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On Viruses, Sex, and Motherhood

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Mammals, being viviparous, pose an interesting immunological dilemma. They have highly adaptive immune systems that fail to recognize their own allogeneic embryos. In a sense, mammalian embryos resemble parasites that must suppress their mothers' immune recognition systems to survive. Mammals are also unique in that their genomes are highly infected with endogenous retroviral agents. As retroviruses are also generally immunosuppressive, I here considered the participation of retroviruses in the immunosuppression by the embryo and the evolution of placental orders.

VIRUS-HOST EVOLUTION

Some viruses have closely coevolved with their hosts. The phylogenetic congruence between these types of virus and their hosts can be very tight (73) and difficult to explain. This has been observed with both small DNA viruses, such as human papillomavirus types 16 and 18 (see reference 2 for a review), and various retroviruses (e.g., human T-cell lymphotropic virus type 2 [HTLV-2] [60]) in that the viral phylogenetic distribution is closely congruent with the host's racial and geographical distributions. This distribution appears in marked contrast to that of the relatively new human immunodeficiency virus type 1 (HIV-1) pandemic, which is geographically distributed, as predicted from mathematical models, based on horizontal transmission and a predator-prey-like relationship of virus to host (1). A similar geographical distribution pattern is seen with primate leukemic viruses (including HTLV-1 and simian T-cell lymphotropic virus type 1 but not the progenitor HTLV-2), which also appear to be spatially distributed into various adjacent primate species, implying multiple interspecies transmissions (85).

CONGRUENCE OF HOST AND ENDOGENOUS RETROVIRUS

In contrast to HIV-1, an extremely close congruence exists between the genetic identities of various endogenous retroviruses (ERVs) and their mammalian hosts. All members of mammalian species appear to be infected with ERVs, some of which are thought to be horizontally acquired (as in rodents) but most being stable proviruses in the germ line. ERVs are particularly prevalent in the germ lines of rodents. These genomic agents are sufficiently unique to identify specific strains of mice (10). Yet these viruses appear to be inefficiently transmitted in wild rodent populations, and many species (including *Mus domesticus*) are often free of the more common endogenous viruses (9, 11). Nevertheless, one family of ERV, the intercisternal A-type particle (IAP), is found in all rodents examined. The human genome is similarly infected with highly conserved ERVs which have a low degree of similarity to any known exogenous retrovirus (49).

IAPs are genomic retroviral agents which are expressed at high levels in various extraembryonic and some lymphoid and transformed mouse tissues. IAPs which can code for *gag*, reverse transcriptase (RT), and *env* gene products are common to all mouse strains and appear to be present in the common ancestor of all rodents (50, 69) (for a review, see reference 10). Other mammalian species also appear to have conserved IAP-like ERVs, such as the related human ERVs (HERVs), which are also found in all primates and expressed in trophectoderm (see references 47 and 49). Two classes of ERVs exist which have either a murine leukemia virus (MuLV)/HERV-E (class I)- or a mouse mammary tumor virus (MMTV)/HERV-K (class II)-like RT gene, but many more deleted defective ERV versions are known (27). However, it has now become clear that full-length IAP-like genomes are present at low copy numbers in all mammals examined, including various species of mice, rats, and Syrian hamsters (13). Various confusing names exist for HERV-like agents found in human genomes (e.g., RTLV, RTVL, RTLH, and HEV; for reviews, see references 47 and 49). It is now established that the low-copy-number nondefective HERV (RTLV-Hp or ERV-3) exists in two RT types (87) which arose early in primate evolution but have been conserved in both Old World and New World primates (77), as well as in marmosets and squirrel monkey lineages which predate the divergence of the New World and Old World primates (87). Although the full-length RTLH copy is conserved (77), defective copies have recently expanded in the genomes of hominoids (25) and are generally numerous in mammalian genomes (14). In contrast to the numerous and rapidly evolving defective copies, both the chromosome location and copy level of known *env*-encoding primate and mouse (IAPE-A) endogenous retroviruses are highly conserved across related species (49, 70, 74).

MAMMAL-SPECIFIC RETROPOSONS

Due to sequence similarity of the HERV-E/MuLV RT gene to these repeated elements, a functional ERV may have been the master genome that was the source, via retrotransposition, of reverse-transcribed mRNA for generation of the roughly 100,000 copies of the long interspersed elements (LINEs) (e.g., LINE-1, with RT-like genes) and mammalian-wide interspersed elements (MIR) and medium reiteration frequency sequences (MER) non-RT retroelements) found in all mammals but not in lower vertebrates such as birds or reptiles (see references 40 and 75 and references cited therein). It is interesting to note that these families of repeated elements are unique to mammals, being especially prevalent in placentals, with each species having only one highly abundant LINE-1 family. Furthermore, all mammal-specific repeated sequences are related to each other, yet no ancestral version of these elements appears to have existed prior to mammalian radiation (40). It is currently difficult to explain why mammalian radiation is so closely associated with these elements or how they evolved.

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THE PREVALENCE OF DISEASE-CAUSING AUTONOMOUS RETROVIRUSES

In rodents, the prevalent autonomous mouse retrovirus, MMTV, has sequence similarity to endogenous mouse IAPs (57), although MMTV distribution is not correlated with host taxonomic distribution (23) as is IAP distribution. Most wild mouse populations (*Mus musculus*, *Mus castaneus*, *M. domesticus*) are MMTV (as well as MuLV) free (37). MMTV, however, is generally nonpathogenic in feral mouse populations, and when virus is isolated from these animals, it is poorly infectious (11). In humans, HTLV was the most prevalent autonomous retrovirus prior to the HIV-1 pandemic, but it shows limited similarity to endogenous HERV-K (42). Similarly, HTLV-2 is nonpathogenic in human populations and poorly transmitted, although HTLV-1 can induce immune and neurological disease (see reference 19 and references cited therein). Yet neither HTLV-2 nor the more recent HTLV-1 is broadly prevalent in the human population. Various class I and class II HERVs (such as HERV-K and HERV-R, which encode *env*) exist; however, no autonomous virus has been observed that resembles these HERVs. A similar situation applies to the baboon endogenous virus (BaEV) and the feline RD114 ERV, which also lack a related autonomous retrovirus. Yet all these ERVs are expressed in placental tissues (see reference 49 and references cited therein). What, therefore, is the selection for the maintenance of these ERVs? Autonomous retroviruses can be found in nature. For example, many primate species are widely infected with foamy virus but have no associated disease. It does appear that the more phylogenetically recent class of retrovirus, such as the HIV lentivirus, is associated with disease, encodes additional regulatory genes, has complex RNA splicing, and has hypervariable *env* genes, but has no proviral counterpart (10, 67), suggesting that these agents are a recent evolutionary occurrence. Feline leukemia virus (FeLV) is also considered a successful horizontally transmitted retrovirus that causes lethal disease and a high incidence of tumors in domestic cats. Yet FeLV persistently infects fewer than 1% of feral or stray cat populations and is seldom isolated from other free-living felid species, whereas all cats have endogenous copies of FeLV-like genomes with variant U3 regions (30). Therefore, most naturally prevalent autonomous retroviruses are relatively nonpathogenic for their natural host populations. Those retroviruses that are pathogenic require very intimate contact for transmission and are otherwise inefficiently transmitted horizontally.

Accordingly, the prevailing view that selection for the endogenous form of a nondefective retrovirus provides some protection from acute disease due to autonomous retrovirus infection cannot account for the conservation of the nondefective endogenous virus since these viruses are seldom sufficiently disease causing in natural populations or settings. Nor are the conserved ERVs often sufficiently similar to existing autonomous retroviruses to provide such protection. In fact, basic genetic principles of virology would predict quite the opposite in that intact endogenous genomes would provide the genetic material to allow recombination during infection with a less adapted or more defective second retrovirus (21). Such genetic behavior is commonly used to rescue various types of defective virus by using cell lines that have part or all of the missing viral gene. It has been reported that an ERV (MMTV) can be protective of acute disease (20, 22), but given that infection by one provirus may actually increase the risk of a second retrovirus infection (15), there appears to be contradicting evidence concerning the protective role of a nondefective ERV. In addition, all these deliberations seem moot if retroviruses seldom induce acute pathogenic disease and if such disease is not a prevalent threat to the survival of natural host populations as the above observations would suggest. Yet as recently reported for the mouse IAPE-A (nondefective *env*-encoding locus), both the copy number and chromosomal position are highly conserved in *Mus* species, yet defective loci are highly evolving (70). Why then is a nondefective version of a retrovirus so highly conserved in mammals?

A METAVIRUS MAY PROVIDE HOST GENETIC FUNCTION

In light of the fact that these low-copy-number complete ERVs are more prevalent and better conserved in their host genomes than their autonomous or defective viral cousins, it is worth considering what biological relationship might account for the high level of conservation and phylogenetic congruence of nondefective ERVs and their mammalian hosts. What then is the biological role of a functional endogenous nondefective provirus in a mammal? The following hypothesis will provide a perspective from which this issue will be considered; it is referred to here as the metavirus hypothesis. The prefix meta derives from Greek and means transcending: the genetic distinction between the host and viral genomes will be lost (transcended) if virus infection is essential for host survival. In other words, virus and host will be phylogenetically identical. It is proposed that because mammals are viviparous and their embryos are sexually produced and antigenically distinct from their mothers, all mammals express ERVs in extraembryonic tissue during embryo implantation and growth in order to suppress the local initiation of recognition by the mother's immune system and allow growth of the embryo. This concept is a generalized hypothesis consistent with the specific proposal by Boyd and colleagues that ERV-3 *env* gene expression in human embryos may mediate immune suppression (86). Nondefective ERVs are therefore absolutely required for viviparous embryonic development. In addition, the mother's immune cells must be susceptible to suppression by these ERVs. As a consequence, a second hypothesis can be proposed: the required production of ERV in an early mammalian embryo also provides an enhanced evolutionary potential for germ line infection by retroviruses and retroposons. The infection of the germ line by various genetic derivatives or recombinants of ERVs to generate genomic derivatives, such as long terminal repeat (LTR)-containing defective ERVs and IAPs or more defective and distant relatives, such as MERs, MIRs, and non-LTR LINEs, would be much more likely if all early embryos must make a functional ERV. In addition, the presence of a functional (but not necessarily contiguous) ERV genome in the germ line and its need to suppress immunity can also allow for the selection of various genetic derivatives, recombinants, and variants which can yield new autonomous infectious lymphotropic retroviruses. This proposed scenario would explain the proposal of Doolittle and colleagues, and later McClure, that all mammalian retroviruses appear to be monophyletic and may have evolved from the ERV progenitors (IAPs and HERVs), as determined on the basis of phylogenetic analysis of mammalian retroviruses and retrotransposons (14, 54). Mammalian genomes are therefore expected to be able to yield autonomous retroviruses that otherwise might not be efficient agents of horizontal transmission (such as MMTV or HTLV-2). The original evolutionary source of most of these autonomous and defective retroviral parasites is therefore a required ingredient of the host genome.

If a functional ERV is an essential host system for transient immune suppression, then it can be expected that various se-

lective conditions (such as the growth of most tumors) can also select for immune evasion, and hence immune suppression, by selecting for ERV or retroviral gene production. If so, following selection for ERV or IAP production in somatic tissues, additional selection (for various host genes that affect virus and cellular growth, such as *env*, *sag* [MMTV], *rex*, *tax* [HTLV], *tat*, *rev* [HIV], etc., as well as altered LTRs such as those with lymphatic or mammary tissue tropism) can eventually yield various retroviral derivatives, now called transforming retroviruses, which sometimes may be transmitted to the same or related species. The in vivo selection for Sag production by a Sag-defective endogenous MMTV to allow milk-borne infection has been observed and could be an example of such selection from an endogenous MMTV provirus (21). Also, the radiation-induced generation of the murine AIDS virus complex from a mutated endogenous retroviral $p12^{gag}$ in the C57Bl/6J mouse is another possible example of autonomous virus generation from endogenous virus (43, 44). A similar proposal could also apply to the possible generation of the HIV *env* gene from HIV-like endogenous EHS-2 sequences that are unique to primates (35), suggesting the existence of an interesting dynamic relationship between virus and host in which the host can be a genetic source for generation of new virus.

THE ERV-HOST RELATIONSHIP

It is therefore crucial to consider the natural biological relationship between nondefective ERVs and their mammalian hosts and to examine evidence relevant to the above-mentioned proposal. When and where are ERVs and IAPs made, and what are the consequences? In fact, ERVs are very efficiently produced in vivo. IAPs are made to a level of greater than 10^5 particles per cell (66), budding from the endoplasmic reticulum into cisternal structures in all wild and domestic early mouse embryos (8, 36, 88), but only in specific highly differentiated cell types (e.g., parietal trophectoderm [36]). In humans and rhesus monkeys, it has been long established that HERV-K and related RT, *gag*, and *env* gene products (and also the ERV-3 *env* gene product) and particles are efficiently produced in placental syncytiotrophoblastic cells and villous trophoblasts which are directly exposed to maternal blood (4, 38, 46, 58, 80, 84, 86). Retroviral particle production is also seen in some normal oocytes (62) and most placentas (51). In fact, most mammalian embryos (trophoblast tissues) examined to date, including those of feral mice (8) and humans, express IAPs to high levels. It was previously thought that IAPs were all deleted in *env* and other genes (52), leading to a confused view that all IAPs were defective, while in fact many defective IAPs (including LINEs) appear to be transcribed in the embryonic cells (5). Subsequently, however, it became clear that complete genomes of human ERVs with nonterminated *pol* (87) and *env* (4, 34, 46, 69, 86) sequences exist and that embryonic (as well as placental and tumor) tissues express all the IAP gene products, including the p15E domain of the endogenous *env* gene, to high levels and also make viral particles (4, 46, 86, 87). The intact RTLV-H genomes in humans and IAPE-A in mice are of relatively low copy number and are phylogenetically conserved, whereas defective copies evolve rapidly (70, 87). If ERV expression is required for transient immune suppression, conservation of functional copies would be positively selected while amplification of ectopically expressed copies would be counterselected. Thus, it appears that the natural site of significant mammalian retrovirus production is the early embryo (especially the trophectoderm and placenta) in all mammals so far examined.

If we consider the established biological activities of the ERV and IAP gene products, a pattern emerges that suggests the existence of a biological role. The IAP group-specific antigen (p73 Gag) has been characterized as a variant of a cellular gene identified as the immunoglobulin E binding factor (45). Thus, p73 IAP *gag* is a cellular gene that regulates various aspects of the humoral immune response. In addition, recent studies using antibodies against synthetic peptides corresponding to the predicted transmembrane domain of IAP p15E now provide clear evidence that endogenous *env* sequences are expressed at a high level in trophoblast tissue and code for the p15E immunosuppressive transmembrane domain (4, 86). This immunosuppressive transmembrane domain region is characterized by a well-conserved sequence of 17 hydrophobic amino acids (CKS-17) found in the *env* genes of most nondefective mammalian retroviruses (4) and may explain why most other retroviruses, such as FeLV, are also immunosuppressive (24). The immunosuppressive function of the CKS-17 *env* region was initially established by FeLV studies in cats. FeLV kills most cats due to immune suppression involving defects in macrophage activation and cytotoxic T-lymphocyte response which allow various lethal secondary infections (see reference 31 for early references). FeLV can also cause fetal abortions and resorption due to placental infection that damages maternal-fetal attachment. This immune suppression can be elicited by soluble FeLV p15E, which abrogates blastogenesis (53). HTLV-1, HTLV-2, and HIV each has an immunosuppressive CKS-17 domain which inhibits T-cell proliferation (72). Most transformed cells (including human [17, 68] and some common canine [33] tumors) are also associated with expression of ERVs or the p15E product. These soluble p15E-like factors from humans can affect monocyte-to-macrophage activation (29). Therefore, there is considerable evidence that p15E is immunosuppressive. Interestingly, other viruses that are poorly immunogenic (e.g., Marburg and Ebola viruses) but are not prevalent in the human population and are able to cause severe acute disease also appear to have the p15E immunosuppressive domain as part of their putative Env proteins (6), suggesting that these agents may also have adopted this putative host immunoregulatory system. However, it seems unlikely that the p15E domain alone can account for the full immunosuppression of most retroviruses because when mink cell focus-forming virus type 13 p15E is expressed in vaccinia virus vectors, immunological reactivity is not always prevented (12). It therefore seems more likely that full immunosuppression by a retrovirus involves additional viral genes, such as *gag*.

THE IMMUNOLOGICAL DILEMMA OF MOTHERHOOD

The lack of a maternal immunological response to the mammalian embryo allograft has been a most perplexing and enduring issue in immunology and evolutionary biology that has defied explanation (for a review of mammalian maternal-embryo immunity, see reference 63). Early mammals (egg-laying species and marsupials) limit maternal-fetal contact with a maternally derived shell membrane that shields the embryo, along with very short gestation periods (65). Embryos of viviparous mammals, however, first differentiate the extraembryonic trophectoderm which will produce the placenta, allowing intimate maternal-fetal contact. This trophectoderm is unique in that it expresses only paternally derived genes (81). Low-level major histocompatibility complex class I expression in mouse embryos has been proposed to prevent maternal recognition, but interferon-mediated induction of major histocompatibility complex class I expression in mouse embryos does not lead to rejection (32). It has recently been suggested by Boyd and colleagues that HERV production is involved in

preventing immune clearance of an embryo by the mother's immune system (86). Given that trophoblast differentiation is required for implantation to occur (39), that differentiating trophoblast tissue (but not inner-mass cells [4, 86]) also makes HERVs and IAPs (29, 39, 80), and that trophoblast cells (but not the zona pellucida) protect inner mass cells from macrophages (76) (possibly by altering the effects of interleukin-1 and other cytokines [28], which are inhibited by leukemia inhibitory factor [79]), this proposal seems well founded. It seems likely that local immune cells, such as macrophages, which are highly prevalent in the placenta and uterus (27) and are responsive to the interleukin produced during implantation, are the likely natural targets of ERVs (IAPs and HERVs). Macrophages and macrophage-derived growth factors normally have an essential role in the initiation of cellular and humoral immune responses. Following embryo implantation, placental macrophages will be in close contact with IAP-producing trophectoderm. Such cocultivation should allow efficient transfer of cisternal IAPs into local monocytes and macrophages, inhibiting their required role in the induction of the immune response and thereby aborting maternal immune recognition of the fetus. However, because mammals give birth to live young and their semiallogeneic embryos have no protective rapidly expressed eggshell such as that of birds, all members of the class *Mammalia* will absolutely require embryonic and placental ERVs to prevent the initiation of a maternal antiembryo immune reaction. Conversely, the immune cells of mammals must by necessity remain susceptible to ERV suppression activity; hence, as proposed, there will be a general tendency for mammalian immune cells to be infectable by retroviruses. However, no evidence for HERV or IAP replication in infected maternal immune or other cells has been reported, suggesting that IAP replication may not be transmitted by maternal cells. Therefore, immune system-suppressing ERVs may be replication defective. It is interesting that about one-third of human pregnancies (see reference 49) are associated with antibodies to HERV given that trophectoderm tissue may express paternally derived genes and that the mouse class I (IAPE-A) *env* gene maps to the Y chromosome (70).

As a consequence of the required high level of ERV production in the early embryo, there will be an increased likelihood of the germ line being infected by variants of the two ERV classes. Therefore, the tendency of mammals, but not other vertebrates, to accumulate defective proviral variants of ERVs (such as LTR-containing elements or more distant and defective LINE-1 or MIR retroposon elements) into their germ lines can be rationalized. This could explain why each placental species possesses its own unique version of these related genetic elements.

A direct prediction of this proposal is that the loss of functional ERV genome expression in an embryo will prevent implantation and be lethal. This prediction is, however, difficult to test because of the very numerous defective copies of ERVs in the mouse genome. In addition, viral genes need not be contiguous for an ERV genome to be functional. As noted by Reuss and colleagues, "coexpression of multiple defective IAP or IAPE genomes in a cell could lead to the production of an infectious virus particle in the absence of complete genomes" (70). However, it is interesting that the mouse embryonic lethal mutant (agouti *y/y*) expresses IAPs in ectopic tissues (56), given that normal nonlymphoid tissues are highly repressed for IAP production (13), possibly due to IAP insertion. The homozygous agouti yellow mouse is prone to obesity as well as to a wide variety of tumors and fails to implant early embryos due to trophectoderm defects (64), suggestive of a role for IAP in implantation and immune suppression. Although intriguing, clearly this issue needs to be tested more directly to be compelling.

Thus, ERV made by the embryo may provide a solution to the immunological dilemma of motherhood. These ERVs, which would be essential for all placental orders, are herein named metavirus and are proposed to be the normal host system for inhibition of the induction of a mother's embryonic immune recognition. It should also be noted that this metavirus hypothesis is distinct from the protovirus model proposed years ago by the late Howard Temin (83) in that ERVs are not proposed to control most normal host development or immune cell differentiation but rather are thought to specifically and transiently repress the local development of maternal immune recognition of an embryo.

A RATIONALE FOR RETROVIRUS-MEDIATED IMMUNOSUPPRESSION

This proposal raises the question of why immune suppression for fetus survival would require a retrovirus system such as an ERV. Why not simply evolve a genetic program in the mother's genome to suppress immune reactions to embryonic antigens? Since the presence of a fetus is an occasional event in the mother, a rather temporary and local mechanism to prevent embryonic immune recognition but allow the mother to react to most pathogens is desirable. It would not be beneficial if the mother's germ line were systemically reprogrammed to be immunologically incompetent. How then could such a transient and perhaps local immune suppression be achieved? This type of immune suppression is similar to that which occurs upon infection with various viruses. Therefore, a way to attain transient suppression would be to affect committed or more highly differentiated maternal immune cells so that they do not initiate immune recognition of the embryo, rather than to affect the ongoing differentiation of other immune cells with other antigenic specificities. A retrovirus would be an ideal way to affect the genetic program of such highly committed initiator cells (such as monocytes or macrophages) without preventing other immune reactions or the subsequent potential for continued immune-cell differentiation. In addition, we now know from animal studies with recombinant retroviruses that primary infections with most nonreplicating recombinant viruses tend to be restricted to local tissues at the site of infection, as most viruses are quickly absorbed. For example, blood infection with recombinant MuLV is short-lived, with a half-life measured in minutes. Defective virus therefore does not generally spread systemically. Since HERVs and IAPs do not appear to propagate after transmission to maternal cells and are repressed in most nonlymphoid somatic tissues (13), ERVs may act much like a defective recombinant retrovirus, affecting only those cells they directly enter, and need not replicate. As high-level IAP production normally occurs only in the embryo (including the placenta and cord blood), the situation resembles that of using a recombinant nonreplicating virus in that the local immune cells will be most affected. Thus, it could be that the initiation of an immune reaction to the fetus is suppressed by the high-level continuous ERV production, not the mother's response to other agents.

THE EMBRYO AS AN INFECTIOUS PARASITE: A PARASITE THAT BEGETS HOST

The need of the embryo of the viviparous mammal to make ERV in order to suppress the mother's immune reaction is very reminiscent of a parasitic event, the embryo being the maternal parasite that must suppress immune recognition by

the mother (the host). In this light, such a relationship resembles the high-level production of the genomic (nonautonomous) polydnavirus that occurs during the differentiation of the eggs of endoparasitoid hymenopteran wasp species (for a review, see reference 48). The fertilized wasp egg, along with a very high concentration of wasp-encoded polydnavirus, is injected via the ovipositor into parasitized host larval species. The polydnavirus then suppresses the host immunity of the parasitized larva, which would otherwise recognize and kill the foreign wasp egg by non-antigen-specific cellular and phagocytic mechanisms. The polydnavirus also profoundly affects host hormonal physiology but does not replicate in the parasitized host larvae; nor are there known free-living versions of polydnaviruses. The entire family of polydnaviruses (consisting of multiple circular double-stranded DNA genomes which may encode separate genes) is found only as endogenous elements of the genomes of endoparasitoid wasp species. Thus, the entire order of endoparasitoid hymenopterans appears to have conserved the ability to produce a species-specific version of genomic polydnavirus. Therefore, the strategy of using a genomic virus (metavirus) to avoid immune recognition may not be unique to mammals.

RETROVIRUSES IN NONMAMMALIAN SPECIES

Other organisms, such as *Drosophila* species (78), also appear to use gypsy-like retrovirus production in eggs in order to affect sexual and developmental events that can alter breeding success, as seen with hybrid dysgenesis (for a review, see reference 41). Even yeasts appear to have employed an infectious retroviral agent for the early evolutionary creation of the mating type locus prior to the *Saccharomyces-Schizosaccharomyces* divergence (see reference 41 and references therein). In plants, low-copy-number nondefective endogenous copia-like retroviruses are also phylogenetically conserved and congruent with host species (16). Therefore, infectious and genomic versions of retroviruses have clearly been prevalent since well before the evolution of viviparous (non-egg-laying) mammals. However, ERVs and their related retroposon derivatives (MIRs and LINEs) are especially prevalent in rodents and other placental orders, and each species has only one highly abundant LINE family element of related but unknown lineage. Although not placental, many bird species appear to harbor various retroviruses, especially those of the avian leukosis virus family (71). In addition, two families of avian leukosis virus-related ERVs (RAV-0 and EAV-0) are known for *Gallus* species (3). These avian ERVs, however, are not found in all *Gallus* species, and the phylogenetic distributions of the host and RAV-0 genomes are not congruent (18). The EAV-0 family of ERVs is found in most *Gallus* species (3), but unlike the situation in mammals, no relative of EAV has been identified in other avian species, such as the goose, quail, or turkey (even under nonstringent hybridization conditions) (26). Thus, these avian ERVs all appear to be recent acquisitions of their host species (61) and are not broadly conserved across species like their mammalian ERV, LINE, or MIR counterparts. In addition, although avian species have conserved non-LTR retroposon-like small repeated sequence elements (such as chicken repeat CR-1 or the related Art-CH [61]), which can also be found in reptiles (7), these retroposon elements are distinctly different from the larger LINEs and MIRs of placental orders (40) in that they lack conservation of the precise 5′ sequence, the *pol* coding sequence, or the 3′ poly(A) track (7). Birds have no analog to the mammalian repeated sequences which are families of species-specific retroposons (40). It therefore appears that although birds harbor retroviruses, the relationship between avian and ERV genomes is very different from that between the genomes of mammals and ERVs.

VIRUS INFECTION AND HOST SPECIATION

I have proposed that an infectious event may have been crucial for the evolution of all placental orders, similar to the evolution of endoparasitic wasp orders. This proposal raises a much larger issue concerning the genetic forces that drive host speciation or the formation of new biological systems because it suggests that a principal driving force for speciation or genetic innovation is a functional virus-like parasitization. Given the enormous rates of evolution that a virus can show relative to those of host genes (a millionfold excess [55]), it seems an impossible task for the host to ever keep pace with such rates of viral adaption. Virus parasitization itself, however, could provide the creative genetic force needed for the host to originate new molecular systems that distinguish self from nonself and provide immunity. Clearly, a virus must distinguish its own genome from that of the host. During infection, a virus can therefore superimpose new systems of molecular genetic identity onto the host. For example, parasitic plasmids of *Escherichia coli* that encode restriction modification enzymes can compel the host to maintain the plasmid in order to avoid host DNA degradation (59). But once infected, these host cells are immune to other nonmethylated plasmid or *E. coli* DNA. These new genetic identities could then lead to new host species, new host systems of immunity to other viruses, and new systems that alter host responses to other parasite or environmental cues (for example, the starvation responses of lysogenic phage in bacterial hosts). Such a process could explain the high degree of congruence between host and some viral phylogenetic data as both host and viral genetic identities are tightly linked. Such parasitic evolution also offers a solution to a major dilemma of biological systems. Most orders increase in genetic complexity with time (82). A process of parasitic evolution should be unrelenting and cumulative, especially if the host itself can be a source of virus and drive the host genome to increase in complexity with evolution. Yet it appears that in order for an organism to utilize such parasitic evolution, it must maintain some degree of vulnerability to virus-like parasitization in order to be able to diversify as rapidly as could be needed. Thus, the rapid radiation of placental orders may be linked to their infectability by retroviruses, suggesting that the genetic identities and fates of the host and the viral parasite may be linked at the most fundamental level.

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REFERENCES

1. **Anderson, R. M., R. M. May, M. C. Boily, G. P. Garnett, and J. T. Rowley.** 1991. The spread of HIV-1 in Africa: sexual contact patterns and the predicted demographic impact of AIDS. Nature **352:**581–589.
2. **Bernard, H. U.** 1994. Coevolution of papillomaviruses with human populations. Trends Microbiol. **2:**140–143.
3. **Boyce-Jacino, M. T., K. O'Donoghue, and A. J. Faras.** 1992. Multiple complex families of endogenous retroviruses are highly conserved in the genus *Gallus*. J. Virol. **66:**4919–4929.
4. **Boyd, M. T., C. M. Bax, B. E. Bax, D. L. Bloxam, and R. A. Weiss.** 1993. The human endogenous retrovirus ERV-3 is upregulated in differentiating placental trophoblast cells. Virology **196:**905–909.
5. **Branciforte, D., and S. L. Martin.** 1994. Developmental and cell type specificity of LINE-1 expression in mouse testis: implications for transposition. Mol. Cell. Biol. **14:**2584–2592.
6. **Bukreyev, A., V. E. Volchkov, V. M. Blinov, and S. V. Netesov.** 1993. The

GP-protein of Marburg virus contains the region similar to the 'immunosuppressive domain' of oncogenic retrovirus P15E proteins. FEBS Lett. **323:**183–187.
7. **Burch, J. B., D. L. Davis, and N. B. Haas.** 1993. Chicken repeat 1 elements contain a pol-like open reading frame and belong to the non-long terminal repeat class of retrotransposons. Proc. Natl. Acad. Sci. USA **90:**8199–8203.
8. **Calarco, P. G.** 1979. Intracisternal A particles in preimplantation embryos of feral mice (*Mus musculus*). Intervirology **11:**321–325.
9. **Callahan, R., D. Gallahan, L. A. D'Hoostelaere, and M. Potter.** 1986. Endogenous MMTV proviral genomes in feral *Mus musculus domesticus*. Curr. Top. Microbiol. Immunol. **127:**362–370.
10. **Coffin, J. M.** 1995. Retrovirus variation and evolution, p. 221–244. *In* G. M. Cooper, R. Greenberg Temin, and B. Sugden (ed.), The DNA provirus: Howard Temin's scientific legacy. ASM Press, Washington, D.C.
11. **Cohen, J. C., and H. E. Varmus.** 1979. Endogenous mammary tumour virus DNA varies among wild mice and segregates during inbreeding. Nature **278:**418–423.
12. **Coppola, M. A., and W. R. Green.** 1994. Cytotoxic T lymphocyte responses to the envelope proteins of endogenous ecotropic and mink cytopathic focus-forming murine leukemia viruses in H-2b mice. Virology **202:**500–505. (Erratum, **204:**487.)
13. **Djaffar, I., L. Dianoux, S. Leibovich, L. Kaplan, R. Emanoil-Ravier, and J. Peries.** 1990. Detection of IAP related transcripts in normal and transformed rat cells. Biochem. Biophys. Res. Commun. **169:**222–231.
14. **Doolittle, R. F., D. F. Feng, M. S. Johnson, and M. A. McClure.** 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64:**1–30.
15. **Durand, J. P., P. Tuppin, P. Maison, G. Galat, A. Galat-Luong, D. Jeannel, and G. de The.** 1995. Increased risk for a second retroviral infection (SIV or STLV type I) for wild African green monkeys already infected by one retrovirus in Senegal (West Africa). AIDS Res. Hum. Retroviruses **11:**985–988.
16. **Flavell, A. J., E. Dunbar, R. Anderson, S. R. Pearce, R. Hartley, and A. Kumar.** 1992. Ty1-copia group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res. **20:**3639–3644.
17. **Foulds, S., C. H. Wakefield, M. Giles, J. Gillespie, J. F. Dye, and P. J. Guillou.** 1993. Expression of a suppressive p15E-related epitope in colorectal and gastric cancer. Br. J. Cancer **68:**610–616.
18. **Frisby, D. P., R. A. Weiss, M. Roussel, and D. Stehelin.** 1979. The distribution of endogenous chicken retrovirus sequences in the DNA of galliform birds does not coincide with avian phylogenetic relationships. Cell **17:**623–634.
19. **Gallo, R. C.** 1995. Human retroviruses in the second decade: some pathogenic mechanisms and approaches to their control, p. 247–269. *In* G. M. Cooper, R. Greenberg Temin, and B. Sugden (ed.), The DNA provirus: Howard Temin's scientific legacy. ASM Press, Washington, D.C.
20. **Golovkina, T. V., A. Chervonsky, J. P. Dudley, and S. R. Ross.** 1992. Transgenic mouse mammary tumor virus superantigen expression prevents viral infection. Cell **69:**637–645.
21. **Golovkina, T. V., J. P. Dudley, A. B. Jaffe, and S. R. Ross.** 1995. Mouse mammary tumor viruses with functional superantigen genes are selected during in vivo infection. Proc. Natl. Acad. Sci. USA **92:**4828–4832.
22. **Golovkina, T. V., J. A. Prescott, and S. R. Ross.** 1993. Mouse mammary tumor virus-induced tumorigenesis in *sag* transgenic mice: a laboratory model of natural selection. J. Virol. **67:**7690–7694.
23. **Golovkina, T. V., A. T. Tikhonenko, N. S. Vassetzky, B. I. Sheftel, and A. V. Gudkov.** 1990. Distribution of mouse mammary tumor virus-related sequences does not correlate with the taxonomic position of their hosts. Virus Genes **4:**85–92.
24. **Good, R. A., M. Ogasawara, W. T. Liu, E. Lorenz, and N. K. Day.** 1990. Immunosuppressive actions of retroviruses. Lymphology **23:**56–59.
25. **Goodchild, N. L., D. A. Wilkinson, and D. L. Mager.** 1993. Recent evolutionary expansion of a subfamily of RTVL-H human endogenous retrovirus-like elements. Virology **196:**778–788.
26. **Grunder, A. A., B. Benkel, P. Sabour, and J. S. Gavora.** 1993. Research note: avian leukosis retroviral genes are not detected in geese. Poult. Sci. **72:**363–367.
27. **Guilbert, L., S. A. Robertson, and T. G. Wegmann.** 1993. The trophoblast as an integral component of a macrophage-cytokine network. Immunol. Cell Biol. **71:**49–57.
28. **Handwerger, S.** 1994. A critical role for interleukin-1 (IL-1) and the type 1 IL-1 receptor in blastocyst implantation. Endocrinology **134:**519–520.
29. **Haraguchi, S., R. A. Good, G. J. Cianciolo, and N. K. Day.** 1992. A synthetic peptide homologous to retroviral envelope protein down-regulates TNF-alpha and IFN-gamma mRNA expression. J. Leukocyte Biol. **52:**469–472.
30. **Hardy, W. D., Jr.** 1980. The virology, immunology and epidemiology of the feline leukemia virus, p. 33–78. *In* W. D. Hardy, Jr. (ed.), Feline leukemia virus. Elsevier/North-Holland, New York, N.Y.
31. **Hardy, W. D., Jr.** 1980. Feline leukemia virus disease, p. 3–31. *In* W. D. Hardy, Jr. (ed.), Feline leukemia virus. Elsevier/North-Holland, New York, N.Y.
32. **Head, J. R.** 1991. Rodent maternal-fetal immune interactions. Curr. Opin. Immunol. **3:**767–771.
33. **Hellmen, E.** 1992. Characterization of four in vitro established canine mammary carcinoma and one atypical benign mixed tumor cell lines. In Vitro Cell. Dev. Biol. **28A:**309–319.
34. **Hirose, Y., M. Takamatsu, and F. Harada.** 1993. Presence of env genes in members of the RTVL-H family of human endogenous retrovirus-like elements. Virology **192:**52–61.
35. **Horwitz, M. S., M. T. Boyce-Jacino, and A. J. Faras.** 1992. Novel human endogenous sequences related to human immunodeficiency virus type 1. J. Virol. **66:**2170–2179.
36. **Huang, T. T. J., and P. G. Calarco.** 1981. Immunoprecipitation of intracisternal A-particle-associated antigens from preimplantation mouse embryos. J. Natl. Cancer Inst. **67:**1129–1134.
37. **Imai, S., M. Okumoto, M. Iwai, S. Haga, N. Mori, N. Miyashita, K. Moriwaki, J. Hilgers, and N. H. Sarkar.** 1994. Distribution of mouse mammary tumor virus in Asian wild mice. J. Virol. **68:**3437–3442.
38. **Jerabek, L. B., R. C. Mellors, K. B. Elkon, and J. W. Mellors.** 1984. Detection and immunochemical characterization of a primate type C retrovirus-related p30 protein in normal human placentas. Proc. Natl. Acad. Sci. USA **81:**6501–6505.
39. **Johnson, P. M.** 1993. Immunobiology of the human placental trophoblast. Exp. Clin. Immunogenet. **10:**118–122.
40. **Jurka, J., E. Zietkiewicz, and D. Labuda.** 1995. Ubiquitous mammalian-wide interspersed repeats (MIRs) are molecular fossils from the Mesozoic era. Nucleic Acids Res. **23:**170–175.
41. **Keeling, P. J., and A. J. Roger.** 1995. The selfish pursuit of sex. Nature **375:**283.
42. **Koralnik, I. J., E. Boeri, W. C. Saxinger, A. L. Monico, J. Fullen, A. Gessain, H.-G. Guo, R. C. Gallo, P. Markham, V. Kalyanaraman, V. Hirsch, J. Allan, K. Murthy, P. Alford, J. P. Slattery, S. J. O'Brien, and G. Franchini.** 1994. Phylogenetic associations of human and simian T-cell leukemia/lymphotropic virus type I strains: evidence for interspecies transmission. J. Virol. **68:**2693–2707.
43. **Kubo, Y., K. Kakimi, K. Higo, H. Kobayashi, T. Ono, Y. Iwama, K. Kuribayashi, H. Hiai, A. Adachi, and A. Ishimoto.** 1996. Possible origin of murine AIDS (MAIDS) virus: conversion of an endogenous retroviral $p12^{gag}$ sequence to a MAIDS-inducing sequence by frameshift mutations. J. Virol. **70:**6405–6409.
44. **Kubo, Y., Y. Nakagawa, K. Kakimi, H. Matsui, K. Higo, L. Wang, H. Kobayashi, T. Hirama, and A. Ishimoto.** 1994. Molecular cloning and characterization of a murine AIDS virus-related endogenous transcript expressed in C57BL/6 mice. J. Gen. Virol. **75:**881–888.
45. **Kuff, E. L., J. A. Mietz, M. L. Trounstine, K. W. Moore, and C. L. Martens.** 1986. cDNA clones encoding murine IgE-binding factors represent multiple structural variants of intracisternal A-particle genes. Proc. Natl. Acad. Sci. USA **83:**6583–6587.
46. **Larsson, E., A. C. Andersson, and B. O. Nilsson.** 1994. Expression of an endogenous retrovirus (ERV3 HERV-R) in human reproductive and embryonic tissues—evidence for a function for envelope gene products. Uppsala J. Med. Sci. **99:**113–120.
47. **Larsson, E., N. Kato, and M. Cohen.** 1989. Human endogenous proviruses. Curr. Top. Microbiol. Immunol. **148:**115–132.
48. **Lavine, M. D., and N. E. Beckage.** 1995. Polydnaviruses: potent mediators of host insect immune dysfunction. Parasitol. Today **11:**368–378.
49. **Lower, R., J. Lower, and R. Kurth.** 1996. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc. Natl. Acad. Sci. USA **93:**5177–5184.
50. **Lueders, K. K., and E. L. Kuff.** 1983. Comparison of the sequence organization of related retrovirus-like multigene families in three evolutionarily distant rodent genomes. Nucleic Acids Res. **11:**4391–4408.
51. **Lyden, T. W., P. M. Johnson, J. M. Mwenda, and N. S. Rote.** 1995. Expression of endogenous HIV-1 crossreactive antigens within normal human extravillous trophoblast cells. J. Reprod. Immunol. **28:**233–245.
52. **Mager, D. L., and J. D. Freeman.** 1987. Human endogenous retroviruslike genome with type C *pol* sequences and *gag* sequences related to human T-cell lymphotropic viruses. J. Virol. **61:**4060–4066.
53. **Mathes, L. E., R. G. Olsen, L. C. Hebebrand, E. A. Hoover, and J. P. Schaller.** 1978. Abrogation of lymphocyte blastogenesis by a feline leukaemia virus protein. Nature **274:**687–689.
54. **McClure, M. A.** 1991. Evolution of retroposons by acquisition or deletion of retrovirus-like genes. Mol. Biol. Evol. **8:**835–856.
55. **McClure, M. A., M. S. Johnson, D. F. Feng, and R. F. Doolittle.** 1988. Sequence comparisons of retroviral proteins: relative rates of change and general phylogeny. Proc. Natl. Acad. Sci. USA **85:**2469–2473.
56. **Michaud, E. J., M. J. van Vugt, S. J. Bultman, H. O. Sweet, M. T. Davisson, and R. P. Woychik.** 1994. Differential expression of a new dominant agouti allele (Aiapy) is correlated with methylation state and is influenced by parental lineage. Genes Dev. **8:**1463–1472.
57. **Mietz, J. A., Z. Grossman, K. K. Lueders, and E. L. Kuff.** 1987. Nucleotide sequence of a complete mouse intracisternal A-particle genome: relationship to known aspects of particle assembly and function. J. Virol. **61:**3020–3029.
58. **Mwenda, J. M., P. M. Maher, G. C. Melling, T. W. Lyden, and P. M. Johnson.** 1994. A murine monoclonal antibody (RV3-27) raised against

isolated human placental endogenous retroviral particles and reactive with syncytiotrophoblast. J. Reprod. Immunol. **26:**75–95.
59. **Naito, T., K. Kusano, and I. Kobayashi.** 1995. Selfish behavior of restriction-modification systems. Science **267:**897–899.
60. **Neel, J. V., R. J. Biggar, and R. I. Sukernik.** 1994. Virologic and genetic studies relate Amerind origins to the indigenous people of the Mongolia/Manchuria/southeastern Siberia region. Proc. Natl. Acad. Sci. USA **91:**10737–10741.
61. **Nikiforov, M. A., and A. V. Gudkov.** 1994. ART-CH: a VL30 in chickens? J. Virol. **68:**846–853.
62. **Nilsson, B. O., P. O. Kattstrom, P. Sundstrom, P. Jaquemin, and E. Larsson.** 1992. Human oocytes express murine retroviral equivalents. Virus Genes **6:**221–227.
63. **Ober, C.** 1992. The maternal-fetal relationship in human pregnancy: an immunogenetic perspective. Exp. Clin. Immunogenet. **9:**1–14.
64. **Papaioannou, V., and R. L. Gardner.** 1979. Investigation of the lethal yellow Ay/Ay embryo using mouse chimaeras. J. Embryol. Exp. Morphol. **52:**153–163.
65. **Parker, P.** 1977. An ecological comparison of marsupial and placental patterns of reproduction, p. 273–286. *In* B. Stonehouse and D. Gilmore (ed.), The biology of marsupials. Macmillan Publishing, New York, N.Y.
66. **Piko, L., M. D. Hammons, and K. D. Taylor.** 1984. Amounts, synthesis, and some properties of intracisternal A particle-related RNA in early mouse embryos. Proc. Natl. Acad. Sci. USA **81:**488–492.
67. **Poss, M., H. L. Martin, J. K. Kreiss, L. Granville, B. Chohan, P. Nyange, K. Mandaliya, and J. Overbaugh.** 1995. Diversity in virus populations from genital secretions and peripheral blood from women recently infected with human immunodeficiency virus type 1. J. Virol. **69:**8118–8122.
68. **Prachar, J., A. Vrbenska, K. Hlubinova, D. Simkovic, and A. Kovarik.** 1987. Retrovirus like particles produced by human embryonal cells and cell lines derived from human malignancies. II. Protein structure. Neoplasma **34:**129–138.
69. **Reuss, F. U.** 1992. Expression of intracisternal A-particle-related retroviral element-encoded envelope proteins detected in cell lines. J. Virol. **66:**1915–1923.
70. **Reuss, F. U., W. N. Frankel, K. Moriwaki, T. Shiroishi, and J. M. Coffin.** 1996. Genetics of intracisternal-A-particle-related envelope-encoding proviral elements in mice. J. Virol. **70:**6450–6454.
71. **Robinson, H. L.** 1978. Inheritance and expression of chicken genes that are related to avian leukosis sarcoma virus genes. Curr. Top. Microbiol. Immunol. **83:**1–36.
72. **Ruegg, C. L., C. R. Monell, and M. Strand.** 1989. Identification, using synthetic peptides, of the minimum amino acid sequence from the retroviral transmembrane protein p15E required for inhibition of lymphoproliferation and its similarity to gp21 of human T-lymphotropic virus types I and II. J. Virol. **63:**3250–3256.
73. **Shadan, F. F., and L. P. Villarreal.** 1996. The evolution of small DNA viruses of eukaryotes: past and present considerations. Virus Genes **11:**239–257.
74. **Shih, A., E. E. Coutavas, and M. G. Rush.** 1991. Evolutionary implications of primate endogenous retroviruses. Virology **182:**495–502.
75. **Singer, M. F., V. Krek, J. P. McMillan, G. D. Swergold, and R. E. Thayer.** 1993. LINE-1: a human transposable element. Gene **135:**183–188.
76. **Sionov, R. V., S. Yagel, R. Har-Nir, and R. Gallily.** 1993. Trophoblasts protect the inner cell mass from macrophage destruction. Biol. Reprod. **49:**588–595.
77. **Sorhaug, H., and B. Grinde.** 1993. Evolution of mouse mammary tumor virus-related sequences in the human genome. Virus Res. **30:**53–61.
78. **Springer, M. S., and R. J. Britten.** 1993. Phylogenetic relationships of reverse transcriptase and RNase H sequences and aspects of genome structure in the gypsy group of retrotransposons. Mol. Biol. Evol. **10:**1370–1379.
79. **Stewart, C. L., P. Kaspar, L. J. Brunet, H. Bhatt, I. Gadi, F. Kontgen, and S. J. Abbondanzo.** 1992. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature **359:**76–79.
80. **Stromberg, K., and R. Benveniste.** 1983. Efficient isolation of endogenous rhesus retrovirus from trophoblast. Virology **128:**518–523.
81. **Surani, M. A., S. C. Barton, and M. L. Norris.** 1987. Influence of parental chromosomes on spatial specificity in androgenetic-parthenogenetic chimaeras in the mouse. Nature **326:**395–397.
82. **Szathmary, E., and J. M. Smith.** 1995. The major evolutionary transitions. Nature **374:**227–232.
83. **Temin, H. M.** 1971. The protovirus hypothesis: speculations on the significance of RNA-directed DNA synthesis for normal development and for carcinogenesis. J. Natl. Cancer Inst. **46:**3–7.
84. **Ueno, H., M. Imamura, and K. Kikuchi.** 1983. Frequency and antigenicity of type C retrovirus-like particles in human placentas. Virchows Arch. A Pathol. Anat. Histopathol. **400:**31–41.
85. **Vandamme, A. M., H. F. Liu, P. Goubau, and J. Desmyter.** 1994. Primate T-lymphotropic virus type I LTR sequence variation and its phylogenetic analysis: compatibility with an African origin of PTLV-I. Virology **202:**212–223.
86. **Venables, P. J., S. M. Brookes, D. Griffiths, R. A. Weiss, and M. T. Boyd.** 1995. Abundance of an endogenous retroviral envelope protein in placental trophoblasts suggests a biological function. Virology **211:**589–592.
87. **Wilkinson, D. A., N. L. Goodchild, T. M. Saxton, S. Wood, and D. L. Mager.** 1993. Evidence for a functional subclass of the RTVL-H family of human endogenous retrovirus-like sequences. J. Virol. **67:**2981–2989.
88. **Yotsuyanagi, Y., and D. Szollosi.** 1981. Early mouse embryo intracisternal particle: fourth type of retrovirus-like particle associated with the mouse. J. Natl. Cancer Inst. **67:**677–685.