A review of HIV restriction factors and viral countering mechanisms

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Abstract

Human immune system is powerful and it has evolved over the past thousands of years to protect us from various foreign pathogens. In fact, very few pathogens can threaten a man with a competent immune system. The notorious Human Immunodeficiency Virus may be among those very few pathogens as it attacks human immune system; however, it does not mean men are left utterly weaponless in the face of HIV infection. The adaptive arm of the human immune system has historically received more attention, given that most vaccines to viral pathogens are based on this type of immune response. In the context of HIV infection, however, attempts to induce antibody responses or cell-mediated immunity with vaccine have yielded little success. As a result, research resource has shifted to look at the first-line protection that precedes the adaptive immunity. Restriction factors are among this innate arm of the immune system. Since 2002, many restriction factors have been described, many in the context of their antiretroviral activities. In this thesis essay, I attempted to cover some of the best-studied HIV restriction factors to date, including their potential antiviral mechanisms and how virus has developed ways to circumvent their inhibition effects. Many restriction factors are now on the verge of being translated into clinical products, so I tried to include some of the latest translational applications of restriction factors in this article. A common theme for most restriction factors is a constant "arm race" between the virus and the host, and therefore, in the end, I have included a short discussion on how, in a human perspective, men managed to stay on the battlefield with the ever-mutating HIV virus.

Primary Reader: Richard Markham, M.D.

Secondary Reader: Gary Ketner, Ph.D., Xiao-Fang Yu, M.D., D.Sc.

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I. Introduction

Acquired Immune Deficiency Syndrome (AIDS) was first described as a new disease in 1981 among a group of young homosexual men. Those young individuals became sick and would soon die of either opportunistic infections or rare malignancies not commonly seen in immune competent people[1]. The etiological agent of AIDS was successfully isolated in 1985[2] and the virus was named Human Immunodeficiency Virus (HIV) shortly after[3].

In the following three decades, the AIDS pandemic has caused more fatalities than any other epidemic in human history. Since the start of the AIDS pandemic, approximately 75 million people have become infected with HIV globally and by 2012, AIDS-related illnesses have claimed over 36 million lives[4]. HIV/AIDS is also predominantly a disease of the poor, disproportionately affecting the world's "bottom billion". Currently seventy percent of the people chronically infected with the HIV virus call the Sub-Saharan Africa region home, where a majority of new HIV infection cases take place each year[4].

Despite of the extensive damage HIV/AIDS has done, our understanding of HIV has come a long way during the past three decades. HIV is among the most well studied viruses within the scientific community and we now have a relatively clear picture of HIV's viral life cycle. The most direct and tangible results from our study of the HIV virus are the discovery of a variety of antiretroviral drugs, targeting an array of viral life stages. The development of Highly Active Antiretroviral Therapy (HAART)[5] [6], employing simultaneous use of multiple antiretroviral agents, can effectively suppress viral replication and minimize the chance of viral drug resistance.

HIV infection has, for individuals who can afford these medications, become a chronic condition resembling asthma and diabetes.

However, HAART does not eradicate HIV virus from one's body and therefore does not provide a cure. With our current knowledge of the HIV latency[7] and the recent failures of HIV vaccine trials[8] [9], it becomes obvious that more basic research needs to be done to help us understand how the virus interacts with the host immune system during infection. Much research effort has been focusing on two levels of viral-host interaction—broadly defined innate immunity at the cellular level involving various restriction factors[10] and adaptive immunity at the organism level involving broadly neutralizing antibodies[11]. In this essay, I am going to review the recent findings on host restriction factors and corresponding viral evasion mechanisms, followed by a discussion on the potential implications for future HIV research.

II. Restriction Factors

Mammalian cells express "a number of diverse, dominantly acting proteins that are widely expressed and function in a cell-autonomous manner to suppress virus replication" [10], These restriction factors, as they have been termed, act as the first line of defense against viral infection at the cellular level, as virus gains entry into the host cell and starts replication, and are thus considered part of innate immunity. Following the discovery of the first restriction factor in 2002[13], more than 30 different anti-HIV-1 host restriction factors have been described[12]. Some of the best-characterized HIV restriction factors include apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G (APOBEC3G)[13], Tetherin (also known as BST-2)[14], Tripartite motif-containing Motif 5 α (TRIM5α)[15] and Sterile Alpha Motif Histidine-Aspartic (HD) domain-containing protein 1 (SAMHD1)[16].

Although restriction factors target different steps of the viral life cycle, there are many shared features among them. They share the capacity to display potent antiviral function as a single gene and in general, they are germline-encoded, IFN-inducble, yet expressed constitutively at low level[10]. Although the majority of restriction factors discovered to date are retrovirus-specific, it is likely a reflection of the lack of research resources on other viruses. Many restrictions factors, such as MxA, which is active against a variety of viruses including Influenza[109] and PKR against poxvirus[17], have been described. Similar experimental approaches have been used to identify most of the restriction factors. For example, APOBEC3G, tetherin and the most recently discovered Mx2 were identified using comparative transcriptomics, which allows high throughput screening of genes that are preferentially expressed in restrictive cells compared to susceptible cells. Those genes

can later be validated if the ectopically expressed gene can transform susceptible cells into restrictive cells[13 14 18]. As in the case of TRIM5α, a more direct screening approach was utilized. A cDNA library derived from the restrictive cells were first created and expressed in the susceptible cells, followed by a selection for cells that acquire viral resistance phenotypes[15]. In contrast, SAMHD1 was uncovered by over-expressing a viral protein Vpx (only in HIV-2 and SIV) in restrictive cell lines and subsequently looking for a host protein that interacts with Vpx with Mass spectrometry[19] or a proteomic screen[16].

Unfortunately, the virus has also come up with mechanisms for counteracting the pressure imposed by restriction factors. In most cases, the viral evasion mechanisms are tied to viral accessory proteins—a group of proteins that play limited roles in HIV infection *in vitro* (in permissive cell lines) yet are absolutely essential for *in vivo* infection. Some viral accessory proteins, such as Vif and Vpx, are able to antagonize host restriction factors by simultaneously binding to the restriction factors and recruiting a cellular ubiquitin ligase complex, which results in the ubiquitylation and eventual proteasomal degradation of the restriction factors.

In the next five sections, I will review the four best-characterized restriction factors and the corresponding viral evasion mechanisms, as well as some other potentially important restrictions factors.

III. APOBEC3G and Vif

APOBEC3 family proteins, and most notably APOBEC3G, were the first discovered group of HIV restriction factors. Initially, the Malim group, among other research teams, found that the HIV accessory protein Vif is required for HIV replication in primary cell types, such as CD4+ cells, while a Vif-defective HIV strain is still able to effectively replicate in certain permissive cell lines, such as 293T cells[20]. It was strongly suspected that a certain host factor(s) was involved in determining the permissiveness of the host cells; however, the nature of this factor was not clear—either a host restriction factor could be limiting viral replication in nonpermissive cells or a host cofactor, present in permissive cells, facilitated viral replication.

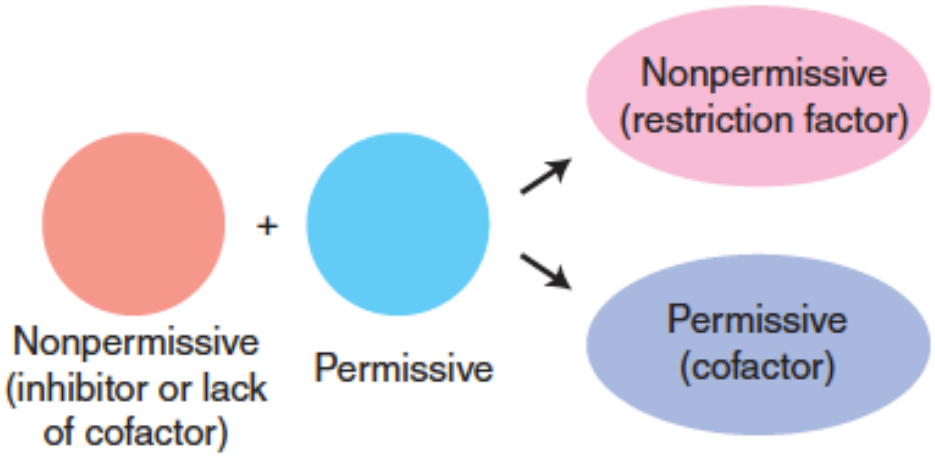

Figure 1. Cell fusion experiment can differentiate between a host restrictive inhibitor and a host cofactor that helps viral replication. Permissive/non-permissive phenotypes can be caused either by the absence/presence of a restriction factor or by the presence/absence of a host cofactor. After the cell fusion, if the permissiveness phenotype is associated with a restriction factor, the fused cell will get this restriction factor from the non-permissive cells, and thus become non-permissive. In contrast, if the permissiveness phenotype is associated with a host cofactor, the fused cell will inherit the cofactor from permissive cells and thus allowing the virus to replicate. Adapted from Malim and Bieniasz. *Cold Spring Harb. Perspect. Med*. 2012.

Subsequently, the Malim group preformed a brilliant series of cell fusion experiments (Figure 1) that confirmed that the permissiveness phenotype is due to a host restriction factor[19]. Not long after that, the same group identified a cellular gene *CEM15*[13]. The expression of CEM15 creates the non-permissiveness phenotype in 293T cells when infected with Vif-defective virus, but the antiviral effect CEM15 confers is overcome by the presence of Vif. A database search of the *CEM15* gene later showed significant similarities between CEM15 and the cytidine deaminase APOBEC1. As a result, CEM15 was named APOBEC3G later and the fact that it belongs to the APOBEC family also shed some light on the potential antiviral mechanism of APOBEC3G. With the successful identifications of host restriction factor APOBEC3G and its corresponding viral antagonist Vif, two major questions remain to be solved—how does APOBEC3G inhibit Vif-defective HIV replication and how does Vif antagonize APOBEC3G.

As mentioned previously, APOBEC3G belongs to the APOBEC family of proteins, which consists of 11 members in humans[21]. The first uncovered APOBEC protein, APOBEC1, was identified as the enzyme expressed in gastrointestinal tissues, where it edits apolipoprotein B mRNA post-transcriptionally to create a premature stop codon[22]. Another example of the APOBEC protein family is activation-induced deaminase (AID), which is expressed in B cells and known to be essential for IgG class switching and somatic hypermutation[23]. One thing in common for those two early members of the APOBEC family is that they all induce the deamination of cytidine and result in a cytidine (C) to uridine (U) conversion. Therefore, researchers suspected that APOBEC3G has similar enzymatic activities, especially since APOBEC3G contains two cytidine deaminase domains, and that turned out to be true. A number of studies done by different research teams found that the expression of

APOBEC3G in permissive cells infected by Vif-defective virus produces APOBEC3G-containing viral progeny[24 25 26]. Those progeny, after infecting new cells, yield reverse transcripts that contain disproportionately large numbers of guanosine (G) to adenosine (A) mutations on the positive strand DNA, often affecting over 10 percent of all guanosines on viral cDNA. Since most mutations are G to A as opposed to C to T, it is believed that APOBEC3G selectively targets the transiently exposed single strand DNA[26] (Figure 2).

	W/O APOBEC3G	With APOBEC3G (on - sense DNA)	With APOBEC3G (on + sense DNA)
+ sense RNA	UAGCGCUGAC	UAGCGCUGAC	UAGCGCUGAC
- sense DNA from	ATCGCGACTG	ATCGUGACTG	ATCGCGACTG
Double strand DNA	ATCGCGACTG- TAGCGCTGAC+	ATCGTGACTG- TAGCAC TGAC+	ATCGCAACTG- TAGCGTTGAC+
mRNA	UAGCGCUGAC	UAGCACUGAC	UAGCGUUGAC
Type of mutation	None	G to A	C to T

Figure 2. APOBEC3G targets transiently exposed single strand DNA. In most cases, C to U conversion takes place on the negative strand DNA as RNase digests away the initial RNA template. This will result in an overall G to A conversion of the viral cDNA. However, there are exceptions. During the later phases of reverse transcription, plus strand DNA of the U3 region of the 5'-LTR and the primer binding site are thought to be transiently exposed and thus could be vulnerable to APOBEC3G activity[27]. In those cases, APOBEC3G activity results in C to T mutations on cDNA.

Two additional characteristics associated with APOBEC3G activity were soon described in a series of studies: 1. APOBEC3G shows a strong preference for specific nucleotide sequences and the most favored site is the 5' -CCCA (the affected C underlined). Interestingly, a C to U conversion at this site on the negative sense DNA corresponds to a tryptophan (TGG) to stop codon (TAG) conversion on the mRNA,

and thus has a good chance of prematurely interrupting viral protein translation[23 26 28]; 2. It is shown that APOBEC3G induced mutations become more likely to occur, going from the 5' to 3' direction of the positive sense DNA[26 27]. This polarity of APOBEC3G-induced mutation has been attributed to the fact that the 5' end of the negative sense DNA is first reverse transcribed and thus exposed (to single-strand-DNA-targeting APOBEC3G) for a longer period of time than the 3' end[27].

By inducing hypermutations on the viral genome, APOBEC3G can effectively inhibit HIV infection via two pathways. Harris *et al*. showed that cellular DNA repair enzymes could potentially recognize uridine residues, leading to degradation of mutated reverse transcripts as a result of abortive repair of unpaired DNA strands[23]. Even if the APOBEC3G edited reverse transcripts can survive the host DNA repair mechanism, the resulting mutation burden is often sufficient to disrupt viral genetic integrity and suppress viral replication[22]. More recently, the Malim group has shown that APOBEC3G may also inhibit elongation of HIV-reverse transcripts via a hypermutation-independent mechanism, although the exact mechanism remains unclear[29]. That is not to say that APOBEC3G is the only protein in the family involved in antiretroviral activity. In fact, it was even suggested that the major restrictor of HIV-1 infection *in vivo* might be APOBEC3F, while APOBEC3G plays a key supporting role[29].

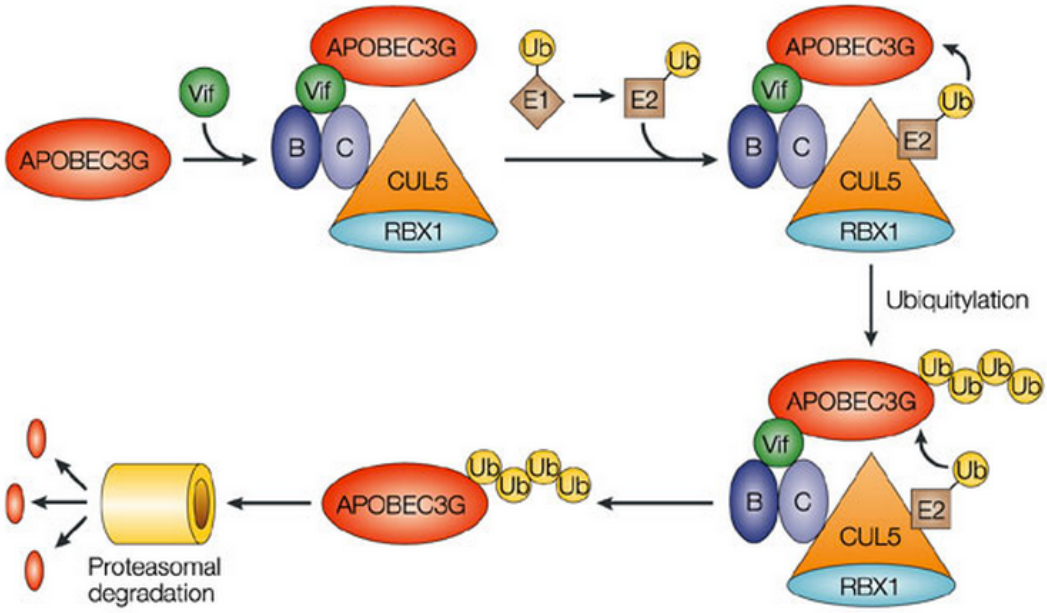

Figure 3. Vif binds APOBEC3G and forms an ubiquitin-ligase complex by recruiting elongin B (B in the figure), elongin C (C in the figure), Cul5 and RBX1. The next step is Cul5-RBX1-dependent ligation of ubiquitin to APOBEC3G by E2 enzyme. The E2-ubiquitin conjugating enzyme receives its ubiquitin from an E1 enzyme. Once APOBEC3G is tagged with poly-ubiquitin tail, it will be destined for proteasomal degradation. Adapted from Harris and Liddament. *Nat. Rev. Immunol.* 2004[30].

As mentioned above, APOBEC3G induces hypermutation on the HIV viral genome and by doing so, effectively inhibits viral replication. Therefore, it is necessary for the virus to develop counter measures. We have learned that Vif can antagonize APOBEC3G, based on previous experimental results that Vif can reverse the non-permissive phenotype conferred by APOBEC3G in a permissive cell line; however, Vif does not have any enzymatic activity and it is unlikely that Vif alone can prevent integration of APOBEC3G into HIV virions. Initially it was found that Vif could directly bind to APOBEC3G and somehow lead to proteosome-mediated degradation of APOBEC3G [31] [32] [33]. A detailed molecular mechanism was subsequently worked out by the Yu group in the same year [34]. Through immunoprecipitation assays followed by mass spectrometry, Vif was shown to recruit the cellular proteins cullin-5 (Cul5), elongin B, elongin C and Rbx1 to form a

ubiquitin-ligase complex. The complex allows Vif to bind APOBEC3G and induce ubiquitination and degradation of APOBEC3G (Figure 3). Similar results were later replicated by Kobayashi *et al.* in 2005[35]. Over the next several years, a number of studies were published that employed mutation analysis to map out the interaction domains among components of the Vif complex and APOBEC3G[36 37 38 39 40 41]. However, all attempts to obtain crystal structure data had not been successful. In 2011, two groups identified a cellular protein cofactor required for Vif function—CBFβ[42 43], which eventually led to the successful crystallization of Vif-Cul5-CBFβ-ElonginB-ElonginC[44] (Figure 4). The structural data, albeit lacking APOBEC3G, provides a structural basis for how Vif antagonizes APOBEC3G and offers many insights for future drug design.

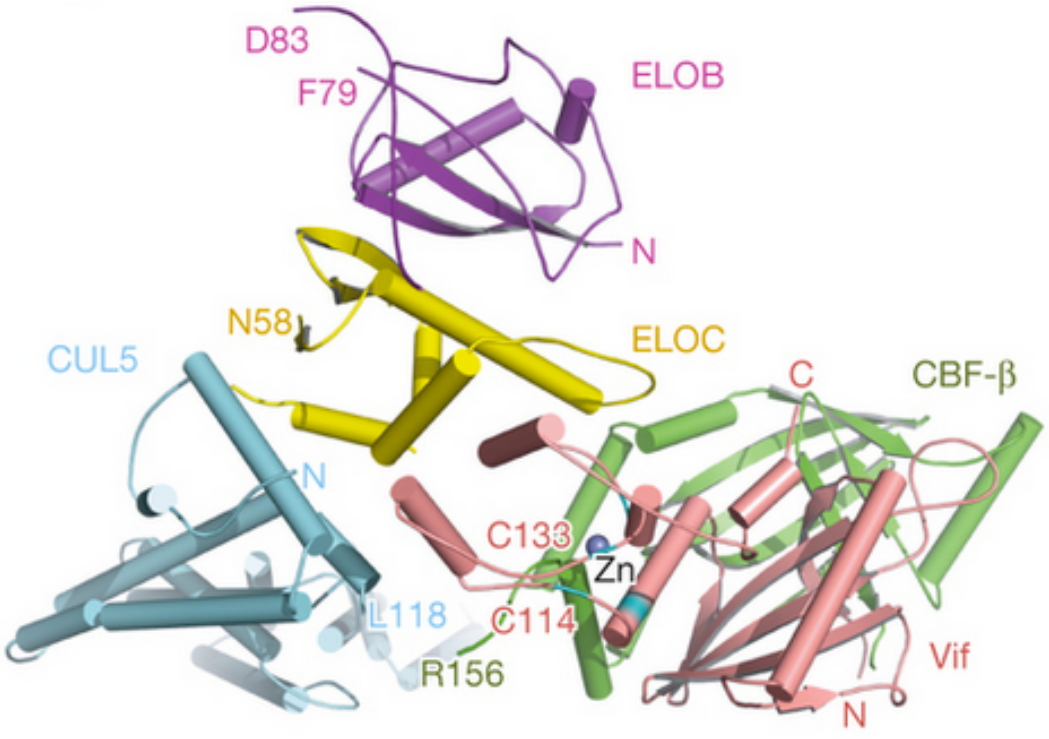

Figure 4. Overall structure of Vif-CBF-β-CUL5-ELOB-ELOC based on crystallography data. Vif interacts directly with ElonginB, Cul5 and CBFβ, offering multiple potential targets for antiviral drug design. Adapted from Guo *et al. Nature*. 2014.

In summary, APOBEC3G and Vif is the first discovered and best-studied pair of restriction factor and the associated viral evasion mechanism. APOBEC3G can suppress HIV replication in the absence of Vif by inducing hypermutation during reverse transcription, while HIV-1 Vif can counteract by inducing proteasomal degradation of APOBEC3G. There seems to a balance between the Vif activity and APOBEC proteins. Mutations may not be particularly bad for the virus at a sub-lethal level. After all, the pathogenicity of HIV-1 depends on its error-prone reverse transcriptase. Studies have shown that, during the course of a HIV infection, Vif does not antagonize APOBEC3 activity completely[13 25 26] and may even utilize the mutation-inducing power of APOBEC3 to generate more genetic diversity to circumvent the host immune system or antiretroviral drugs[23].

IV. TRIM5α

In 2004, TRIM5α was identified as a result of large-scale screen of rhesus macaque genes that could restrict HIV-1 replication when expressed in human cells[15]. Unlike APOBEC3G, tetherin and SAMHD1, TRIM5α is not known to be actively targeted by any viral accessory proteins. TRIM5α is 56kDa cytoplasmic protein that belongs to the family of TRIM proteins. All TRIM proteins are characterized by their tripartite motif (TRIM), which consists of RING, B-Box type 2 and coiled coil domains[45]. TRIM5α, in addition to the TRIM domain, possesses a C-terminal B30.2 or PRYSPRY domain[46]. Additionally, TRIM5α is ubiquitously expressed throughout the human body, including in T cells[47]. The expression level is generally low but can be upregulated by IFN[48]. Another unique feature that distinguishes TRIM5α from other restriction factors is that in general, endogenous TRIM5α proteins are poor inhibitors of retroviruses that are found in the same host species but can inhibit retroviruses infecting other species. For example, while TRIM5α from Rhesus macaques (rhTRIM5α) is a strong inhibitor of HIV-1, it does not restrict replication of the SIV strain commonly infecting Rhesus macaques, SIVmac[15]. Likewise, human TRIM5α (hTRIM5α) is a strong inhibitor of N-tropic murine leukemia virus (N-MLV) and equine infectious anemia virus (EIAV) but is essentially inactive against HIV-1[49 50]. It is also reported that TRIM5α can provide mild restriction of HIV-2, which may account for why HIV-2 is more difficult to transmit and less progressive than HIV-1[51].

TRIM5α is believed to act following the entry of the retroviral nucleocapsid into the cytoplasm of host cells and is responsible for the failure of viral cDNA synthesis[15]. The exact mechanism is not as well characterized as the other restriction

factors possibly due to the limitation of biochemical and biophysical techniques[10]. It is, nonetheless, clear that TRIM5α can bind directly to the HIV-1 capsid via its PRYSPRY domain[52]. Because previous study has shown that interaction between TRIM5α and capsid (CA) monomers is weak[53], the researchers soon recognized the importance of the coiled coil domain, because it drives the formation of TRIM5α dimers[45]. TRIM5α can then continue on to form hexamers from dimers, a process enhanced in the presence of incoming viral capsid and may involve the B-Box type 2 domain[54], and the high-avidity binding to CA conferred by the hexamers was later shown to be essential for efficient restriction[55]. However, aside from the binding of the TRIM5α multimer to capsid in a polyvalent manner, no other activities associated with TRIM5α have been shown to be absolutely necessary for its restriction effect on HIV. The current consensus among the scientific community is that TRIM5α may be capable of suppressing viral replication via at least two pathways[10], a redundancy that is commonly encountered among pathways of human innate immunity (Figure 5). The RING domain of TRIM5α proteins possesses E3 ubiquitin-ligase activity and therefore, the initial postulate was that by binding to CA in the cytoplasm, TRIM5α could lead to accelerated and disrupted uncoating of the virus[48 51 56]. Experimental results further suggested that TRIM5α's RING and B-Box type 2 domains allow the TRIM5α-virus complex to autoubiquitinate and undergo proteasomal degradation[57].

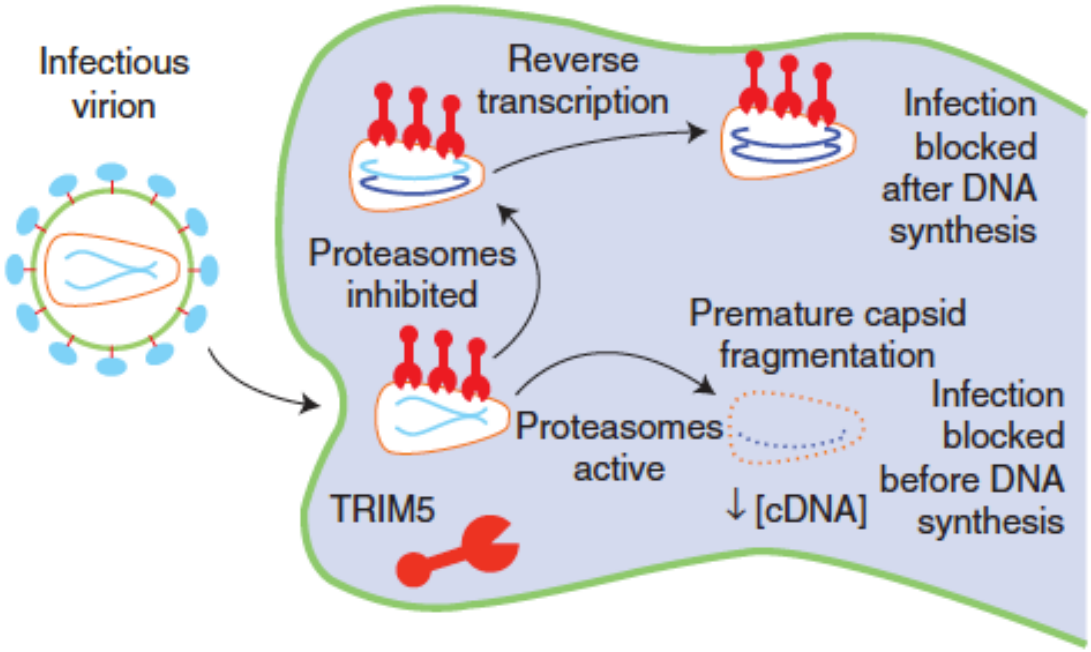

Figure 5. Two pathways that may be responsible for TRIM5α's restriction activity upon binding to viral capsid, one proteasome-dependent and the other proteasome-independent. Refer to the text for further details. Adapted from Malim and Bieniasz. *Cold Spring Harb. Perspect. Med*. 2012.

Because the reverse transcription has to take place in the capsid environment, premature capsid fragmentation induced by TRIM5α will presumably lead to early termination of the viral life cycle. However, it wasn't long before researchers realized that TRIM5α could even suppress HIV-1 replication in the presence of proteasome inhibitors or in cell lines deprived of active ubiquitin activating E1 enzymes[58]. Inhibiting proteasome activity can protect viral the nucleocapsid from premature disassembly and restore viral reverse transcript level, yet cannot reinstitute infectivity[59]. In addition to directly inhibit HIV-1 replication in cells, TRIM5α may also be involved in the activation of NFκB signaling and innate immune responses[60]; however, more research remains to be done in that area.

That a particular TRIM5α can selectively inhibit certain retroviruses but not others is most likely the result of virus-host co-evolution. Presumably, the host-specific TRIM5α is a hurdle that a retrovirus has to overcome when crossing host species barriers. Most species-specific differences in TRIM5α's restrictive capabilities are attributable to the CA sequence variation among retroviruses and host-specific TRIM5α's ability to recognize those sequences, predominantly in the PRYSPRY domain. Researchers have shown that either substitution of hTRIM5α PRYSPRY with rhTRIM5α sequence or a single amino acid change at the 322 position of hTRIM5α is sufficient to activate restriction of HIV-1[61 62]. There are even TRIM5α polymorphisms within species. Preliminary studies on human TRIM5α polymorphisms have shown reduced HIV-1 infection susceptibility among individuals with high TRIM5α levels[63] and accelerated disease progression among individuals with the homozygous 43Y TRIM5α genotype compared to 43H heterozygotes and homozygotes[64]. It is, however, unclear if TRIM5α will become an ideal candidate for HIV prophylaxis. Although HIV-1 lacks an accessory protein that is able to counteract the restrictive effects of TRIM5α, it is obvious that potential mutations on CA may quickly render TRIM5α ineffective since a similar mutation event must have taken place when SIV crossed the host species barrier to infect humans.

V. Tetherin and Vpu

The third addition to the restriction factor family is tetherin. Initially, studies indicated that the Vpu accessory protein was required for virion release from some cell lines but not the others[65], and the absence of Vpu rendered HIV-1 sensitive to an IFN-α-induced tetherining mechanism that trapped viral progeny on the surface of the infected cells[66 67]. Finally, tetherin was identified to be the protein responsible for this previously described tethering mechanism[14 68].

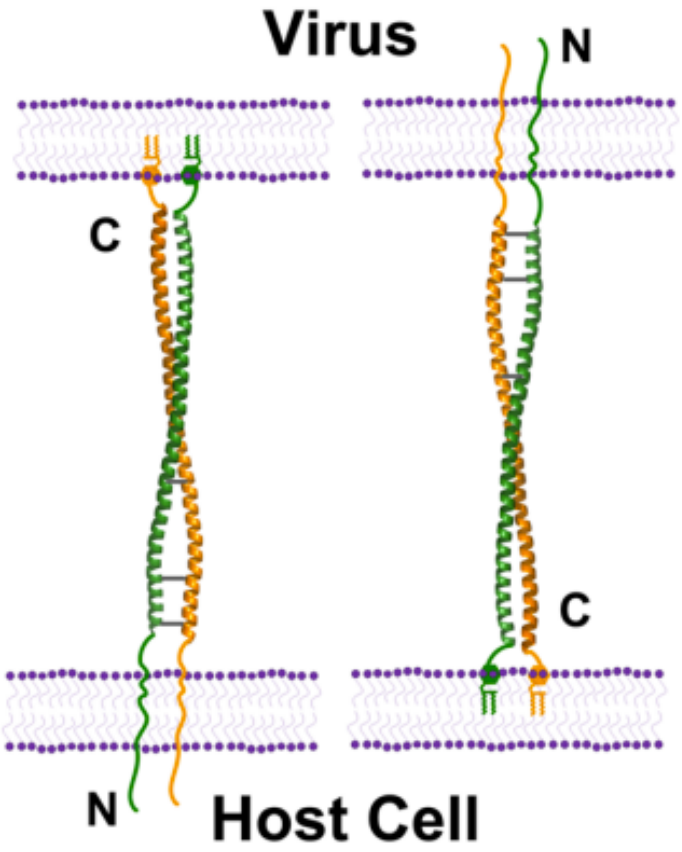

Figure 6. A structural basis for the anti-viral effect of restriction factor tetherin. Tetherin dimer is able to trap enveloped nascent virion at the cell surface during release by infiltrating a pair of membrane anchors into the viral envelope, while retaining the other pair in the infected cell membrane. C represents C-terminal of the protein, while N, the N-terminal. Adapt from Venkatesh and Bieniasz. *PLoS Pathog.* 2013.

Tetherin is a type II single-pass transmembrane protein, containing a short cytoplasmic tail followed by a N-terminal transmembrane domain, a C-terminal Glycosyl-phosphatidylinositol (GPI) anchor, and a ~110 amino acid ectodomain (ED) in between[69]. The extracellular part alone forms a single long α-helix and interacts with a second tetherin molecule to adopt a canonical coiled-coil configuration[70]. Three disulfide bonds are formed between two tetherin molecules at C53, C63, and C91 position[69].

The exact antiviral mechanism of tetherin is rather unspecific, given that tetherin is active against members from at least four virus families: retroviruses, filoviruses, arenaviruses and Herpesviruses[71], and some viruses have even evolved a counteractive mechanism[72]. But essentially in all cases, tetherin is a damage control mechanism for the host, terminating viral spread as a last-line of defense. Based on the protein structure of tetherin[69 73], its anti-viral function is fairly self-explanatory. The tetherin dimer has two potential membrane-associated domains at both ends (one transmembrane domain and one GPI anchor) and, by attaching one end to the infected cell and the other end to the enveloped virion, tetherin prevents nascent virions from being released. Although it was shown that two tetherin ED could associate into a tetramer by forming an anti-parallel four-helix bundle at their N terminus *in vitro*, the tetrameric structure is not necessary for tetherin's antiviral activity[69 74]. Most recently, it was shown that tetherin adopts an "axial" configuration in its functional state (Figure 6) with one pair of membrane domains in infected cell plasma membrane and the other pair in viral envelope[69]. While either end of the tetherin dimer can be inserted into a virion (N-terminal transmembrane domain or GPI anchor), Venkatsh and Bieniasz observed a preference for the insertion of tetherin's C-terminal GPI anchor into the virion envelope, possibly because this position offers some advantages

for host cell signaling since the N-terminal cytoplasmic tail remains inside the host cell[69].

Given the promiscuous nature of the anti-viral activity exerted by tetherin, it is foreseeable that as long as the overall protein structure remains intact, the specific amino acid sequence does not matter much. In fact, a completely artificial tetherin-like protein, assembled from structurally similar but unrelated protein domains, can mimic tetherin's activity[10]. Likewise, because tetherin does not target a specific viral protein but rather interacts with the largely host-derived viral envelope, it would seem impossible for the virus to evade by avoiding interaction. The viruses have to come up with a way to avoid the localization of tetherin to the plasma membrane altogether. HIV's solution is its accessory protein Vpu (Figure 7).

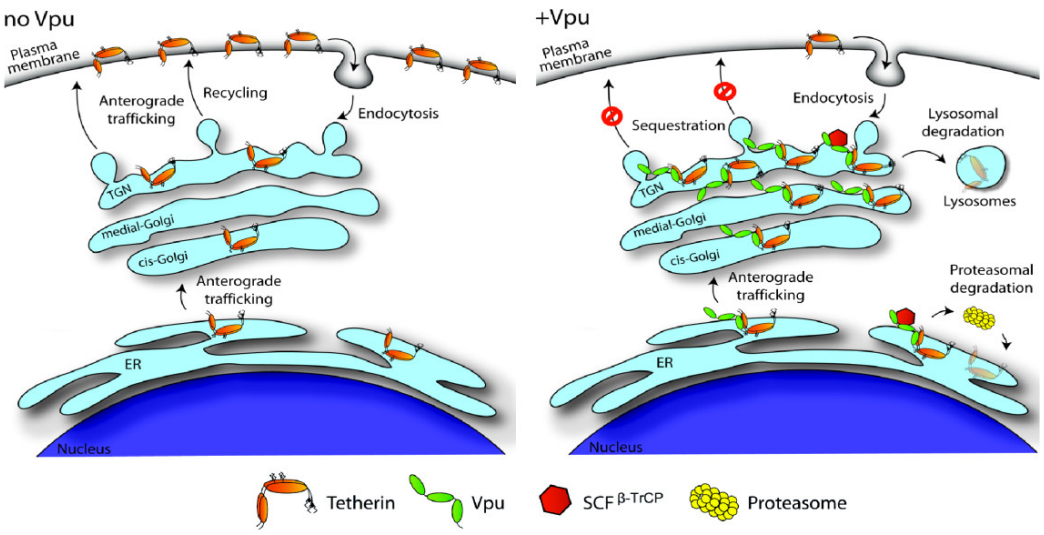

Figure 7. A working model portraying multiple mechanisms of Vpu-mediated down-regulation of cell surface tetherin level. $SCF^{\beta\text{-}TrCP}$ (shown in red) is a E3 ubiquitin ligase. Adapted from Dube *et al. Retrovirology*. 2010.

Vpu is a ~14 kDa protein consisting of a single transmembrane helix and a small cytoplasmic domain[10]. Early studies have shown that Vpu and tetherin can be

co-immunoprecipitated and give positive results in bimolecular fluorescence complementation assays. Additionally, the presence of Vpu reduces tetherin level at cell surface[67 75 76]. Most recently, McNatt *et al*. were able to demonstrate the direct interaction of Vpu and Tetherin in the cell membranes via their transmembrane domains[77]. Although an accurate molecular mechanism of Vpu as an antagonist is still lacking, it is highly possible that Vpu employs multiple mechanisms. It has been shown that Vpu may directly induce proteasomal degradation of tetherin by recruiting host proteins to form an SKP1-Cullin1-βTRCP ubiquitin ligase[78 79]. Alternatively, Vpu might induce sequestration of tetherin in the *trans* golgi network, which may later undergo lysosome-mediated degradation. Vpu might also saturate tetherin GPI anchors at the viral assembly site[76 80].

Finally, it may be interesting to mention that most SIV do not encode Vpu protein at all[81] and have developed a different tetherin evasion mechanism involving the Nef protein [82]. In some cases, primate lentiviruses may even utilize the Env protein as a tetherin antagonist[83]. These findings may have significant implications when considering both the origin of HIV and the design of future HIV drugs targeting Vpu.

VI. SAMHD1 and Vpx

The first important clue that preceded SAMHD1 discovery was an observation made on HIV-1 viral tropism. It has been long established that HIV-1 failed to infect a number of immune cell types that possess the necessary cell receptor and co-receptors for HIV-1, namely dendritic cells[84], macrophages[85] and resting CD4+ T cells[86 87]. This inability to infect certain immune cells was suspected to be attributable to the absence of the genomic Vpx protein in HIV-1, because HIV-2 and SIVmac, both encoding the accessory protein Vpx, could effectively infect those cells but failed to do so with a defective Vpx[88 89]. Furthermore, HIV-1 infection in monocyte-derived macrophages (MDM) can be achieved by pre-loading the macrophage with Vpx via a virus-like particle (VLPs) delivery system[82 83]. The specific molecular mechanism underlying this function of Vpx was also defined when researchers demonstrated that Vpx activity is dependent on a functional proteasome[82]. It was determined that Vpx recruits cellular proteins to form an E3 ubiquitin ligase complex $CRL4^{DCAF1}$, consisting of DCAF1, DDB1 (damage-specific DNA binding protein1), Cullin4 (Cul4) and Rbx1[90 91 92].

The only missing piece of this puzzle was the host protein Vpx targets and this piece finally came in 2011[16 18]. Hrecka *et al*. used a proteomic screening approach looking for a cellular protein that is associated with the $CRL4^{DCAF1}$ ubiquitin ligase only in the presence of Vpx[16]. In contrast, Laguette *et al*. took a somewhat similar but more direct approach. They directly pulled down Vpx from a monocyte cell line stably expressing Vpx and subjected the pull-down eluates to SDS-PAGE and mass spectrometry[18]. In both cases, SAMHD1 was identified and both groups further showed that knocking out SAMHD1 enhances HIV-1 infectivity and renders it Vpx

independent[16 18]. Later in the year, an elegant study from Berger *et al*. nicely complemented the results from Hrecka *et al*. and Laguette *et al*. by demonstrating CD14 positive monocytes isolated from individuals with Aicardi-Goutières Syndrome (AGS), who are known to have no endogenous SAMHD1 expression, are highly susceptible to HIV-1 infection[93].

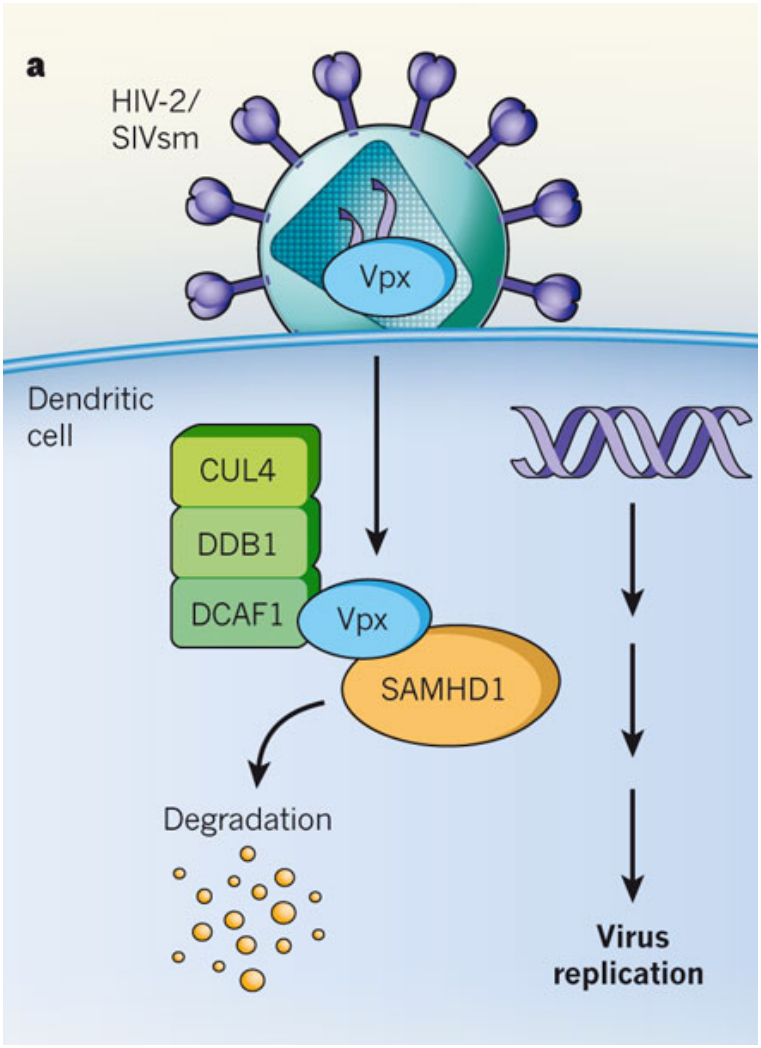

Figure 8. a. Upon infecting dendritic cells (the same mechanism applies to macrophages), HIV-2 and SIVsm deliver their Vpx protein into the cell cytoplasm, where it then binds to SAMHD1. Vpx recruits Cul4-DDB1-DCAF1 to form an E3 ubiquitin ligase, leading to proteasomal degradation of SAMHD1. In contrast, HIV-1 does not encode Vpx and SAMHD1 thus can inhibit HIV-1 replciation by mechanism(s) that remains unclear. Adapted from Lim & Emerman. *Nature*. 2011[94].

SAMHD1 is expressed at basal levels in most tissues[95] but is highly expressed in dendritic cells and other cells of the myeloid lineage[18]. Similar to many other restriction factors, SAMHD1 too is upregulated following type I IFN treatment[91]. The mechanisms of SAMHD1's anti-HIV-1 activity are still intensively studied today. The main enzymatic activity described to date is SAMHD1's dGTP-dependent dNTPase activity, which allows it to deprive the cellular dNTPs pool available for viral replication [96] [97] [98] (Figure 8). SAMHD1 converts dNTPs to the constituent deoxynucleoside and inorganic triphosphate, and data derived from crystallography suggested a tetrameric configuration is needed for full enzymatic activity[93] [95].

However, dNTPase activity is unlikely the only function of SAMHD1 as a restriction factor. Recent studies have shown that SAMHD1 is regulated by phosphorylation[99] [100] [101]. A phosphorylation event that takes place at a theronine residue near the C-terminal of SAMHD1 (C592) was shown to inhibit SAMHD1's restriction activity, while maintaining the dNTPase activity. Moreover, it was even demonstrated that SAMHD1 possesses exonuclease activity[102]. It is thus obvious that more research remains to be done on SAMHD1's anti-viral mechanism.

VII. Others

As mentioned earlier, rigorous investigations have revealed over 30 host-restriction factors associated with anti-HIV activities since the initial discovery of APOBEC3G. It is likely that those may only be the tip of the iceberg considering the complexity of the human innate immune system. Other than APOBEC3G and other proteins in the APOBEC3 family, TRIM5α and other proteins in the TRIM family, tetherin (BST-2) and SAMHD1, which are relatively well studied, a variety of other host proteins were identified in recent years to suppress HIV-1 replication in a cell-autonomous manner, many of which are IFN-inducible and non-HIV-1 specific. In this section, I will list three examples: Schlafen 11, TREX1 and MxB.

In a study published in Nature in 2012, a group of researchers from the University of California at San Diego described the anti-viral mechanism of a cellular Type I IFN-inducible protein Schlafen 11 (SLFN11)[103]. SLFN11 belongs to a large family of proteins initially described in 1998[104] that are preferentially expressed in lymphoid tissues. I It was shown that a cell line expressing SLFN11 produces fewer virions than a cell line that does not express SLFN11, even though the viral life cycle up to the stage of integration of viral DNA into the host genome remains unaffected[101]. Based on the fact that SLFN11 binds to all tRNAs *in vitro* and on a previously unexplained observation made by Coccia *et al*. that viral protein synthesis was inhibited in HIV-infected cells following IFN treatment[105], the authors proposed that SLFN11 can interfere with viral protein synthesis to achieve viral replication inhibition, specifically by depleting certain rare tRNAs. Viruses that have rare a codon bias (an HIV genome that is particularly A-T rich) may be especially vulnerable to this inhibition[101].

Strictly speaking, TREX1 is not a restriction factor, but rather a pro-virus host factor. However, it is believed to be important for HIV-replication by inhibiting innate immune responses to HIV cDNA[106]. TREX1 is a 3' exonuclease that contains three well-conserved exonuclease motifs at its N-terminus and a hydrophobic region at the C-terminus that is responsible for TREX1's localization to the cytoplasm and endoplasmic reticulum. TREX1 binds to and digests excessive cytosolic HIV DNA so that viral DNA will not activate IFN expression via a pathway involving TBK1, STING and IRF3. In contrast, in cells derived from TREX1 knockout mouse and human CD4+ T cells and macrophages in which RNAi inhibited TREX1 expression, cytosolic HIV DNA accumulated and HIV infection induced type I IFN production.

Human dynamin-like myxovirus resistance 2 (Mx2 or MxB) is the most recently added member to the repertoire of restriction factors. Late in 2013, three groups reported a cell-autonomous anti-HIV-1 restriction factor, previously known as MxB[17 107 108]. Although the protein was first identified in the 1980s[109], it was not associated with any anti-viral activity then and was therefore quickly overshadowed by the other protein in the family—MxA, which has long been recognized as a broadly acting inhibitor of many viruses, including influenza A virus[110]. Although the anti-HIV mechanism of MxB is currently under investigation, it was shown that MxB blocks the HIV-1 life cycle at a late post-entry step, inhibiting both nuclear cDNA accumulation and integration[17 105 106]. Additionally, the researchers isolated an escape HIV[17 111 112]strain from serial passage of HIV-1 through an MxB-expressing cell line and found a single mutation on the viral CA protein. Because the A88 residue is known to be indispensable for interacting with a host protein peptdyl-prolyl-isomerase cyclophilin A (CypA), the authors suspected that CypA and CA are involved in the anti-viral activity of MxB, which might be a reasonable guess since

previous studies on MxA[114], which is structurally superimposable on MxB, have shown that MxA can oligomerize and interact with the nucleocapsid of many viruses[113 114 115].

VIII. Implications of HIV Viral-Host Interactions

The repeated failure to produce an effective HIV vaccine is a humbling reminder that a full understanding of the immune response to HIV is still lacking[116]. Understanding how a virus with extremely limited coding capacity can evolve in a way that enables it to circumvent and bypass our powerful and intricate immune system is not only a biologically interesting but also a medically significant question. The identification of host restriction factors and corresponding viral evasion mechanism may merely be the first step, allowing us to know what we are dealing with (Figure 9). After all, a natural retroviral infection does not set off the production of cytokines or IFNs, whereas most restriction factors are IFN-inducible. Although the history of restriction factors is short, the scientific community has already been able to transform some of our knowledge on restriction factors and viral antagonists to the next level.

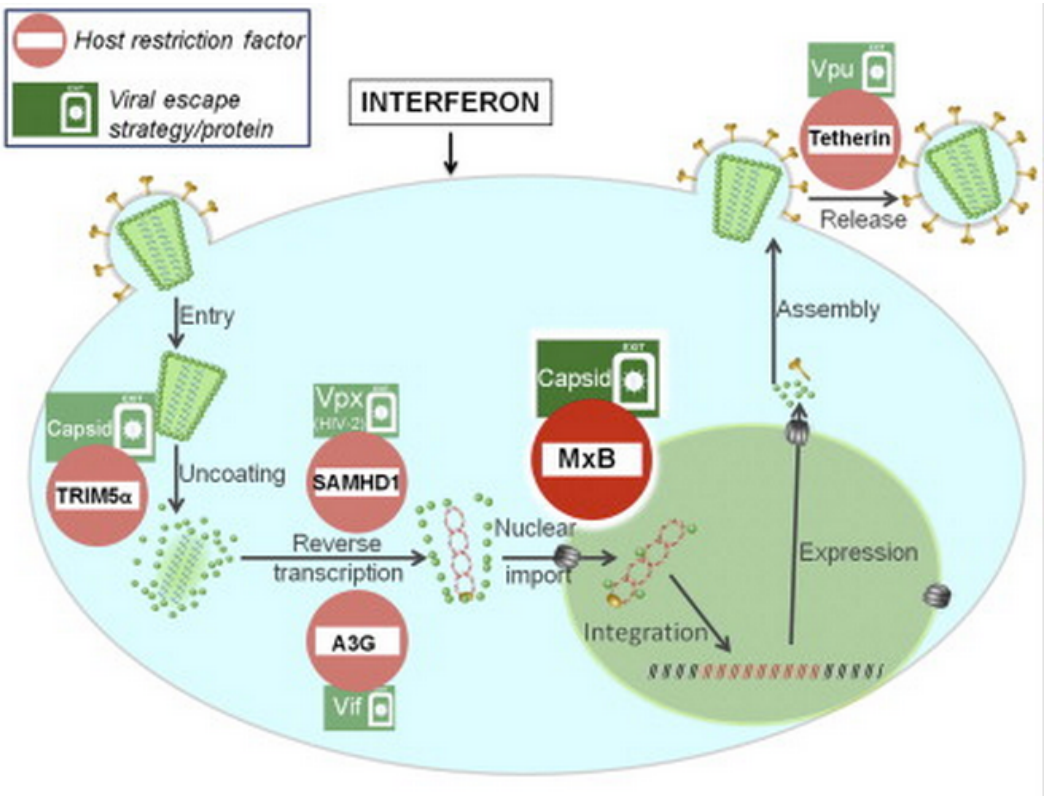

Figure 9. An illustration of the HIV-1 life cycle targeted by different host restriction factors. The virus has also developed mechanisms to evade restriction factors in order to produce progeny. Studying this dynamic HIV-1-host interaction may provide insights for future antiretroviral treatment design and also shed light on the origin of HIV virus. Adapted from Haller. *Cell Host Microbe*. 2013.

For instance, our knowledge on host restriction factors and their corresponding antagonists have played an important role in allowing us to trace back the evolutionary history of SIV because lentiviral interspecies transmission are partly driven by the evolution and capacity of viral accessory genes, including vpx, vpr, vpu, and vif, to antagonize host antiviral factors, such as APOBEC3, SAMDH1 and tetherin. We now know that HIV-1 resulted from cross-species transmission of SIVcpz, a simian immunodeficiency virus that naturally infects chimpanzees. SIVcpz itself is the result of an earlier recombination event between two SIV stains from Old World monkeys. The passage to chimpanzees may have served as an intermediate step for SIV that facilitates its adaptation to humans[117 118].

Although deciphering the evolution of the SIV-HIV transition may help explain why the virus produces such different disease progression patterns in primates and human respectively, the most tangible benefits from studying HIV-host interactions at the basic science level is the potential to apply that knowledge clinically. Currently, clinical applications can be divided into two conceptually promising areas: 1. Drug design that disrupts viral protein antagonism or mimics host restriction factors still capable of resisting viral infection; 2. Population level studies that aim at associating long-term non-progressors (LTNPs) or elite controllers (ECs), individuals who are able to maintain low plasma viremia and high CD4+ cell count without antiretroviral treatment (ARV) for a relatively long time, to certain restriction factor polymorphisms[119].

APOBEC3G was the first discovered restriction factor and therefore we now have the most knowledge about it, as well as other APOBEC3 family proteins. Population-based studies have been published to examine if APOBEC3G or APOBEC3G contribute to LTNPs' ability to control viral replication[118]. The results are ambiguous and inconsistent, possibly due to the generally small sample size and the fact that the ability to achieve long-term control of HIV viremia is likely multi-factorial. It might also be possible that A3G's hypermutation-independent antiviral activity was not accounted for, for most studies measured the hypermutation level as the readout for APOBEC3G activity level[120]. Nevertheless, an APOBEC3G polymorphism identified (rs8177832) in African-Americans and the 6892C allele present in European-American populations were associated with accelerated disease progression[121 122 123]. A recent study that examined 19 ARV-naïve individuals (12 LTNPs and 7 not) showed that the deaminase-dependent antiviral activity might only

contribute to viral replication control above a threshold level while the deaminase-independent activity does not require a threshold level[124].

Scientists have also made progress on exploiting the APOBEC3 family proteins for development of new treatments. However, any strategies involving upregulating APOBEC3 level needs to be approached cautiously—in host cells, APOBEC protein expression and activity must be strictly regulated in order not to backfire on the host genome. Previous studies have shown that over-expression causes genome damage and leads to cancer[125]. Regardless of the side effects, APOBEC3G expression enhancement can be achieved by stimulating CCR5 and CD40 with CCL3 and CD40L chemokines, respectively, as well as with heat shock protein 70 (HSP70), which is present in HIV virions[126] and was previously shown to bind directly to CCR5[127]. One study has successfully demonstrated the use of HSP70 as a preventive method in rhesus macaques[128]. In addition, the Vif-APOBEC3G, Vif-Cul5, Vif-CBFβ interfaces are also promising sites for the development of new antiretroviral drugs, such as RN-18[129], which targets the Vif protein directly. With the recent addition of APOBEC3G[130 131] and Vif-Cul5-ElogBC-CBFβ[43] complex high-resolution structural data, new ARVs should be in the pipeline soon. Alternatively, there have also been attempts to enhance incorporation of APOBEC3G into nascent virions by deploying a APOBEC3G-viral (for example, Vpr and Nef) protein fusion delivery system[132 133].

TRIM5α is another candidate under rigorous investigation, mainly as a gene therapy option, since there is no known viral antagonist. At the population level, several SNPs of huTRIM5α were identified, but only two were studied for their effect on disease progression[134 135]. It was suspected that the TRIM5α H43Y polymorphism occurs at the RING domain of TRIM5α and may affect its E3 ubiquitin ligase activity,

but it could not be fully confirmed in *in vitro* assays[132]. The other polymorphism, R136Q, occurs at the domain involved in TRIM5α oligomerization and thus potentially may influence its antiviral activity. However, epidemiological studies gave inconsistent results[63 136]. TRIM5α gene therapy research, however, is rather productive. Since huTRIM5α does not itself target HIV-1, researchers have tried engineering chimeric TRIM5α using the PRYSPRY domain from the rhesus macaque[137] and chimeric TRIM5αCyp combining huTRIM5α and huCypA[138] (a idea inspired by TRIM5Cyp from new world owl monkey), were both quite successful in animal models. Additionally, the recent discovery that a single nucleotide mutation of huTRIM5α can prevent HIV-1 evasion may allow development of gene therapy with no immunogenicity problems[60 61]. Meanwhile, because the crystal structure of the rhesus macaque TRIM5α PRYSPRY domain (where it interacts with CA) was already available[139], there may be space for drug development targeting the viral CA protein.

Both tetherin and SAMHD1 have been discovered fairly recently, therefore, few clinical studies have been done on either of them. Since both restriction factors are IFN-inducible, an obvious way to exploit their antiviral effects is immunotherapy (IFN treatment). However, it turned out that not only is IFN-α treatment accompanied by some side effects, it is also associated with increased risk of progression to AIDS[140]. An alternative approach is to design molecules to prevent vpu binding to tetherin at the transmembrane domain[141]. In the case of SAMHD1, clinical studies are still lacking but potential drug development can target the SAMHD1-Vpx interface, again with the help of available biophysical data, or inhibit phosphorylation (deactivation) of SAMHD1. Other than the four well-studied restriction factors, polymorphisms in TREX1, specifically the single nucleotide polymorphism rs3135945 was significantly associated with HIV infection[142]. A recent study looking

at the host restriction factor expression profile among HIV-1 elite controllers in a UCSF cohort has successfully identified schlafen 11 as a potential signature of HIV-1 elite controllers. The mRNA and protein expression of schlafen 11 were elevated among elite controllers who tend to have a low cellular activation level and low restriction factor levels.

Finally, because almost all known viral evasion mechanism involves ubiquitin mediated proteasomal degradation, a drug that can potentially target HIV-infected cells and inhibit proteasome activity could be an ideal ARV candidate because it will be able to target multiple stages of the viral life cycle, serving the same purpose as HAART.

IX. Conclusion

Since the discovery of APOBEC3G in 2002, our knowledge on HIV-1 viral-host interaction has expanded exponentially. A group of proteins termed restriction factors were found to inhibit the replication of virus in host cells. Unlike the classical innate immunity, which is mediated by special cell types such as Natural Killer cells, Dendritic cells and polymorphonuclear leukocytes (PMNs), restriction factors are germline-encoded and IFN-inducible proteins that provide intrinsic immunity against viral replication in individual host cells. Many restriction factors target specific virus or viruses in certain family, such as TRIM5α, while others possess rather unspecific antiviral mechanisms, such as tetherin. Despite of restriction factors' potent antiviral activity, viruses have evolved ways to get around the host defense. Most well-studied restriction factors are associated with viral antagonists. Some of the examples mentioned in this article include Vif and APOBEC3, SAMHD1 and Vpx, tetherin and Vpu. The widespread of HIV-1 virus is certainly a clear indication of the equally strong potency of viral antagonists. The very existence of host restriction factors and viral antagonist clearly points to "an evolutionary 'arm race' that drives continuous rounds of selection for beneficial mutations in the genes encoding restriction factors and their viral antagonists, through evolutionary pressure for both host survival and virus replication"[143].

In the context of HIV, which has only circulated in human host for 50 years, the question thus becomes why do those restriction factors exist in the first place before HIV has come alone and whether human can keep up with the HIV virus in this evolutionary arm race.

To answer the first question, we need to first understand that virus in general has been a significant presence throughout the vertebrate evolution and this arm race between the virus and the host is an ancient concept[144]. In fact, it has been shown that many human restriction factors have been evolving under episodic positive selection throughout primate evolution[141]. Thus the existence of many restriction factors may be the result of co-evolution of human and many other ancient viruses, some of which may have successfully achieve co-existence with its host by incorporating their genetic material into host genome. The co-evolution of host and virus is a classical example of the "Red Queen" Hypothesis[145], in which two conflicting entities undergo continuous adaptation to maintain the status quo. At the end of the "Red Queen" competition, often a balance is reached when the virus becomes avirulent enough that it does not cause any significant morbidity or mortality in host while remaining the capability of reproducing its genetic material. This long-term balance is obviously not reached for human and HIV. The majority of the human host has only been exposed to the virus for three decades and thus it is unlikely that the restriction factors can evolve within such a short time period. Although some of the restriction factors can inhibit HIV replication, it is likely they evolved to restrict replication of some ancient viruses (possibly retrovirus) but possess cross-reactivity to HIV.

Another possibility is that some of the restriction factors were involved in other cellular pathways before the HIV virus comes along and happens to inhibit viral replication. A great example is SAMHD1, which functions as not only a restriction factor but also an innate immune response mediator to non-viral events. Genetic mutations in SAMHD1 are associated with autoimmunity in humans called Aicardi-Goutières Syndrome[146], potentially because SAMHD1 can prevent the accumulation of inappropriate retrotransposons by-products, such as single-stranded DNA[147].

Likewise, APOBEC3G has been suggested to also play a similar role in control excessive endogenous retrotransposons by inducing hypermutation[148]. Additionally, restriction factors may play role in the immune signaling pathways. In many ways, restriction factors are similar to pattern-recognition receptors because they recognize certain structure pattern of the virus[141]. Specifically, TRIM5α, upon binding to the retroviral capsid, activates NF-κB signaling and a distinct innate immune response. In the absence of retrovirus, TRIM5α is shown to still function as a constitutive signaling intermediate in the NF-κB cascade[60 149]. Similarly, tetherin has been shown to activate NF-κB, in addition to its antiviral activity against enveloped virus[150].

Clearly further research on restriction factors will help us better understand our innate immune system and how restriction factors talk to the other components of the immune system. But for now, a more urgent question would be what will be the outcome of this evolutionary arm race between the human host and the HIV virus. In the case of primates and SIV, we know they eventually co-exist. But how is it possible? If single nucleotide changes were the only mechanism driving the co-evolution of human and HIV, the host would be at an enormous disadvantage since RNA virus mutate much faster rate than the human host, especially considering that in reality a host are simultaneously being challenged by a variety of pathogens. The answer seems to lie in the nature of genetic material utilized by virus and human. Because virus has a densely packed genome, it has many overlapping reading frames, as well as certain secondary RNA structures crucial in the viral life cycle. Due to the limited coding capacity, many viral proteins serve multiple functions. These factors severely constrain the viruses' ability to evolve even though viral mutants are relatively easy to generate. In contrast, the human host has tremendous potential for evolution because it has two alleles for each gene. Many genes in human are also

duplicated, for example the APOBEC3 protein family, so that each alone can undergo mutations without compromising the function of this gene.

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Curriculum vitae

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NATIONALITY	Chinese
DATE OF BIRTH	05.1991
GENDER	Male
LANGUAGE	English (near native) and Mandarin (native)

Research Experience

DATES	2013-2014
POSITION	Research Assistant
PRINCIPLE INVESTIGATOR	Xiao-fang Yu, M.D., D. Sc. Johns Hopkins Bloomberg School of Public Health
PROJECT CONTENT	HIV viral pathogenesis and human restriction factors specifically concerning Vif-APOBEC3G, Vpx-SAMHD1 and Mx2.

DATES	2011-2012
POSITION	2012 iGEM Team Grinnell Member
PRINCIPLE INVESTIGATOR	Lisa Bowers, Ph. D. Grinnell College
PROJECT CONTENT	Gold-medal-winning project on the utilization of protease Esp-secreting *Caulobacter crescentus* in inhibiting *Staphylococcus aureus* biolfim.

DATES	2012
POSITION	Research assistant
PRINCIPLE INVESTIGATOR	Ashley Pearcy Ph. D. University of Witwatersrand Organization of Tropical Studies
PROJECT CONTENT	A preliminary assessment of the Mutale River and its suitability for Nile Crocodiles.

Curriculum vitae

Education and Training

DATES	2014 (expected)
QUALIFICATION AWARDED	Master of Health Science
PRINCIPAL STUDIES	Molecular Microbiology and Immunology
INSTITUTION	Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

DATES	2013
QUALIFICATION AWARDED	Bachelor of Arts
PRINCIPAL STUDIES	Biology with honors
INSTITUTION	Grinnell College, Grinnell, IA

DATES	2012
QUALIFICATION AWARDED	Off-campus studies
PRINCIPAL STUDIES	Grinnell in Washington Semester
INSTITUTION	Grinnell College, Washington, DC

DATES	2012
QUALIFICATION AWARDED	Summer Program
PRINCIPAL STUDIES	Global Health Issues in South Africa
INSTITUTION	Organization for Tropical Studies, Skukuza, South Africa Duke University, Durham, NC

DATES	2012
QUALIFICATION AWARDED	Off-campus Studies
PRINCIPAL STUDIES	African Ecology and Conservation Semester
INSTITUTION	Organization for Tropical Studies, Skukuza, South Africa Duke University, Durham, NC

Curriculum vitae

Work Experience

DATES 2012
POSITION Environmental Health Intern
ORGANIZATION Physicians for Social Responsibility, Washington, DC
PRIMARY RESPONSIBILITY Performed background research, produced synthesis, and reported to the director on environmental health policies. Compiled and designed information pamphlets on shale gas drilling and off-shore wind energy campaigns.

DATES 2012
POSITION Intern at Doctors for America
ORGANIZATION Center for American Progress, Washington, DC
PRIMARY RESPONSIBILITY Conducted daily internet research on states' stands on the Affordable Care Act. Maintained membership/ volunteer database and provided administrative support.

DATES 2012
POSITION Intern
ORGANIZATION Tshulu Trust, Limpopo, South Africa
PRIMARY RESPONSIBILITY Developed, initiated and improved a computer literacy program for community members. Designed and organized a system of monitoring feedback on Tshulu's projects. Attended community consultation meetings and assisted in the preparation of Tshulu Impact Assessment.

DATES 2009-2011
POSITION Teaching Assistant
ORGANIZATION Grinnell College, Grinnell, IA
PRIMARY RESPONSIBILITY Served as laboratory teaching assistant for senior level biology course for one semester. Tutored Chinese at the Chinese Lab for two and a half years.

Curriculum vitae

Publications

Evans SL, Schön A, **Gao Q**, Han X, Zhou X, Freire E, Yu XF. 2014. HIV-1 Vif N-temrinal motif is required for recruitment of Cul5 to suppress APOBEC3. *Retrovirology*. 11:4. doi: 10.1186/1742-4690-11-4.

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