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SERINC5 as a New Restriction Factor for Human Immunodeficiency Virus and Murine Leukemia Virus

Claudia Firrito,[1] Cinzia Bertelli,[1] Teresa Vanzo,[1] Ajit Chande,[2] and Massimo Pizzato[1]

[1]Centre for Integrative Biology, University of Trento, 38123 Trento, Italy; email: claudia.firrito@unitn.it, cinzia.bertelli@unitn.it, teresa.vanzo@alumni.unitn.it, massimo.pizzato@unitn.it

[2]Department of Biological Sciences, Indian Institute of Science Education and Research Bhopal, Bhopal 462 066, Madhya Pradesh, India; email: ajitg@iiserb.ac.in

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Abstract

SERINC genes encode for homologous multipass transmembrane proteins with unknown cellular function, despite being highly conserved across eukaryotes. Among the five SERINC genes found in humans, SERINC5 was shown to act as a powerful inhibitor of retroviruses. It is efficiently incorporated into virions and blocks the penetration of the viral core into target cells, by impairing the fusion process with a yet unclear mechanism. SERINC5 was also found to promote human immunodeficiency virus 1 (HIV-1) virion neutralization by antibodies, indicating a pleiotropic activity, which remains mostly unexplored. Counteracting factors have emerged independently in at least three retrovirus lineages, underscoring their fundamental importance during retrovirus evolution. Nef and S2 of primate and equine lentiviruses, and glycoGag of gammaretroviruses, act similarly by targeting SERINC5 to endosomes and excluding it from virions. Here, we discuss the features that distinguish SERINC5 from other known restriction factors, delineating a yet unique class of antiviral inhibitors.

1. INTRODUCTION

Efficient retroviral replication requires a complex interaction network between virus and host, which translates into an arms race in which viruses hijack cellular functions to the benefit of their replication and persistence and the host develops barriers to counteract infection. A first line of defense in the host is the innate immune system, which, upon recognition of the pathogen by pathogen recognition receptors (PRRs), mounts a proinflammatory response, anticipating the activation of the specific adaptive immunity.

Restriction factors are important components of the innate immune system. The notion of restriction factors initially came from the concept that viral tropism is restricted in nonpermissive cells, posing a barrier against cross-species transmission or within species spread. After the discovery of the Friend virus susceptibility factor 1 (FV-1), which targets murine leukemia virus (MLV) in rodent cells (1), restriction factors have been discovered in different species over the last 15 years. These proteins interfere with various steps of virus replication including capsid uncoating (2, 3), genome duplication (4, 5), nuclear entry (6), virus release (7, 8), and fusion (9). Restriction factors often act as PRRs by alerting the immune system (10, 11), and their expression levels can be further induced by interferon to raise the level of protection. In this arms race, reciprocal selective pressure has forced viruses to evolve mechanisms to counteract restriction factors and the host to favor mutations that maximize the efficacy of its antiviral defenses, resulting in positive selection of antiviral genes. Indeed, interferon induction and positive selection are two hallmarks common to most restriction factors.

Recent studies have revealed the antiretroviral roles of SERINC proteins, a multipass transmembrane protein family, which in humans includes five members. SERINC5 has been shown as the most powerful antiviral inhibitor within the family, counteracted by retroviral factors evolved independently in human immunodeficiency virus (HIV), MLV, and equine infectious anemia virus (EIAV). Here, we recount the current knowledge surrounding the SERINC genes and their antiviral roles.

2. THE SERINC GENES

SERINC genes are conserved in all eukaryotes and encode homologous transmembrane proteins, which remain orphan of a known molecular function. SERINCs are named after a putative role of serine incorporators in phospholipids (12). Five SERINC genes are present in the human genome, encoding homologous proteins with a similar predicted transmembrane topology. The first to be identified was SERINC3, which was found overexpressed in testicular tumors developed in polyomavirus large T-antigen transgenic mice (13). A related human sequence was later cloned from cytotrophoblasts and in human promyelocytic leukemic cells differentiated into macrophages (14). Although SERINC3 was found expressed in all mouse tissues (13) and in several human cell lines (15), higher expression was reported in some tumor-derived tissues; hence the gene family was initially named TDE (tumor differentially expressed gene) (13). Additional homologous gene sequences were later identified, postulating the existence of a family of genes encoding closely related transmembrane spanner (TMS) proteins (16). A transcript encoding SERINC5 was first identified in cultured rat pro-oligodendrocytes, differentiated into myelinating oligodendrocytes, whereas SERINC1 was retrieved in a mouse expressed sequence tag (EST) database and in human lung and liver cancers (17, 18). As well as being overexpressed in tumor cells, SERINC transcripts were also detected in differentiated PC12 adrenal chromaffin cells (19), in various parts of the mouse brain (16), and in mouse brain following axotomy (20), indicating an important role in the central nervous system.

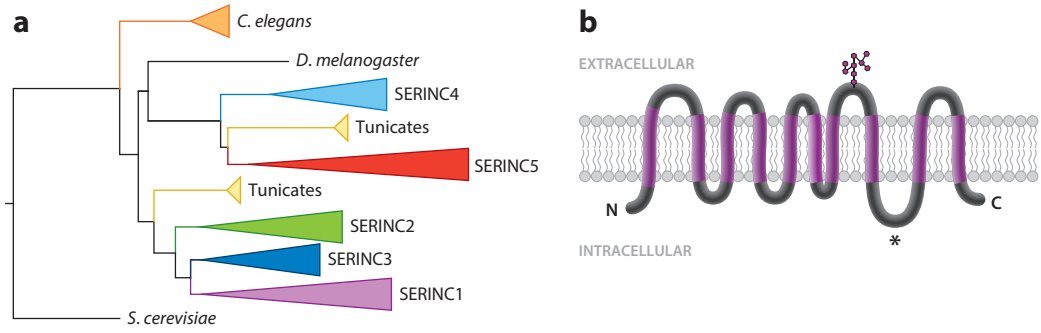

Figure 1

The SERINC family of genes and proteins. (*a*) Phylogenetic relationship of SERINC genes. A single copy of the SERINC gene family is found in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. Mammals have five copies divided in two clusters derived by a duplication that occurred in an early ancestor and gave rise to a cluster including SERINC1, SERINC2, SERINC3, and another cluster including SERINC4 and SERINC5. Colors indicate different branches of SERINC genes. The lengths of the triangles are proportional to the number of nucleotide substitutions that have taken place in a particular branch. The tree was generated using Ensembl. (*b*) Schematic topology of the SERINC5 protein. Similar topologies are also predicted for the remaining SERINC1 through SERINC4 proteins encoded by the longest splice isoforms of each gene. A predicted glycosylation site for SERINC5 is represented in the fourth extracellular loop. The fourth intracellular loop, between the eighth and ninth transmembrane domains, was recently found to govern the sensitivity of SERINC5 to Nef (*asterisk*). Abbreviations: C, C terminal; N, N terminal.

By mining the available sequence databases, SERINC genes can be observed in all eukaryotes, including plants and yeast. Although only one SERINC gene is found in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*, higher eukaryotes have five copies (**Figure 1*a***). The increased copy number can be attributed to two rounds of whole-genome duplication events that occurred in vertebrates (21, 22). Such expansion was maintained in almost every vertebrate species, suggesting a diversification of their function. SERINCs appear to have split into two phylogenetically related groups, with SERINC4 and SERINC5 most closely related (**Figure 1**). Multiple splice isoforms were reported for each human SERINC gene, but only the largest SERINC5 variant was found to encode a stable protein (23), with 10 transmembrane domains and intracellular N and C termini, as was recently experimentally confirmed (24) (**Figure 1*b***).

The universal presence of SERINC genes in eukaryotes suggests that these genes have a fundamental role or roles for survival and evolution of complex organisms. However, their functions remain unknown. TMS1 (YDR105C), the only SERINC gene in *S. cerevisiae*, is not essential for cell growth and survival in culture (16). Only a slight perturbation of the yeast vacuolar fragmentation was documented in a TMS1 *S. cerevisiae* deletion mutant (25), indicating a possible alteration of membrane dynamics (25). Accordingly, Inuzuka and colleagues (12) proposed a role of these proteins in the biosynthesis of phospholipids by supporting the synthesis of serine-containing lipids (hence the name SERine INCorporator). However, such function was recently challenged by two studies indicating that ablation of SERINC1 (26) and SERINC5 (27) does not result in qualitative nor quantitative alterations of the cellular lipidome.

High expression in the brain (16), in differentiating neuronal cells and oligodendrocytes (19, 28) and in neurons following axotomy (20), supports the notion of an important role of SERINC proteins in the central nervous system. A role of SERINC genes in tumor progression was also postulated based on high expression in cancer cell lines and tumors (13–18, 29, 30). SERINC3 overexpression associates with loss of contact inhibition as well as with decreased apoptosis in rat and human cells, and supports the growth of tumor xenografts in nude mice, suggesting a potential

protumorigenic role (29). In addition, SERINC1 is preferentially expressed in hepatocarcinoma cell lines and is required for cell cycle progression, possibly by preventing transcriptional activation of p21 (17). However, the physiological functions of SERINC genes and their roles in tumor progression remain speculative, in need of insight into their molecular structure and the identification of their binding partners.

3. THE DISCOVERY OF SERINC5 AND SERINC3 AS RESTRICTION FACTORS FOR THE HUMAN IMMUNODEFICIENCY VIRUS

The discovery of the antiretroviral activity of SERINC5 is linked to the study of Nef in primate lentiviruses. The *nef* gene encodes a 27-kDa myristoylated protein, which is dispensable for viral replication in most in vitro conditions but is crucial for disease progression in vivo. Among its several activities, Nef modulates the activation status of cells, manipulates the cell cytoskeleton, alters the surface expression of receptors, and increases the intrinsic infectivity of virus particles (31). Depending of Nef expression in producer cells, virion particles were observed to acquire a defect that impairs an early stage of the infection process in target cells (32). Crucially, it was observed that such a phenotype is strictly dependent on the producer cell type, suggesting the presence of a cell-type-specific inhibitor of infectivity counteracted by Nef (33). Another prominent activity of Nef, documented in the early days, is the ability to downregulate cell-surface molecules, notably the receptor CD4 and major histocompatibility complex class I, by targeting them to the vesicular machinery (34). Evidence emerged that the function of Nef on virion infectivity depends on the ability to engage the endocytosis machinery as optimal infectivity was observed to require the ability of Nef to interact with AP2 (35) and dynamin 2 (36). Altogether, these observations supported the notion that Nef could target a membrane-associated cell-type-specific activity inhibiting virus particle infectivity.

In one of the most fascinating examples of convergent findings, such activity was identified concurrently by two complementary strategies carried out independently by separate research groups. By comparing transcriptomes of different types of producer cells, our group identified SERINC5 as the transcript that best correlates with the requirement of Nef for virus infectivity. SERINC5 was found to associate with virus particles and to inhibit infectivity only in the absence of Nef (37). Simultaneously, by comparing the proteomes of cell-free virions produced in the presence and absence of Nef and glycoGag from murine leukemia virus, the Göttlinger group (38) revealed that Nef-defective virus particles are enriched in SERINC3. SERINC5 was then again identified as the SERINC protein with the most powerful inhibitory activity against the virus. Their high hydrophobicity together with the presence of cysteine-rich regions make SERINC proteins particularly difficult to investigate, given their propensity to aggregate and cross-link. Such technical difficulties perhaps contribute to explaining the long time required to identify them as restriction factors following the initial report describing the Nef effect on infectivity (39).

4. INHIBITION OF INFECTIVITY BY SERINC5

SERINC5 is known to have the strongest antiviral activity among the human SERINC gene products; on the opposite end, SERINC2 lacks any ability to alter HIV-1 infectivity. SERINC1 and SERINC3 were observed to exert a modest inhibition of HIV-1, and it was recently revealed that SERINC4 carries also strong antiviral activity (24), which is consistent with its phylogenetic vicinity to SERINC5 (**Figure 1**). However, the role of SERINC4 awaits further insights because, after interrogating the gene-expression data sets available to date, we failed to identify any tissue where mRNA encoding the protein is expressed. In contrast, SERINC5 appears to be highly

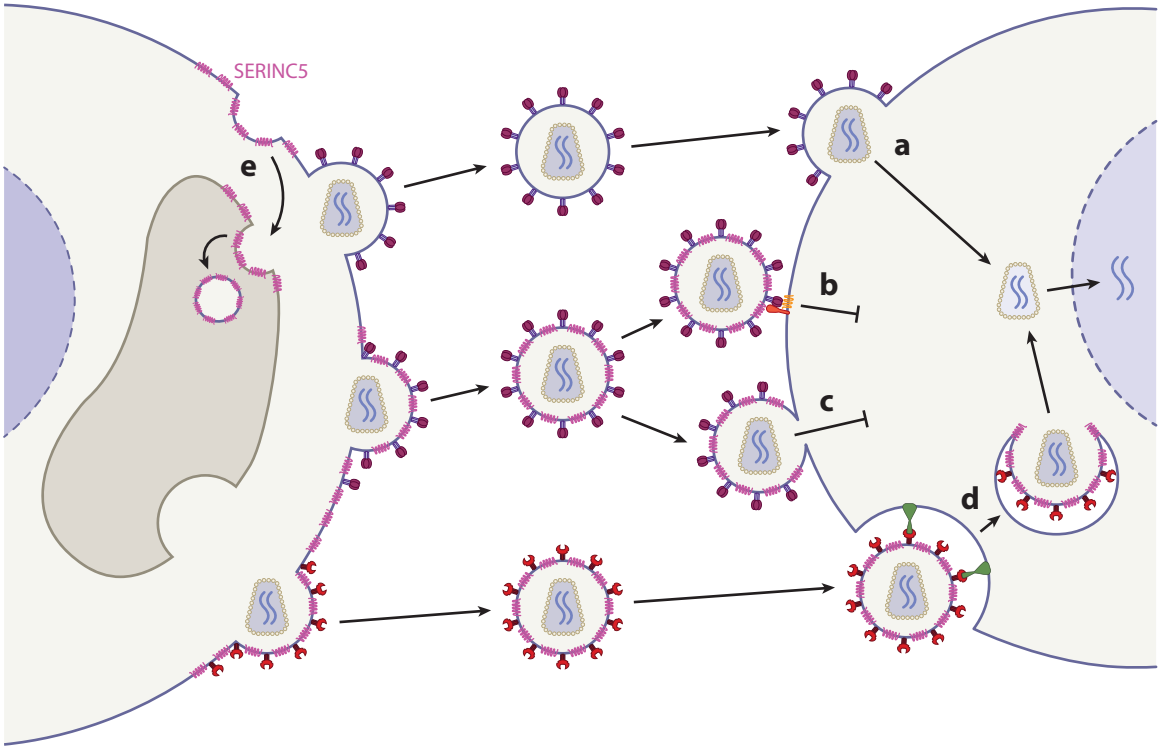

Figure 2

A model for SERINC5 inhibition of retrovirus infectivity and the counteracting activity of retroviral factors. Successful infection (*a*) is impaired following incorporation of SERINC5 into retrovirus particles, which inhibits virus-cell fusion either by preventing the initial fusion (*b*) or by interfering with the expansion of the fusion pore (*c*). (*d*) SERINC5 does not affect infection following virus uptake into an intracellular vesicle. (*e*) Counteracting retroviral factors prevent SERINC5 incorporation into virions by targeting it to the endosomal compartment.

expressed in several tissues, including lymphoid lineages, consistent with an antiviral role against the infection of lymphotropic and myelotropic viruses, such as HIV, MLV, and EIAV, which have evolved counteracting factors. Only the larger SERINC5 splice isoform encoding for a 10-transmembrane domain protein (**Figure 1*b***) was reported to be highly expressed and to carry antiviral activity (23).

Based on initial reports (37, 38), a simple model illustrates SERINC5 activity on retrovirus infectivity (**Figure 2**). Incorporation of SERINC5 into a virion particle impairs the delivery of the viral core to target cells. Nef, by mediating the endocytosis of SERINC5, counteracts its inhibitory effects by preventing its virion incorporation. Below, we illustrate and discuss this model in detail.

4.1. The Incorporation of SERINC5 into Virions

A hallmark of SERINC5 is its ability to associate with virion particles. Accordingly, the protein is more readily detected in virion pellets than in cell lysates (37, 38), suggesting an enrichment of the host factor in virus particles. Although different SERINC paralogs may be found incorporated into virions, a recent study reported that virus association is superior for SERINC5 as compared to

SERINC2 (24). It remains unclear whether SERINC5 relies on a specific mechanism to associate with virions, perhaps by interacting with viral proteins. Unlike SERINC2, SERINC5 was found to preferentially localize into detergent-resistant domains of the plasma membrane, where virus budding could occur (40), indicating that incorporation into virions could be a passive mechanism enhanced by the colocalization with the budding virions. A clarification of this issue is awaiting further studies because a physical interaction of SERINC5 with other viral proteins could not yet be detected.

4.2. The Stage of Virus Infection Inhibited by SERINC5

It remains formally unclear whether virion incorporation of SERINC5 is absolutely required for its effect on infectivity. However, a linear correlation between the level of incorporation into virions and inhibition of infectivity strongly supports the notion that virus-laden SERINC5 contributes to particle infectivity inhibition (41). The step of the virus penetration process affected by SERINC5 and the molecular mechanism of the inhibition remain obscure.

SERINC5 affects a stage that prevents reverse transcription of HIV in the cytoplasm (37), correlating with the viral core being undetected in the cytoplasm shortly after infection (24). The notion that SERINC5 impairs virus-cell fusion was deduced from effects of SERINC5 on the cytoplasmic delivery of reporter proteins (37, 38). Further supporting an inhibition of fusion, SERINC5 was also found to inhibit fusion from without and syncytia formation promoted by cell-free virions (42). However, by monitoring the cytoplasmic translocation of a fluorescent protein using fluorescent microscopy, SERINC5 was shown to inhibit the formation of fusion pores as small as 4 nm (42). Moreover, the host factor was also observed to inhibit infection to a greater extent than fusion (37, 38, 42). This indicates another block following the initial fusion pore formation, such as fusion pore expansion. Therefore, SERINC5 might act by increasing the energy barrier required for successful fusion, and according to the amount of virion-associated protein, it could impair only pore expansion, which requires a high level of energy (44), and/or the formation of the fusion pore itself.

4.3. Possible Mechanisms of Fusion Inhibition by SERINC5

How does SERINC5 inhibit the fusion process? A few hypotheses have been formulated to answer the question (**Figure 3**).

Given a role of SERINCs in the synthesis of phospholipids as initially suggested (12), SERINC5 might indirectly increase the rigidity of virus particles by altering the lipid composition of the viral envelope (**Figure 3*b***). The increased stiffness of the viral membrane would lead to increased energy required for fusion. However, a recent study (27) indicates that SERINC5 expression in producer cells does not contribute to the viral lipidome, making this hypothesis unlikely. Nevertheless, the rigidity of the envelope could also be affected by the crowding of SERINC5 molecules on virion particles (**Figure 3*c***), interfering with fusion or with delivery of the capsid into the cytoplasm.

Alternatively, a direct effect of SERINC5 on Env functionality, resulting in conformational changes and in an impairment of its fusion-promoting activity, could also be postulated based on observations that SERINC5 can inactivate the HIV-1 Env glycoprotein (42) and change Env sensitivity to neutralizing antibodies (24, 42, 45), as discussed below. Given that SERINC5, like the HIV Env glycoprotein, has been reported to localize in lipid rafts, one possibility is that the restriction factor could interfere with functional Env clustering on the cell surface at virus assembly sites and on the virus particle itself (**Figure 3*d***). It was reported that only 7 to 14 Env trimers are present on the virus particle (46, 47), forming a cluster upon virion maturation

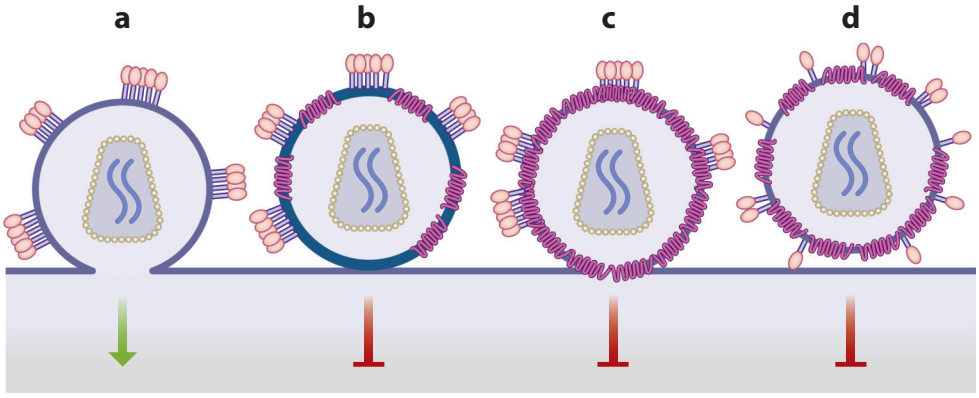

Figure 3

Possible models of SERINC5 inhibition of retrovirus fusion with target cells. Productive virus-cell fusion could be (*a*) hindered by an increased stiffness of the virus particle caused by a modification of the lipid composition induced by SERINC5 or (*b*) directly caused by the enrichment of the restriction factor on the virus envelope (*c*). Finally, SERINC5 could impair the structure or functional clustering of the envelope glycoprotein trimers (*d*).

(48, 49). Given the low number of Env spikes in the viral membrane, Env clustering is thought to allow a crucial cooperative interaction necessary to promote efficient fusion and entry. It is therefore tempting to speculate that the presence of SERINC5 in the virus particle could interfere with the distribution of Env spikes and therefore impair the efficiency of the virus entry process. This is a suggestive hypothesis that correlates well with the effect of SERINC5 on fusion and entry. However, although Env clustering was reported to require the cytoplasmic tail of Env and viral matrix (50), the susceptibility of virus particles to the activity of Nef does not depend on either (43). Therefore, a complete understanding of the mechanism by which SERINC5 inhibits the early stages of retrovirus infection requires additional insight.

5. COUNTERACTION OF SERINC5 BY NEF OF PRIMATE LENTIVRUSES

Parallel to the ability of Nef to exclude SERINC5 from virions is its capacity to alter SERINC5's cell-surface expression. This was confirmed by a functional proteomics screen, which found SERINC5 and SERINC3 among the plasma membrane proteins affected by HIV-1 Nef during infection (51). Decreased SERINC5 surface expression is associated with its accumulation in the late endosomes, indicating that the restriction factor is endocytosed when Nef is expressed. Confirming early research on Nef (35), intravesicular trafficking of SERINC5 was found to be required for counteraction because depleting clathrin adaptors in producer cells abrogates the Nef antagonizing activity (36, 37). Accordingly, Nef's ability to engage the vesicular biogenesis machinery plays a crucial role for SERINC5 counteraction, as residues required for Nef interaction with clathrin adaptors, as well as residues that regulate interaction with dynamin 2 and the N-terminal myristoylation site, are required for Nef function. Such requirements are also important for CD4 downregulation, indicating that the accessory protein engages a similar mechanism to alter SERINC5 and CD4 surface expression levels. In addition, in line with the rate-limiting nature of endocytosis, the ability of Nef to counteract SERINC5 can be readily saturated by artificially overexpressing the host factor in producer cells.

A recent report observes that, in the presence of Nef, the steady-state level of ectopically expressed SERINC5 declines, suggesting that after internalization the protein is degraded. Accordingly, colocalization with the lysosome marker LAMP1 and restoration of SERINC5 steady-state levels by treating cell cultures with bafilomycin A1 and ammonium chloride support the notion that internalized SERINC5 is degraded into lysosomes (53). Furthermore, SERINC5 degradation was shown to involve K48- and K63-linked polyubiquitination, suggesting that the protein is targeted into multivesicular bodies. However, such a sorting process mediated by Nef remains unclear because ubiquitination of SERINC5 appears to occur independently of Nef itself. Whether degradation of SERINC5 following Nef-mediated endocytosis occurs in different cell types and with the endogenously expressed protein also remains to be established. Although several indications point strongly toward the essential role of vesicular trafficking, the possibility that Nef counteracts SERINC5 via an alternative mechanism was also raised based on observations that antagonism by Nef can be observed in conditions where endosomal retargeting, reduced surface expression, and virion exclusion of SERINC5 are not obvious (41).

Because no reagent is currently available for detection of endogenously expressed SERINC5, artificial overexpression of tagged proteins is required to study the antiviral activity of the protein and the mechanism of Nef counteraction. This poses a challenge for achieving an ectopic expression capable of mimicking the endogenous expression of SERINC5.

Given the great efficiency of Nef in mediating SERINC5 downregulation, it is tempting to speculate that the lentiviral protein has evolved the ability to directly interact with the host protein. Accordingly, a bimolecular fluorescence complementation assay has recently revealed a close association between the two proteins, compatible with a direct interaction (53).

The antiretroviral activity of SERINC5 has no evident species specificity as murine, equine, fish, and amphibious orthologs can all efficiently inhibit HIV-1 (52, 54, 55). By contrast, it was recently revealed that the counteracting activity of Nef from different HIVs and simian immunodeficiency viruses (SIVs) can discriminate between human SERINC paralogs and SERINC5 from different species (54). Göttlinger's group (38) observed that, unlike other *nef* alleles that can counteract both human SERINC5 and SERINC3, Nef derived from HIV-1_{SF2} is active against SERINC5 but does not antagonize SERINC3. The sensitivity of different SERINC5 orthologs to Nef was also found to diverge because amphibian SERINC5 is resistant to different SIV and HIV Nef proteins but is antagonized by Nef derived from SIVmac239. The differential sensitivity to Nef was found to map in the largest intracellular loop of SERINC5, located between the eighth and ninth transmembrane domains (**Figure 1**). Accordingly, susceptibility to Nef-mediated downregulation and exclusion from virus particles could be transferred from Nef-sensitive to Nef-resistant SERINC5 molecules. Two hydrophobic residues in the same region of human SERINC5 (Leu350 and Ile352) were found to play a crucial role because their conversion to alanine is sufficient to render SERINC5 mostly resistant to Nef. Although proof of a direct interaction between SERINC5 and Nef awaits biochemical confirmation, it is tempting to speculate that the region of SERINC5 containing these hydrophobic residues identifies the interaction site with Nef. Mutations of residues Leu350 and Ile352, which render SERINC5 Nef resistant, do not alter the ability of the host factor to inhibit HIV, paving the way to a strategy to create novel SERINC5-inspired antiviral molecules, which could be used to combat HIV.

6. OTHER RETROVIRAL FACTORS COUNTERACTING SERINC5

SERINC genes are widely conserved in the animal kingdom, and unlike most other restriction factors, the ability of SERINC5 to inhibit retrovirus infectivity does not appear to be species specific (54, 55). Accordingly, SERINC5 appeared to have forced the evolution of counteracting

factors in retroviruses other than primate lentiviruses. A serendipitous discovery, driven by the presence of gammaretroviruses in different cell lines (56), allowed the observation that glycoGag of MLV can functionally replace the activity of Nef for infectivity (33). glycoGag is a type-II transmembrane protein translated from an alternative initiation codon located upstream of the gag open reading frame (57). Different reports now confirm that SERINC5 is a strong inhibitor of MLV (37, 38) by impairing the entry process with a mechanism that depends on the nature of the Env glycoprotein (58), as for HIV-1. glycoGag, like Nef, acts as antagonist of SERINC5, despite not sharing sequence homology. Similar to Nef, glycoGag was shown to encode for a putative clathrin adaptor interaction motif essential for the activity on infectivity (59). Accordingly, like Nef, glycoGag causes efficient endosomal relocalization of SERINC5 and prevents its incorporation into virion particles, indicating an antagonizing mechanism that is specular to the activity of Nef.

The impact of SERINC proteins on the evolution of retroviruses was further demonstrated when a third SERINC5 retroviral antagonizing factor was identified in the genome of EIAV (52). EIAV S2 is a 65-amino-acid accessory protein that remained orphan of a known molecular function after its discovery. Like MLV glycoGag, S2 is capable of functionally replacing the activity of Nef on infectivity by counteracting SERINC5 and SERINC3 (52). Recapitulating the features observed for Nef and glycoGag, S2 promotes the intracellular accumulation of the host factor and prevents its virion incorporation. Highlighting its mechanistic convergence with Nef and glycoGag, the SERINC5 counteracting activity of S2 requires a functional endocytic machinery and a putative conserved sorting signal predicted to engage the clathrin adaptor protein AP2. In addition, like Nef, S2 is potentially myristoylated and therefore capable of localizing to the cell membrane, another feature that seems to be essential for the anti-SERINC5 activity of all antagonizing viral factors (52).

Nef, glycoGag, and S2 share no sequence homology and are derived from distinct locations of the retroviral genome (**Figure 4**). The SERINC5 antagonizing activity was therefore acquired independently by distinct retrovirus genera, a sign that this restriction factor has shaped the evolution of diverse retroviruses.

The selective constraint, which forced different retroviruses to acquire factors targeting SERINC5, remains to be fully understood. As explained below, the envelope glycoprotein is a crucial variable, which can render virus particles resistant to the action of SERINC5 on infectivity. Unexpectedly, virions carrying Env glycoproteins derived from Moloney murine leukemia virus (MoMLV) and EIAV remain mostly unaffected by SERINC5 even in the absence of glycoGag and S2 (33, 52). Therefore, the evolution of counteracting factors capable of targeting SERINC5 appears in some cases unrelated to the inherent susceptibility of the retroviral particles to the effect of SERINC5 on infectivity. As we discuss below, it is possible that the antiviral activities of SERINC5, other than their effect on infectivity, could have provided the selective force required for the evolution of antagonizing factors.

As a transmembrane, it is possible that SERINC proteins inhibit enveloped viruses other than retroviruses; thus, other viruses could have evolved antagonizing mechanisms. However, these possibilities await experimental insights.

7. THE IMPORTANCE OF ENV FOR VIRION SUSCEPTIBILITY TO SERINC5

HIV pseudotyped with heterologous glycoproteins derived from viruses, such as vesicular stomatitis virus (VSV-G) and Ebola (Ebola GP), or other retroviruses, such as avian leukosis virus (ALV) and MoMLV, has been known to no longer require Nef for optimal virus infectivity (33, 60–62). Accordingly, it has now been confirmed that susceptibility of the virus particle to SERINC5 is

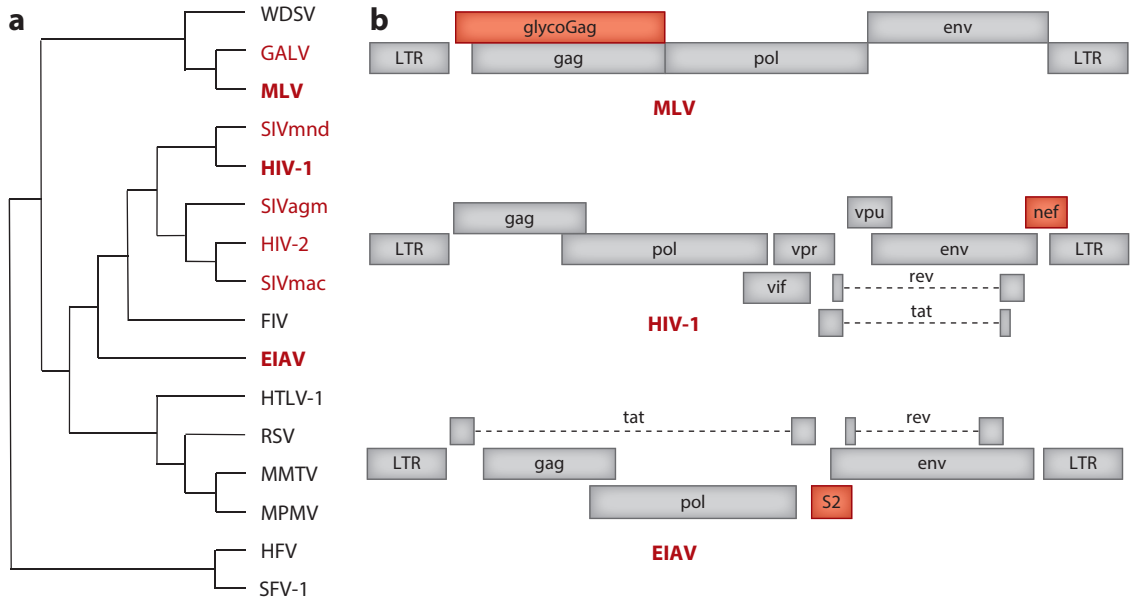

Figure 4

Parallel emergence of SERINC5 counteracting activity in retrovirus genomes. (*a*) The phylogenetic relationship of selected retrovirus species belonging to different genera is shown, as well as the (*b*) genomic positioning of the SERINC5 counteracting factors evolved in HIV-1, EIAV, and MLV. The retrovirus species that encode, or are predicted to encode, SERINC5 counteracting factors are highlighted in red. Abbreviations: EIAV, equine infectious anemia virus; env, envelope glycoprotein; FIV, feline immunodeficiency virus; gag, group-specific antigen; GALV, gibbon ape leukemia virus; glycoGag, type-II transmembrane protein; HFV, human foamy virus; HIV-1 and -2, human immunodeficiency virus 1 and 2; HTLV-1, human T-lymphotropic virus; LTR, long terminal repeat; MLV, murine leukemia virus; MMTV, mouse mammary tumor virus; MPMV, Mason-Pfizer monkey virus; nef, negative factor; pol, polymerase; rev, regulator of expression of virion proteins; RSV, Rous sarcoma virus; SFV-1, simian foamy virus type 1; SIVagm, simian immunodeficiency virus African green monkey; SIVmac, simian immunodeficiency virus rhesus macaque; SIVmnd, simian immunodeficiency virus mandril; tat, transactivator; vif, viral infectivity factor; vpr, viral protein R; vpu, viral protein U; WDSV, walleye dermal sarcoma virus.

determined by the envelope glycoprotein (37, 38, 45, 52, 58) because VSV-G; Ebola GP; and Env from EIAV, ALV, and even from certain HIV isolates can render virions resistant to this restriction factor. How these glycoproteins alter susceptibility to SERINC5 remains unknown. Unlike Nef, glycoGag, and S2, these glycoproteins do not affect the incorporation level of SERINC5 into virus particles but turn virions inherently unresponsive to SERINC5. One hypothesis formulated on different occasions (60, 62, 63) derives from the observation that the heterologous glycoproteins, which render HIV resistant to SERINC5, share a similar ability to trigger endosome-dependent cell entry pathways. Accordingly, VSV, Ebola, ALV, and EIAV glycoproteins were all shown to require conformational events triggered by low pH within an intracellular vesicle to promote fusion with the cell membrane. Env from MoMLV, which is not a strictly pH-dependent envelope glycoprotein, is nevertheless known to mediate entry preferentially via endosomal uptake, although in a cell-type-specific manner (64, 65). The route of viral entry mediated by the uptake of an intracellular vesicle could therefore force the virus through a pathway that overrides the inhibition of the restriction factor (**Figure 2*d***). However, how the endosomal uptake would allow the virus to escape the block imposed by SERINC5 remains to be explained. If SERINC5 acts by increasing the energy barrier required to promote the fusion process, it is possible that the maturing endosome could provide a favorable environment that crucially enhances fusion efficiency. For example,

the low pH in the endosome could lead to protonation of the acidic headgroups of phospholipids, which neutralizes the negative charges and thus lowers the repulsion between membranes and the energy required for fusion in the presence of SERINC5.

As observed for VSV-G and Ebola GP, Env from several primary HIV isolates can also alter the susceptibility to SERINC5 (37, 38, 42, 43, 45, 66) without altering its incorporation into virions. The ability to confer resistance to SERINC5 does not correlate with coreceptor usage of HIV-1 Env because both CXCR4- and CCR5-tropic glycoproteins can equally sensitize virion particles to the restriction factor (66). However, interestingly, the molecular features associated with the different response of HIV-1 Env to the restriction factor were found to lie exclusively within gp120 and to involve the V1, V2, and V3 loops (45, 66). How can the extracellular subunit of Env modulate the sensitivity to SERINC5? Given that the V1, V2, and V3 loops together cooperate for a functional interaction with the coreceptor, one possible explanation could be that some Envs may provide a tighter binding with the cell surface, allowing an endocytic vesicle to engulf the virus and drive it into an endosomal compartment (67). Accordingly, the possibility that HIV-1 entry involves endocytosis has been proposed (68, 69), indicating that HIV could in theory override SERINC5 inhibition by promoting a SERINC5-resistant entry pathway, as discussed above. However, further research is needed to clarify this option because infection of the relevant target cells by HIV-1 was found not to involve an endocytosis-mediated entry pathway (70).

An alternative possibility is that the variable sensitivity to the effect of the host factor could reflect the inherently different abilities of HIV Env glycoproteins to promote entry, linked to conformational differences. It was observed that the ability of HIV-1 Env to confer resistance to SERINC5 is associated with resistance to neutralization by monoclonal antibodies targeting the V3 loop, which correlates with a higher propensity of the glycoprotein to adopt a closed and more stable quaternary conformation (66). Accordingly, Env glycoproteins that are sensitive to SERINC5 are also more prone to undergo spontaneous inactivation in cell-free virus particles, accelerated by the host factor itself (42). Susceptibility to SERINC5 seems therefore to associate with the stability of Env trimers, which is thought to represent a major determinant of the efficiency of fusion (42, 66, 71). Thus, inhibition of infectivity could be the result of an imbalance between the energy provided by Env toward fusion and an increased energy requirement imposed by SERINC5 (**Figure 3**). Accordingly, treatment with the CCR5 inhibitor maraviroc, which increases the energy required for fusion by decreasing the level of the cell-surface coreceptor available, renders virions sensitive to SERINC5 (45). The presence of SERINC5 on the virus particle could crucially increase the requirement of energy necessary for successful fusion to a threshold level that cannot be provided by some Env glycoproteins.

Therefore, it appears that HIV can adopt two distinct strategies to evade SERINC5 inhibition of infectivity—mediated by Nef or by the Env glycoprotein. Because it cannot be saturated by excessive amounts of SERINC5 expression, antagonism by Env appears to be more robust than counteraction by Nef (45). Consequently, it could be expected that viruses with an Env that turns them resistant to SERINC5 were not required to evolve or to maintain an additional mechanism to antagonize the host factor. However, the observations reported so far indicate that this is not the case. Nef was found to maintain a similar ability to counteract SERINC5 irrespectively of whether the specific HIV-1 strain had evolved an Env capable of protecting virus infectivity from the host factor (45). MoMLV also appears to have evolved glycoGag as a powerful SERINC5 counteractor, alongside an Env glycoprotein that provides full protection of virion infectivity (33, 58). Similarly, despite possessing a SERINC5-resistant Env, the lentivirus EIAV has evolved additional SERINC5 counteracting activity with the accessory protein S2 (52). These examples indicate that the two antagonizing mechanisms are not only distinct but also independent. The question therefore arises, Why did retroviruses evolve and maintain redundant mechanisms to

evade the inhibition exerted by SERINC5 on infectivity? One likely possibility is that, in addition to the effect on virion infectivity, SERINC5 exerts other antiviral functions that require its surface expression.

8. THE EFFECT OF SERINC5 ON THE SUSCEPTIBILITY TO ENV NEUTRALIZATION

If the Env quaternary structure seems to determine the susceptibility of the virus particle to SERINC5, the host factor in turns appears to affect Env functionality. This is suggested not only by the ability of SERINC5 to promote inactivation of HIV-1 Env (42) but also by its ability to alter the susceptibility of virus particles to broadly neutralizing antibodies (bNAbs). HIV-1 resistance to some bNAbs targeting gp41 was initially found to be elevated by Nef (43), but such an observation remained orphan of a molecular culprit until susceptibility to these bNAbs was found to depend on the expression of SERINC5 in producer cells (42, 45). SERINC5 renders HIV-1 more vulnerable to antibodies (e.g., antibodies 4E10 and 10E8) targeting highly conserved epitopes, such as the gp41 membrane-proximal external region (MPER), and the gp41-gp120 interface within the trimeric complex (35O22). Conversely, SERINC5 appears to have no or less effect on the susceptibility of the virus to antibodies targeting gp120 (42, 43, 45), suggesting a specific effect on the accessibility of only certain epitopes. Accordingly, SERINC5 could act directly by altering the Env structure and favoring the exposure of the MPER residues. The ability of SERINC5 to promote inactivation of HIV-1 Env (42) indicates an effect of SERINC5 on the structural stability of Env, consistent with the higher susceptibility of HIV-1 to the 35O22 antibody, which targets the quaternary structure of gp120-gp41 interface (45). In addition, the effect of SERINC5 on Env structure could also result in a slower refolding, leading to the formation of the six-helix bundle and exposing the virus to the effect of agents targeting intermediate fusion conformations, as observed by Sood and coworkers (42). However, a direct effect of SERINC5 on Env structure would postulate an interaction of the host factor with the Env glycoprotein, which has not yet been documented.

Although a full understanding of this activity of SERINC5 awaits further investigation, it appears clear that the antiretroviral activity of SERINC5 is not limited to the effect on intrinsic virion infectivity and that it is a more complex phenomenon, involving the responsiveness of the virus particle to the host's immune environment. Accordingly, HIV Env glycoproteins capable of resisting inhibition of infectivity due to virion-associated SERINC5 remain sensitive to the effects of the host factors on susceptibility to antibody neutralization. Therefore, although HIV can antagonize the effect on infectivity by developing a SERINC5-resistant Env, downregulation and exclusion from virions remain requirements for the protection of HIV from other potentially deleterious effects of SERINC5, such as an increased susceptibility to neutralization.

The Env glycoproteins of different retroviruses share structural similarities and are expected to promote an analogous fusion mechanism. Accordingly, a hydrophobic MPER domain targeted by neutralizing antibodies is not only present in HIV-1 Env but is also present in the Env TM subunits of other lentiviruses, including EIAV and feline immunodeficiency virus (72, 73), and gammaretroviruses (74). The ability of SERINC5 to target the susceptibility of Env to neutralization might not be restricted to the glycoprotein of HIV-1. The evolution of factors capable of targeting SERINC5 in EIAV and MoMLV, which already encode SERINC5-resistant Env, could have been driven by the requirement to escape the antiretroviral activities of SERINC5, such as its effect on neutralization. Therefore, the latter could at least have contributed to keeping the selective pressure to maintain the SERINC5 downregulating activity.

9. THE RELEVANCE OF SERINC5 FOR RETROVIRUS SPREAD

Three retrovirus species, which were found to evolve SERINC5 counteracting activity, replicate preferentially in blood cells. This correlates with the high expression of SERINC5 in lymphoid and myeloid cells, as deduced from public gene-expression databases (75) and from gene-expression analyses on cell lines and primary cells (23, 37). Thus, the antiviral activity of SERINC5 could represent a significant antiviral barrier for retroviruses that are adapted to replicate and persist in hematopoietic lineages. How important is such a barrier? The evidence that three different retroviruses have independently evolved a similar antagonizing mechanism is a strong indication that SERINC5 poses a strong selective pressure on retroviruses and therefore that the antagonizing activity is crucial for persistence in the host population.

Further insightful information comes from comparing the SERINC5 counteracting activity of *nef* alleles derived from natural infections of HIV and SIV. An earlier study had already indicated that the activity of Nef on infectivity is highly conserved and preserved under strong selective pressure within an individual during the infection (76). It has now been observed that the anti-SERINC5 activity is indeed highly conserved across *nef* alleles from all primate lentivirus species (55). Accordingly, anti-SERINC5 activity is detected in all HIV-1 and HIV-2 lineages as well as in all SIV species. In addition, during HIV-1 evolution, Nef appears to have gained anti-SERINC5 activity potency after transmission of SIVs from monkeys to chimpanzees. Interestingly, inversely correlating with pathogenicity, the counteracting activity of HIV-2 Nef appears attenuated compared to HIV-1 Nef. A positive correlation was instead reported between the potency of anti-SERINC5 activity and the prevalence of SIV in the respective monkey and ape species, suggesting that SERINC5 antagonism could determine the ability of primate lentiviruses to spread within the natural host species and confirming the importance of the antagonizing activity for retrovirus evolution (55). Although the effect of SERINC5 on infectivity is the phenotype most prominently visible in culture, it remains to be determined whether other activities, such as the effect on the susceptibility to neutralization or other antiviral activities yet to be uncovered, contribute to the requirement of antagonizing SERINC5 in vivo. Since *nef* alleles from HIV and SIV appear to equally efficiently counteract orthologs from different primate species, SERINC5 is unlikely to represent a barrier against cross-species transmission, at least between primates (76).

10. UNUSUAL FEATURES OF SERINC5 AS A RESTRICTION FACTOR

What we now call restriction factors constitute a class of molecules that target different stages of the viral life cycle and provide an immediate barrier to the invading virus complex. Most of these host factors play a bigger role in the context of the innate immune system, regulated by PRRs and interferons. Interferons are endowed with pleiotropic functions, including the activation of professional phagocytes and the induction of genes with antiviral functions, including most of the currently known restriction factors. In turn, some restriction factors have been shown to function as pattern recognition receptors by sensing the presence of retrovirus components and contributing to proinflammatory signaling and the establishment of the antiviral state. Another feature common to most restriction factor genes is the acquisition of distinct evolutionary genomic signatures resulting from a positive selection pressure exerted by the counteracting activity of the virus (77). Despite having restrictive activity on retrovirus replication, SERINC genes do not appear to have these common attributes.

Expression of SERINC genes is not induced by interferon nor by strong interferon inducers, such as lipopolysaccharide, in cells known as highly responsive to proinflammatory stimuli, such

as primary dendritic cells (37, 38). Since the interferon system came into existence in vertebrates after whole-genome duplication during the course of evolution, the already present invertebrate SERINC might have escaped this regulatory gene layer (78). SERINC appears to function as a restriction factor intrinsically expressed in crucial cell types, such as the hematopoietic lineages.

Adding to the atypical characteristics for a restriction factor, no evidence for positive selection in primates was detected among SERINC5 and SERINC3 genes of primates, which seem subjected to purifying selection (79). The lack of positive selection is in line with the evidence that the retroviral factors counteracting SERINC5 lack the fine species specificity observed with other restriction factors.

The evidence that SERINC gene orthologs are universally conserved in eukaryotes and traced to distant species is also a unique feature that has not been observed in any other known restriction factor. This suggests that the SERINC family plays an important conserved function beyond viral restriction, which could have posed a dominant evolutionary constraint. Whether these host factors interplay with the immune system by acting as PRRs and contributing to the host's ability to sense the retrovirus infection remains to be determined. Altogether, such unusual features delineate a rather unique class of host antiviral factors.

11. CONCLUSIONS

Given their unusual features that deviate from the canons delineated previously, there could be a question as to whether SERINC proteins should be formally classified as restriction factors. Nevertheless, SERINC5, and possibly other SERINC genes, must play a fundamental role in regulating the interaction with the host because three retrovirus species have independently evolved the ability to target SERINC5. Moreover, the SERINC5 counteracting ability and the downregulation activity of CD4 are the most conserved features of Nef from HIV and SIV. Such conservation is in striking contrast with the variable susceptibility of different isolates to the effect of SERINC5 on infectivity, dictated by the Env glycoprotein. Hence, SERINC5 is likely to play a pleiotropic function, the inhibition of infectivity being only one of its activities. Future research will hopefully reveal the full implications of the SERINC genes in the interaction of viruses with their hosts and the possibility of exploiting their antiviral activity to combat pathogenic retrovirus infections.

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