpzPtt in wild chimpanzees in Cameroon.* Virology, 2007. **368**(1): p. 155-71.
109. Neel, C., et al., *Molecular epidemiology of simian immunodeficiency virus infection in wild-living gorillas.* J Virol, 2010. **84**(3): p. 1464-76.
110. Etienne, L., et al., *Gene Loss and Adaptation to Hominids Underlie the Ancient Origin of HIV-1.* Cell Host Microbe, 2013. **14**(1): p. 85-92.
111. Hirsch, V.M., et al., *Vpx is required for dissemination and pathogenesis of SIV(SM) PBj: evidence of macrophage-dependent viral amplification.* Nat Med, 1998. **4**(12): p. 1401-8.
112. Lenzi, G.M., et al., *Kinetic variations between reverse transcriptases of viral protein X coding and noncoding lentiviruses.* Retrovirology, 2014. **11**(1): p. 111.
113. Laguette, N., et al., *Premature activation of the SLX4 complex by Vpr promotes G2/M arrest and escape from innate immune sensing.* Cell, 2014. **156**(1-2): p. 134-45.
114. Blondot, M.L., et al., *How SLX4 cuts through the mystery of HIV-1 Vpr-mediated cell cycle arrest.* Retrovirology, 2014. **11**(1): p. 117.
115. Lemey, P., A. Rambaut, and O.G. Pybus, *HIV evolutionary dynamics within and among hosts.* AIDS Rev, 2006. **8**(3): p. 125-40.
116. Holmes, E.C., *Error thresholds and the constraints to RNA virus evolution.* Trends Microbiol, 2003. **11**(12): p. 543-6.
117. Parkin, N.T., M. Chamorro, and H.E. Varmus, *Human immunodeficiency virus type 1 gag-pol frameshifting is dependent on downstream mRNA secondary structure: demonstration by expression in vivo.* J Virol, 1992. **66**(8): p. 5147-51.

118. Pollard, V.W. and M.H. Malim, *The HIV-1 Rev protein.* Annu Rev Microbiol, 1998. **52**: p. 491-532.
119. Schwartz, O., et al., *Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein.* Nat Med, 1996. **2**(3): p. 338-42.
120. Garcia, J.V. and A.D. Miller, *Serine phosphorylation-independent downregulation of cell-surface CD4 by nef.* Nature, 1991. **350**(6318): p. 508-11.
121. Planelles, V., et al., *Vpr-induced cell cycle arrest is conserved among primate lentiviruses.* J Virol, 1996. **70**(4): p. 2516-24.
122. Stivahtis, G.L., et al., *Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology.* J Virol, 1997. **71**(6): p. 4331-8.
123. Berger, G., et al., *G2/M cell cycle arrest correlates with primate lentiviral Vpr interaction with the SLX4 complex.* J Virol, 2015. **89**(1): p. 230-40.
124. Guenzel, C.A., C. Herate, and S. Benichou, *HIV-1 Vpr-a still "enigmatic multitasker".* Front Microbiol, 2014. **5**: p. 127.
125. Sharifi, H.J., A.M. Furuya, and C.M. de Noronha, *The role of HIV-1 Vpr in promoting the infection of nondividing cells and in cell cycle arrest.* Curr Opin HIV AIDS, 2012. **7**(2): p. 187-94.
126. Ma, D., et al., *SIVagm infection in wild African green monkeys from South Africa: epidemiology, natural history, and evolutionary considerations.* PLoS Pathog, 2013. **9**(1): p. e1003011.
127. Gottlinger, H.G., et al., *Effect of mutations affecting the p6 gag protein on human immunodeficiency virus particle release.* Proc Natl Acad Sci U S A, 1991. **88**(8): p. 3195-9.
128. Garrus, J.E., et al., *Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding.* Cell, 2001. **107**(1): p. 55-65.
129. Newman, R.M., et al., *Balancing selection and the evolution of functional polymorphism in Old World monkey TRIM5alpha.* Proc Natl Acad Sci U S A, 2006. **103**(50): p. 19134-9.
130. King, R.C.S., W.D. Mulligan, P.K., *A Dictionary of Genetics*. Seven ed. 2006: Oxford University Press.
131. Wolfhiem, J., *Primates of the World: Distribution, Abundance, and Conservation*. 1998, Seattle: University of Washington Press.
132. Perelman, P., et al., *A molecular phylogeny of living primates.* PLoS Genet, 2011. **7**(3): p. e1001342.
133. Allan, J.S., et al., *Species-specific diversity among simian immunodeficiency viruses from African green monkeys.* J Virol, 1991. **65**(6): p. 2816-28.
134. Poss, M. and J. Overbaugh, *Variants from the diverse virus population identified at seroconversion of a clade A human immunodeficiency virus type 1-infected woman have distinct biological properties.* J Virol, 1999. **73**(7): p. 5255-64.
135. Regier, D.A. and R.C. Desrosiers, *The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus.* AIDS Res Hum Retroviruses, 1990. **6**(11): p. 1221-31.

136. Goldstein, S., et al., *Comparison of simian immunodeficiency virus SIVagmVer replication and CD4+ T-cell dynamics in vervet and sabaeus African green monkeys.* J Virol, 2006. **80**(10): p. 4868-77.
137. Sunseri, N., et al., *Human immunodeficiency virus type 1 modified to package Simian immunodeficiency virus Vpx efficiently infects macrophages and dendritic cells.* J Virol, 2011. **85**(13): p. 6263-74.
138. Edgar, R.C., *MUSCLE: multiple sequence alignment with high accuracy and high throughput.* Nucleic Acids Res, 2004. **32**(5): p. 1792-7.
139. Johnson, W.E., *Rapid adversarial co-evolution of viruses and cellular restriction factors.* Curr Top Microbiol Immunol, 2013. **371**: p. 123-51.
140. Cribier, A., et al., *Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1.* Cell Rep, 2013. **3**(4): p. 1036-43.
141. Laguette, N., et al., *Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein.* Cell Host Microbe, 2012. **11**(2): p. 205-17.
142. Belshan, M., et al., *Vpx is critical for SIVmne infection of pigtail macaques.* Retrovirology, 2012. **9**: p. 32.
143. Gibbs, J.S., et al., *Progression to AIDS in the absence of a gene for vpr or vpx.* J Virol, 1995. **69**(4): p. 2378-83.
144. VanderKuyl, A.C., J.T. Dekker, and J. Goudsmit, *St Kitts green monkeys originate from West Africa: Genetic evidence from feces.* American Journal of Primatology, 1996. **40**(4): p. 361-364.
145. Delport, W., et al., *Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology.* Bioinformatics, 2010. **26**(19): p. 2455-7.
146. Pupko, T., et al., *A fast algorithm for joint reconstruction of ancestral amino acid sequences.* Mol Biol Evol, 2000. **17**(6): p. 890-6.
147. Yan, J., et al., *Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection.* J Biol Chem, 2013. **288**(15): p. 10406-17.
148. Lahouassa, H., et al., *SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates.* Nat Immunol, 2012. **13**(3): p. 223-8.
149. Beloglazova, N., et al., *Nuclease activity of the human SAMHD1 protein implicated in the Aicardi-Goutieres syndrome and HIV-1 restriction.* J Biol Chem, 2013. **288**(12): p. 8101-10.
150. Ahn, J., et al., *HIV/simian immunodeficiency virus (SIV) accessory virulence factor Vpx loads the host cell restriction factor SAMHD1 onto the E3 ubiquitin ligase complex CRL4DCAF1.* J Biol Chem, 2012. **287**(15): p. 12550-8.
151. Srivastava, S., et al., *Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection.* PLoS Pathog, 2008. **4**(5): p. e1000059.
152. Zhang, Y., et al., *Arabidopsis DDB1-CUL4 ASSOCIATED FACTOR1 forms a nuclear E3 ubiquitin ligase with DDB1 and CUL4 that is involved in multiple plant developmental processes.* Plant Cell, 2008. **20**(6): p. 1437-55.

153. Kim, B., et al., *Tight interplay among SAMHD1 protein level, cellular dNTP levels, and HIV-1 proviral DNA synthesis kinetics in human primary monocyte-derived macrophages.* J Biol Chem, 2012. **287**(26): p. 21570-4.
154. Courgnaud, M.P.a.V., *Overview of primate lentiviruses and their evolution in nonhuman primates in Africa.* HIV Sequence Compedium, 2002: p. 2-23.
155. Cohen, E.A., et al., *Human immunodeficiency virus vpr product is a virion-associated regulatory protein.* J Virol, 1990. **64**(6): p. 3097-9.
156. Yuan, X., et al., *Human immunodeficiency virus vpr gene encodes a virion-associated protein.* AIDS Res Hum Retroviruses, 1990. **6**(11): p. 1265-71.
157. Yu, X.F., et al., *A naturally immunogenic virion-associated protein specific for HIV-2 and SIV.* Nature, 1988. **335**(6187): p. 262-5.
158. Fletcher, T.M., 3rd, et al., *Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIV(SM).* EMBO J, 1996. **15**(22): p. 6155-65.
159. DePaula-Silva, A.B., et al., *Determinants for degradation of SAMHD1, Mus81 and induction of G2 arrest in HIV-1 Vpr and SIVagm Vpr.* Virology, 2015. **477**: p. 10-7.
160. Morellet, N., et al., *NMR structure of the HIV-1 regulatory protein VPR.* J Mol Biol, 2003. **327**(1): p. 215-27.
161. Belzile, J.P., et al., *HIV-1 Vpr-mediated G2 arrest involves the DDB1-CUL4AVPRBP E3 ubiquitin ligase.* PLoS Pathog, 2007. **3**(7): p. e85.
162. DeHart, J.L., et al., *HIV-1 Vpr activates the G2 checkpoint through manipulation of the ubiquitin proteasome system.* Virol J, 2007. **4**: p. 57.
163. Hrecka, K., et al., *Lentiviral Vpr usurps Cul4-DDB1[VprBP] E3 ubiquitin ligase to modulate cell cycle.* Proc Natl Acad Sci U S A, 2007. **104**(28): p. 11778-83.
164. Schrofelbauer, B., Y. Hakata, and N.R. Landau, *HIV-1 Vpr function is mediated by interaction with the damage-specific DNA-binding protein DDB1.* Proc Natl Acad Sci U S A, 2007. **104**(10): p. 4130-5.
165. Wen, X., et al., *The HIV1 protein Vpr acts to promote G2 cell cycle arrest by engaging a DDB1 and Cullin4A-containing ubiquitin ligase complex using VprBP/DCAF1 as an adaptor.* J Biol Chem, 2007. **282**(37): p. 27046-57.
166. Le Rouzic, E., et al., *HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase.* Cell Cycle, 2007. **6**(2): p. 182-8.
167. Schwefel, D., et al., *Structural basis of lentiviral subversion of a cellular protein degradation pathway.* Nature, 2014. **505**(7482): p. 234-8.
168. Cassiday, P.A., et al., *Understanding the molecular manipulation of DCAF1 by the lentiviral accessory proteins Vpr and Vpx.* Virology, 2015. **476**: p. 19-25.
169. Biasini, M., et al., *SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information.* Nucleic Acids Res, 2014. **42**(Web Server issue): p. W252-8.
170. Goujon, C., et al., *Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 Vpx function in human myeloid cells.* J Virol, 2008. **82**(24): p. 12335-45.
171. Horton, R., P. Spearman, and L. Ratner, *HIV-2 viral protein X association with the GAG p27 capsid protein.* Virology, 1994. **199**(2): p. 453-7.

172. Wyma, D.J., et al., *Coupling of human immunodeficiency virus type 1 fusion to virion maturation: a novel role of the gp41 cytoplasmic tail.* J Virol, 2004. **78**(7): p. 3429-35.
173. DeLucia, M., et al., *HIV-2 and SIVmac accessory virulence factor Vpx down-regulates SAMHD1 enzyme catalysis prior to proteasome-dependent degradation.* J Biol Chem, 2013. **288**(26): p. 19116-26.
174. Schwefel, D.B., V. Chritodoulou , E. Walker, P. Stoye, J. Bishop, K. Taylor, I., *Molecular determinants for divergent restriction factor recognition by the lentiviral accessory protein Vpx.* Cell Host Microbe, 2015.
175. Jauregui, P., et al., *Degradation of SAMHD1 by Vpx is Independent of Uncoating.* J Virol, 2015.
176. Aghokeng, A.F., et al., *Widely varying SIV prevalence rates in naturally infected primate species from Cameroon.* Virology, 2006. **345**(1): p. 174-89.
177. Pertel, T., C. Reinhard, and J. Luban, *Vpx rescues HIV-1 transduction of dendritic cells from the antiviral state established by type 1 interferon.* Retrovirology, 2011. **8**: p. 49.
178. Dewhurst, S., et al., *Sequence analysis and acute pathogenicity of molecularly cloned SIVSMM-PBj14.* Nature, 1990. **345**(6276): p. 636-40.
179. Coleman, C.M., C.S. Gelais, and L. Wu, *Cellular and viral mechanisms of HIV-1 transmission mediated by dendritic cells.* Adv Exp Med Biol, 2013. **762**: p. 109-30.
180. Wu, L. and V.N. KewalRamani, *Dendritic-cell interactions with HIV: infection and viral dissemination.* Nat Rev Immunol, 2006. **6**(11): p. 859-68.
181. Miller, C.J. and R.J. Shattock, *Target cells in vaginal HIV transmission.* Microbes Infect, 2003. **5**(1): p. 59-67.
182. Pandrea, I., et al., *Mucosal simian immunodeficiency virus transmission in African green monkeys: susceptibility to infection is proportional to target cell availability at mucosal sites.* J Virol, 2012. **86**(8): p. 4158-68.
183. Stieh, D.J., et al., *Vaginal challenge with an SIV-based dual reporter system reveals that infection can occur throughout the upper and lower female reproductive tract.* PLoS Pathog, 2014. **10**(10): p. e1004440.
184. Rinaldo, C.R., *HIV-1 Trans Infection of CD4(+) T Cells by Professional Antigen Presenting Cells.* Scientifica (Cairo), 2013. **2013**: p. 164203.
185. Spragg, C.J. and M. Emerman, *Antagonism of SAMHD1 is actively maintained in natural infections of simian immunodeficiency virus.* Proc Natl Acad Sci U S A, 2013. **110**(52): p. 21136-41.
186. Zhu, C., et al., *Structural insight into dGTP-dependent activation of tetrameric SAMHD1 deoxynucleoside triphosphate triphosphohydrolase.* Nat Commun, 2013. **4**: p. 2722.
187. Souquiere, S., et al., *Simian immunodeficiency virus types 1 and 2 (SIV mnd 1 and 2) have different pathogenic potentials in rhesus macaques upon experimental cross-species transmission.* J Gen Virol, 2009. **90**(Pt 2): p. 488-99.
188. Luban, J., *Innate immune sensing of HIV-1 by dendritic cells.* Cell Host Microbe, 2012. **12**(4): p. 408-18.
189. Manel, N., et al., *A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells.* Nature, 2010. **467**(7312): p. 214-7.
190. Duvall, M.G., et al., *Polyfunctional T cell responses are a hallmark of HIV-2 infection.* Eur J Immunol, 2008. **38**(2): p. 350-63.

191. Doitsh, G., et al., *Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection.* Nature, 2014. **505**(7484): p. 509-14.
192. Lamkanfi, M. and V.M. Dixit, *Manipulation of host cell death pathways during microbial infections.* Cell Host Microbe, 2010. **8**(1): p. 44-54.
193. Fink, S.L. and B.T. Cookson, *Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells.* Infect Immun, 2005. **73**(4): p. 1907-16.
194. Eckstein, D.A., et al., *HIV-1 actively replicates in naive CD4(+) T cells residing within human lymphoid tissues.* Immunity, 2001. **15**(4): p. 671-82.

CURRICULUM VITAE

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EDUCATION:

University of Washington
Ph.D. in Molecular and Cellular Biology — May 2015
National Science Foundation Graduate Research Fellowship
NIH Viral Pathogenesis Training Grant
GPA 3.7

Emory University
B.S. in Biology — Aug 2006-Dec 2009
GPA 3.96
John Emory Scholarship
Phi Beta Kappa

RESEARCH EXPERIENCE:

Graduate student in the laboratory of Dr. Michael Emerman — Aug 2010 - present
Fred Hutchinson Cancer Research Center, Seattle, WA

My work in the Emerman lab has focused on the evolution of the lentiviral accessory proteins Vpx and Vpr and their molecular interactions with SAMHD1, a host antiviral protein that inhibits infection of dendritic cells, macrophages, and resting CD4+ T cells.

- **Project 1**: I identified extensive polymorphism in the restriction factor SAMHD1 in four species of African green monkeys naturally harboring Simian immunodeficiency virus infections. I determined that these polymorphisms make SAMHD1 resistant to antagonism by heterologous SIVagm Vpr, though SAMHD1 variants are always sensitive to autologous Vpr. I concluded that in natural infections of primate hosts, there is selective pressure for lentiviruses to maintain the ability to antagonize SAMHD1. This work is published in Proceedings of the National Academy of Sciences.

- **Project 2**: I have used a structural and evolutionary approach to create functional, chimeric Vpx proteins and designed a lentiviral construct that will package these proteins into virions. I have used these chimeras as a tool to map the amino acids responsible for SAMHD1 antagonism in several highly divergent Vpr/x proteins. I plan to submit this work for publication before graduation.

Techniques and skills:
Tissue culture: lentiviral production, viral titering, T cell infections
Molecular biology: cloning, PCR, RT-PCR, SDM, Western blotting
Phylogenetic analysis
ELISA
Flow cytometry (basics)
Student mentoring
Presentation skills

UNDERGRAD RESEARCH EXPERIENCE

Gerardo lab, Population Biology, Evolution, and Ecology, Emory University, Aug 08 - Dec 09

Pea aphids are hosts to an array of bacterial symbionts that offer benefits such as protection from heat stress and natural enemies. I developed the lab protocol to infect aphids with *Zoopthera occidentalis* fungus, and I found that the symbiont *Regiella insecticola* confers protection to this aphid specific fungus. This work was continued by a graduate student and published in Applied and Environmental Biology. My protocol was also used in an immune gene expression study done by a postdoc in the lab.

Marcus lab, Hematology and Oncology, Emory University Winship Cancer Institute Apr 09 – Dec 09

I used live cell confocal microscopy to study how the cytoskeleton protein vimentin contributed to the ability of cells to become motile. I found that the vimentin filament structure broke down and rebuilt when cells transitioned from an immobile state sitting on a plate to a migrating state moving across it. I found that in motile cells, filaments move faster and are more directly targeted to the leading edge of the cell.

PRESENTATIONS

Conference on Retroviruses and Opportunistic Infections Boston, 2014
Oral Presentation
Selection for Active SAMHD1 Antagonism in Natural Infections of SIVagm

West Coast Retrovirus Meeting Palm Springs, 2013
Oral presentation
Coevolution of the restriction factor SAMHD1 and the viral antagonist Vpr
Awarded for best talk by a graduate student

Conference on Retroviruses and Opportunistic Infections Seattle, 2012
Poster presentation

Characterizing the role of a novel transport factor in the life cycle of HIV-1

PUBLICATIONS

Spragg, CJ and Emerman, M. (2013). Antagonism of SAMHD1 is actively maintained in natural infections of Simian immunodeficiency virus. Proc Natl Acad Sci U S A. 110(52):21136-41

Parker BJ, Spragg CJ, Altincicek B, Gerardo NM. (2013). Symbiont-mediated protection against fungal pathogens in pea aphids: a role for pathogen specificity? Appl Environ Microbiol. 79(7):2455-8

Gerardo, N. et al. (2010). Immunity and other defenses in pea aphids, Acyrthosiphon pisum. Genome Biology 11(2):R21

The International Aphid Genomics Consortium. (2010). The genome of the pea aphid, Acyrthosiphon pisum. PLOS Biology 8(2):e1000313

MENTORING AND TEACHING EXPERIENCE

Research Mentor for Undergraduate Student Jun 2012-Aug 2013

Served as student mentor, designed project, and taught basic laboratory techniques

- The student mapped the amino acid responsible for differential SAMHD1 degradation between two highly similar Vpr proteins
- She gave a presentation at the University of Washington Undergraduate Research Symposium

Research Mentor for Undergraduate Student Jun-Sep 2014

Served as student mentor, designed project, and taught basic laboratory techniques

- The student mapped SAMHD1 protein degradation to one amino acid in SIVagm Vpr

Teaching Assistant for Introductory Biology Lecture and Laboratory Spring 2012

Bio200 under Dr. Jennifer Neuhauser and Dr. Mark Cooper, University of Washington
Taught a 24 student lab section twice a week and assisted in lecture class

Teaching assistant for Introductory Biology lecture course Aug 2007-May 2008

Biology 141 and 142 under Dr. Rachelle Spell, Emory University
Assisted in lecture course and led a weekly 2 hour discussion and review session

SCIENTIFIC OUTREACH

Presenter coordinator for Seattle Expanding Your Horizons Oct 2011 – present

Responsible for recruiting and organizing 40+ individuals working in STEM fields to present workshops at the annual SEYH conference, which aims to engage over 400 middle school girls in science, math, engineering, and technology.

References

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