Review article
Viruses as symbionts

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Abstract

Up to present most symbiologists have not considered viruses as potential symbionts. In large part, this is the result of a refusal to see viruses as living organisms, which in turn derives from misunderstandings about viral complexity, behavior and evolution. The consequences have been detrimental to evolutionary biology and a wide diversity of symbiogenetic evolution has been ignored or misinterpreted. To resolve this problem I propose a formal definition of the role of viruses in symbiosis. My review complements Villarreal's paper (2007). I also proffer advice to colleagues who wish to explore this exciting field of symbiogenesis and recommend the setting up of a methodological database to assist future study.

1. Introduction

Virus-host partnerships, including genomic fusions, are commoner than many biologists realize and play an important and underestimated role in evolution. A topical example is *Elysia chlorotica,* a sacoglossan sea slug, that inhabits the coastal salt marshes from Nova Scotia to Florida. Its bright green color derives from plastids of the xanthophyte, *Vaucheria litorea,* which are ingested and sequestered within the body of the juvenile slug in specially developed digestive diverticula (West, 1979; Pierce et al., 1999). The slug-chloroplast association has been called "kleptoplasty", since the chloroplasts are no longer independent. This "unique symbiotic association" of algal chloroplast and molluscan cells, by means of which the sea slug acquires a new metabolic capability, photosynthesis, is how Mary Rumpho describes it (Rumpho et al., 2000). Whether borrowed or symbiogenetically incorporated, the ingested plastids photosynthesize actively throughout the slug's life. However, the endurance of the association requires synthesis of plastid proteins for eight to nine months after ingestion (Pierce et al., 1996). In *Vaucheria* a number of these proteins are coded by the xanthophyte nuclear genes, which requires a dynamic integration of plastid and xanthophyte nuclear genomes for continued photosynthesis. Comparable integration of plastid and nuclear genomes must explain the enduring photosynthesis in the slug. But any such continued photosynthesis would require the transfer of key genes and/or entire genetic pathways from the xanthophyte to the slug nucleus. There is gathering evidence that this transfer is enabled by viruses, putatively retroviruses (these have proved difficult to type although they possess the enzyme reverse transcriptase), that have been found in both the cytoplasm and nucleus of the slug tissues (Pierce et al., 1999; Rumpho et al., 2006).

The virus-slug association may be even more intimate. The hermaphroditic adults lay egg masses in the spring, after which they all die. Death invariably correlates with the appearance of viruses within the dying slugs' tissues. The role of the viruses in the demise of the slug population is currently under investigation (Pierce, personal communication). But if a causative role is confirmed, the *Elysia*-retroviral association may be an example of "aggressive symbiosis" (Ryan, 1996/1997).
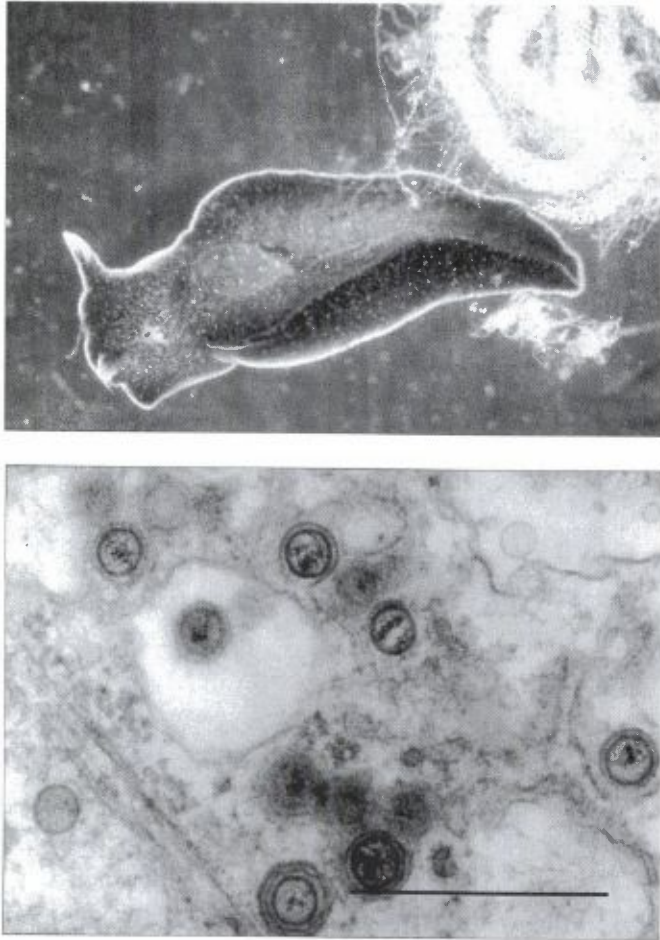
Figure 1. A: *Elysia chlorotica*; B: its associated unspecified virus (magnification 85,000, scale bar = 500 nm). A: Courtesy of Sidney K. Pierce; B: from Pierce et al. (1999), reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA.

2. Examples of Virus-Host Symbiogenesis

In studies of emerging plagues, I reviewed many examples in which the causative pathogen, whether virus, bacterium or protist, culled the population of a virgin host, thereby selecting a genotype that would survive its continuing presence. This "population gene pool culling" enabled subsequent co-evolution of pathogen and host. Aggression *per se* may thus be seen to play an important role in the origins of some evolutionary partnerships. Moreover, this capacity for aggression, initially directed at the partner, may subsequently be directed at a rival or prey of the partner after the co-evolutionary association appears. The virus-host dynamics in the myxomatosis epidemic is an illustrative example of this. The wild European rabbit, *Oryctolagus cuniculus*, was first introduced into Australia in 1859 as a source of food for British settlers. Lacking

natural predators, the rabbit population underwent an explosive expansion, leading to widespread destruction of agricultural grassland. In 1950, in a deliberate act of biological control, feral rabbits in the Murray Valley, in southeastern Australia, were infected with myxoma virus of the genus *Leporipoxvirus* (Fenner and Kerr, 1994). In this way the innate aggression of a plague virus was employed to control a "plague of rabbits".

Although no evolutionary experiment was planned, the methodology and outcome had obvious evolutionary implications. The myxoma virus normally co-evolves with the Brazilian cotton tailed rabbit, *Sylvilagus brasiliensis*, in which it causes no more than minor skin blemishes. It is spread by many different species of biting mosquitoes. By releasing infected rabbits into the wild, biologists created a situation analogous to virgin contact between the two rabbit species in nature. After proliferation of mosquitoes during a wet spring, the epidemic exploded. Within three months, 99.8% of the rabbits of southeast Australia, a land area the size of Western Europe, were exterminated. Had this been the result of the two rabbit populations competing for resources, viral aggression would have decimated the Brazilian rabbit's rival, thus allowing the virus-bearing Brazilian rabbit to dominate the ecology. In this man-induced scenario no evolutionary rival took advantage of the cull, but the evolutionary dynamics of virus and host continued. In the tiny surviving rudiment of the *Oryctolagus* population, the virus had selected a minority genotype capable of surviving its presence. Culling was followed by co-evolution between the virus and its new host, *Oryctolagus,* so that, within seven years, the lethality of what was now a persisting endemic infection was reduced to 25%. Co-evolution between rabbit and virus has continued to the present, when the rabbit population, now almost completely resistant to the former lethality of its persisting viral partner, has reoccupied the ecology in large numbers. Plague dynamics in other species, including humans, suggests that aggressive symbiosis, involving epidemic and endemic pathogens, has played a similar role in host evolution.

Other examples of aggressive symbiosis include the ants that protect plants by attacking browsing animals, or the toxin-producing fungi in grasses that discourage herbivores. A familiar example is that of the parasitoid wasps, which include approximately 25,000 species in symbiotic partnerships with approximately 20,000 species of polydnaviruses. In many such examples the viral genome has been integrated into the wasp genome (Whitfield, 1990). These 'unique symbiotic viruses', with a segmented genome of circular double-stranded DNA, replicate from integrated proviral DNA in the wasp ovary (Marti et al., 2003; Wyler and Lanzrein, 2003) and are injected along with the wasp egg into the host, where they are essential for the wasp larval survival. Genetic analysis of a polydnavirus has revealed a complex organization (Espagne et al., 2004)
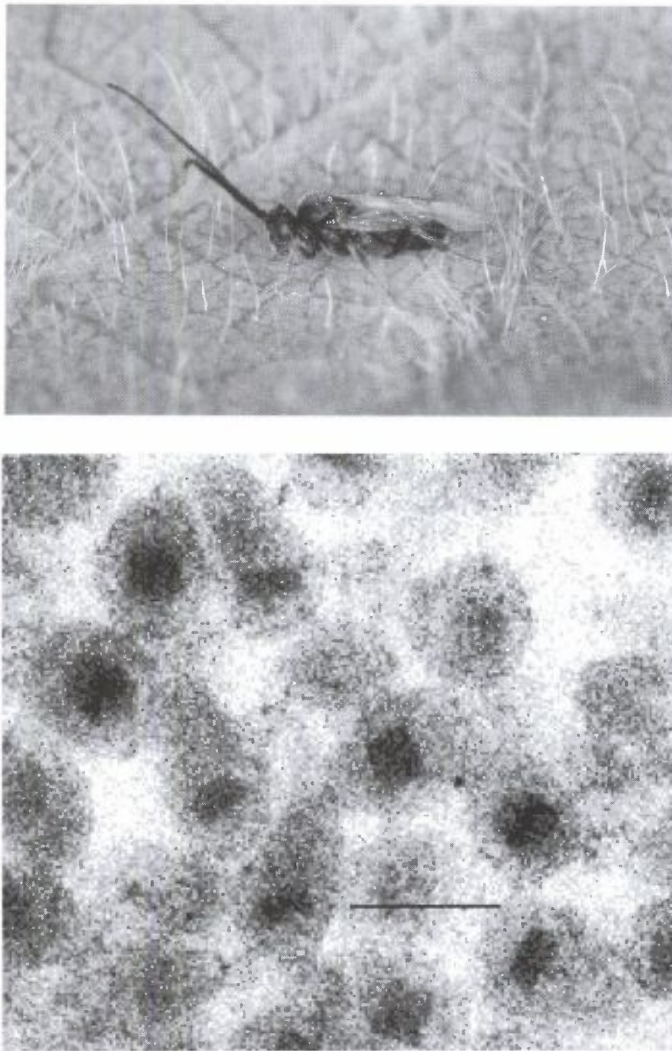
Figure 2. A: Parasitic wasp of the *Cotesia* genus; B: bracovirus from oviduct of *Chelonus inanitus* (scale bar 100 nm). A: Courtesy of James B. Whitfield; B: courtesy of Beatrice Lanzrein.

and a conserved gene family has been found in viruses of different wasp subfamilies, leading Provost and colleagues to propose a common phylogenetic origin (Provost et al., 2004). Estimations of the first establishment of the endosymbiosis, based on mitochondrial and nuclear sequencing, suggest a unique integration event 73.7 ± 10 million years ago (Belle et al., 2002; Whitfield, 2002), illustrating powerful selection pressure for stability of the virus-wasp partnership over time.

Intimate co-evolution, with or without genetic integration, of viruses with their host groups has been described in many taxa. From D'Herelle (1926) onwards, a number of biologists have used the term symbiosis in relation to viruses, but these earlier authors did not define or develop the concept. Since 1996, I have attempted to do so, expanding on my original proposal of viruses as symbionts, and developing the conceptual basis for including viruses within the definition of symbiogenesis. In 1878 De Bary defined symbiosis as "the living together of unlike named organisms" (Sapp, 1994). By "unlike named organisms" we now interpret different species. There can be no doubt that virus-host associations begin as "living together of unlike organisms". It is also clear that in the categories of symbiogenesis advanced by Margulis, all putative viral symbiogenesis is at "genetic" level (Margulis, 1993).

The origins and phylogeny of viruses and their hosts have been extensively analyzed by Villarreal (2004; 2005). His molecular genetic and phylogenetic analyses support the view that virus-host interaction has played a major role in both the origins of life and its subsequent diversification (Villarreal, 2007). This has obvious implications for evolutionary biology and for symbiosis in particular. Why then have evolutionary biologists been slow to realize the major contribution of viruses to their discipline?

3. Viruses as Life Forms

The term "symbiogenesis" was first conceptualized by Constantin Merezhkowsky as "the origin of organisms by the combination or by the association of two or several beings which enter into symbiosis" (Sapp, 1994; 2002). Thus, whether we set our standard by de Bary's definition of symbiosis, or Merezhkowsky's definition of symbiogenesis, the key question is whether or not viruses should be considered "organisms" or "beings". To examine this important question we need to disabuse ourselves of misleading assumptions about the nature of viruses.

The arguments against viruses as life forms

– Viruses, being genetic parasites of living organisms, could not have come into existence until the cellular forms, the prokaryotes, had evolved.
– As obligate parasites, and thus incapable of independent metabolism outside their hosts, viruses are not life forms.
– Viruses evolve by borrowing genes from their hosts.
– Some of the most important families of viruses originated as genetic offshoots of their host genomes.
– From the above, no meaningful evolutionary phylogenies can be depicted for viruses.
– Viruses are not cellular and thus, from a cellular definition of life, they should be dismissed from any consideration as life forms.

The arguments for viruses as life forms

Modern virology recognizes that genetic replicators, even at the prebiotic stage of evolution, would be

susceptible to parasitism by other replicators. Thus, in the Swiftean sense of fleas on the backs of other fleas, 'The tendency for replicators to become parasitized, and even for the parasitic replicators themselves to be parasitized, is a well-established phenomenon in virology' (Villarreal, 2005). The same pattern was observed when Eigen and Winkler (1992) attempted to reproduce prebiotic evolution through the concept of "hypercycles" and "quasispecies", or when the mathematician John von Neumann used computer modeling to create artificial life programs (Levy, 1992). The computer and mathematical models were inevitably parasitized by genetic self-replicators. As Villarreal concludes, viruses owe their origins not to the prior evolution of prokaryotic hosts but to the innate evolutionary nature of self-replication.

Today, most biologists accept that organelles such as mitochondria and plastids were formerly independent living organisms (Margulis, 1970). Nucleotide sequence analysis suggests that one of the closest known modern relatives to the ancestor of mitochondria is *Ricketssia prowazeki,* the cause of epidemic typhus (Andersson et al., 1998; Gray, 1998). In size and genetic complexity the *Rickettsiae* occupy a biological position that is intermediate between smaller bacteria and larger viruses. But the recently discovered nucleocytoplasmic large DNA viruses, the *Mimiviridae,* are, at 400 nanometers, as large as mycoplasma and, with genomes in excess of 1,200 genes, more complex genetically than many *Rickettsiae* (Raoult et al., 2004).

With the exception of *R. quintana*, all *Rickettsiae* are also obligate intracellular parasites. Are we then to dismiss the *Rickettsia* as life forms on the basis of their obligate intra-cellular parasitism? If so we will also be obliged to dismiss the *Chlamydiales,* Coxiella, Ehrlichia, Cowdria and Simkania, as well as the obligate symbiotic bacteria such as

Figure 3. Mimivirus. Courtesy of Didier Raoult.

Buchnera, Wolbachia, Wigglesworthia glossinidia and *Sodalis glossinidius.* It seems logically inconsistent to claim on the one hand that viruses are non-living because they lack the genomic imperatives for an independent existence, meanwhile accepting that these bacterial taxa, which lack the same imperatives, are alive.

In a keynote presentation at the Ninth Symposium of the Society for General Microbiology, in London, April 1959, the distinguished virologist and future Nobel Laureate, Salvador E. Luria, summarized the prevailing views on virology (Luria, 1959). While acknowledging the links to pathology and medicine, he reported that the major theoretical advances since 1952 reflected the growing importance of viruses, and phage viruses in particular, as tools in the investigation of cellular biology and genetics. Nine years earlier, Luria had pioneered the concept of phage infection as genetic parasites (Luria, 1950). But now he contradicted his earlier views, as well as those of D'Herelle, in concluding, 'we now consider phages as segments of bacterial DNA which possess a specific viral function, that is, a genetically determined potentiality for active release and transfer to other cells.' This downgrading of viruses to genetic offshoots of host genomes was subsequently adopted by two other distinguished scientists and Nobel Laureates, André Lwoff and Howard M. Temin. In Temin's Nobel speech (1975), he declared: 'This distribution is consistent with an hypothesis (the protovirus hypothesis) I originally proposed in 1970 to explain the origin of ribodeoxyviruses – ribodeoxyviruses evolved from normal cellular components.' This derivative perspective, advanced by such distinguished scientists, influenced future generations. Today, however, the overwhelming evidence would suggest the opposite (Villarreal, 2007; Suttle, 2005; Hambly and Suttle, 2005). Viruses, in particular bacteriophages and retroviruses, do not derive from host genomes. Rather they have their own evolutionary lineages, much as any other life form. And while there is substantial genetic exchange, loosely referred to as horizontal gene transfer, within viral-host relationships, the bulk of the creativity, and transfer, is from virus to host.

The cellular definition is not the only definition of life. In Chapter III of *The Bacteriophage and its Behaviour* (1926), D'Herelle concluded that life, in its very essence, was not readily definable. But its presence could be recognized as a composite of two fundamental properties, 'the power of true assimilation... and the power of adaptation'. He went on to conclude that 'the bacteriophage corpuscle, conforming to this criterion in every respect, is a living being'. He was supported in this by many of the foremost pioneers of his day, including Prausnitz, Beijerinck, Fabry and van Beneden. Some eighty years later we are no closer to a universally accepted definition of life (Hazen, 2006). Some might even define viruses as a variant of cellular life, possessing as they do an enclosing
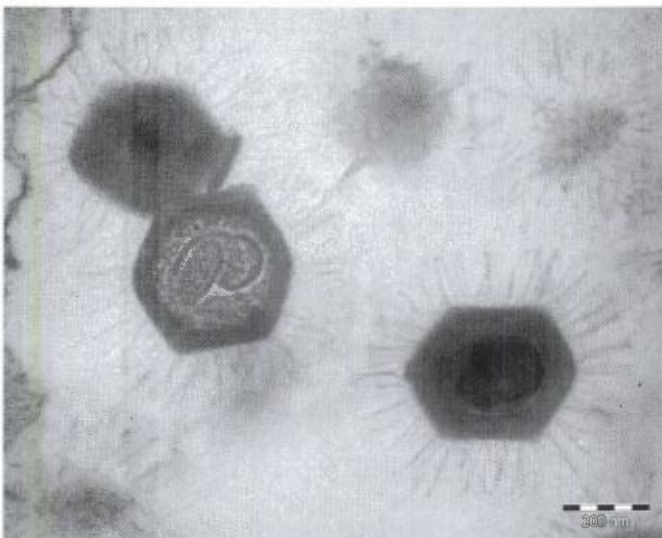
capsid and all the genetic and biochemical make-up necessary to fulfill their lifecycle within their appropriate ecology. I have argued elsewhere that, were we to propose the ideal size, physical structure, physiology, biochemistry and genetics of a life-form designed to parasitize the landscape of the genome, we would end up with a virus (Ryan, 2002/2003). Viruses have definite lifecycles, as any virologist, or physician, would recognize. They inhabit specific target organs or tissues in specific hosts, where they show recognizable patterns of behavior, pathology and evolutionary development. And as evolutionary biologists have long recognized, viruses are the ultimate expression of evolution through natural selection.

To dismiss viruses from the evolutionary dynamics of symbiosis is outmoded and prejudicial. In my view, viruses, in their universal interaction with the genomes of their hosts, will be seen for what they truly are – the ultimate symbionts. I shall explore some of these implications for human evolution.

4. Horizontal Gene Transfer versus Symbiogenesis

The nodulation and fixation genes of the rhizobium, *Mesorhizobium loti,* are encoded in the bacterial genophore as a 500-kb "symbiosis island" that can be transferred from symbiotic to non-symbiotic strains of rhizobium because of an enzyme (P4 phage integrase) intrinsic to the symbiosis island (Sullivan and Ronson, 1998). The presence of the phage integrase is usually attributed to "horizontal gene transfer". But how useful, conceptually and mechanistically, is such a statement? "Horizontal gene transfer" is valid in broad terms but says nothing about the dynamics of the transfer, and little about the evolutionary implications. Each mode of "horizontal gene transfer" merits the same objective analysis as any other process in evolutionary biology. Indeed, when the evolutionary dynamics of virus-host union is considered, it becomes clear that simple gene transfer is wholly inadequate to explain what actually takes place.

5. Retroviral Symbiogenesis

The genomes of retroviruses are composed of RNA. To insert themselves into the DNA-coded genome of their hosts, they employ a key enzyme, reverse transcriptase, that copies the viral RNA to its complementary DNA, which is then integrated into the host chromosomes. If the viral genome integrates into germ cells, the union of the two disparate genomes, virus and host, gives rise to a novel holobiontic genome. Since viruses are small, and their genomes are minuscule when compared to those of their hosts, it is easy to underestimate the contribution a virus makes to such a union.
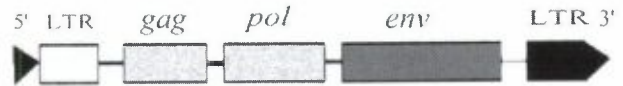
Genomic structure of retroviruses, including HIV-1 and HERV

Figure 4. Basic genome of retroviruses, including HERVs.

All retroviruses are identified by their pathognomonic genes, *env*, *gag* and *pol* (Fig. 4). Each of these genes codes for multiple genetic potentials. For example *gag* codes for matrix and core shell proteins, *pol* codes for enzymes such as reverse transcriptase, protease, ribonuclease and integrase, while *env* codes for the surface and transmembrane glycoproteins. *Env* also codes for proteins that cause host cell fusion and immunosuppression. The retrovirus also brings to the union flanking control regions, known as long terminal repeats, or LTRs, essential for genomic integration. Once integrated into the holobiontic genome, the LTRs contain promoter and enhancer elements as well as polyadenylation signals capable of controlling not only the expression of viral genes but also those derived from the former host. The LTRs may replicate throughout the genome, thereby amplifying their influence. Regulatory sequences from the viral contribution may include splice donor and acceptor sites and primers that bind specific tRNA molecules required to initiate reverse transcription. These virus-derived genes and control sequences increase the "genomic creativity" of the holobiont (Ryan, 2006).

It is of critical importance to examine the role of natural selection in the evolution of such virus-host genomic unions. John Maynard Smith (Smith, 1991) reasoned that in symbiogenesis selection acts to an important degree at the level of the partnership, adapting it to a stable evolutionary strategy. In retrovirus-host mergers, the symbiogenetic event is the initial union of virus and host genomes. From then on, natural selection working at holobiontic level, will adapt both the viral and original host elements to the new partnership. Where viral integrity is important to the holobiont, the viral genome will be conserved. This is what we see in the parasitoid wasps and the polydnaviruses. Where viral integrity is deleterious to the holobiont, viral independence is eliminated. This is seen in human-retrovirus symbiogenesis, where the HERVs lose their capacity for horizontal transmission. Positive selection preserves viral or host genes, viral or host translational sequences and viral or host control and developmental sequences, that contribute to the evolving holobiont.

Retroviruses with this potential for genomic fusion are widespread in nature, with the exception of angiosperms, which support a related group of viruses, the pararetroviruses (these also replicate using reverse transcriptase). Retroviruses are ubiquitous in animals, particularly vertebrates, and their endogenous counterparts

(ERVs) are found in the genomes of fish, reptiles, amphibians and birds (Martin et al., 1997). The evolution of the placental mammals was accompanied by an explosive and lineage-dependent increase in ERV colonization (Villarreal, 2005). We are only beginning to explore the implications of such viral symbiogenesis in human evolution.

6. Retroviruses and the Holobiontic Nature of the Human Genome

Given the ubiquity and importance of retroviruses, the recognition of their relevance to human disease and human evolution is surprisingly recent. Human T-cell leukemia was first described by Takatsuki and colleagues in Japan in 1977 (Uchiyama et al., 1977), and the causative retrovirus, human T-cell leukemia virus type I, or HTLV-I, was first identified by Gallo and co-workers in 1980 (Poiesz et al., 1980). This led in rapid succession to the recognition of HTLV-II, and even more so to the human immunodeficiency viruses, HIV-1 and HIV-2, which brought about the present AIDS pandemic (Ryan, 1996/1997). *Exogenous* retroviruses, like HIV-1, replicate within the somatic tissues of infected individuals and spread horizontally in the human population through sexual intercourse and contaminated blood products. They also spread vertically from mother to child, by passage of virus transplacentally or during parturition, or via breast milk. *Endogenous* retroviruses are inherited according to Mendelian principles through the germ line as an integral component of the genome. Approximately 8% of the human genome consists of human endogenous retroviruses, or HERVs, and, if we include HERV fragments and derivatives, the retroviral legacy amounts to roughly half our DNA (Bannert and Kurth, 2004; Medstrand et al., 2002).

Endogenous retroviruses arise through germ cell endogenization of formally exogenous retroviruses. A contemporary endogenization through retroviral invasion of the koala genome has been described (Tarlington et al., 2006) and there is growing evidence of a recent endogenization in sheep (Palmarini, personal communication) and in humans (Turner et al., 2001), where HERV-K113, found on chromosome 19 in just 29% of people of mainly African, Asian and Polynesian descent, appears to have entered the human genome after the last great migration from Africa (less than 150,000 years ago). A virus designated HERV-H/RGH-2, circumstantially associated with multiple sclerosis, is still horizontally transmissible (Christensen et al., 2002). The fact that almost all present day humans are carrying the same endogenous retroviruses suggests that the exogenous forebears of HERVs were highly contagious pandemic infections, following transmission pathways akin to HIV

and HTLV, and their spread through the original African-based population of primates and subsequently hominids may well have been associated with repeated plague culling. There is circumstantial evidence for such gene pool culling in primates from the comparison of mitochondrial and tissue compatibility genes in humans and chimpanzees (Gagneux et al., 1999; Zhao et al., 2000; De Groot et al., 2002).

Virus-host integrations probably played a key role in the origins of both non-adaptive and adaptive immunity (Villarreal, 2005). In mammals there is growing evidence that endogenized retroviruses sometimes protect the animal from exogenous viral infection. For example, Palmarini and his colleagues have found that a high degree of genital expression of the endogenous Jaagsiekte sheep retrovirus (enJSRV) in ewes may prevent genital infection with a related pathogenic exogenous retrovirus (Palmarini et al., 2004; Mura et al., 2004; Murcia et al., 2007). Selective pressure exerted by the blocking effects of enJSRVs may thus have favored the development of lung tropism for the JSRV that causes the Jaagsiekte endemic disease in sheep (Palmarini, personal communication). HERVs in the human placenta may play some role in reducing cross-placental maternal immune reactivity to fetal antigens in addition to reducing the potential of infection with other retroviruses, perhaps even mitigating the risk of cross-placental spread of HIV-1. We have seen that the *env* gene has the ability to fuse host cells, which is a familiar pathological feature of HIV-1 infection, resulting in multinucleated inflammatory aggregates. Fusion of cells is also an important histological feature of the syncytium, the placental membrane that forms the physiological barrier between human maternal and fetal circulations. The endogenous retroviruses, HERV-W and HERV-FRD, play an important role in the construction and physiological function of the human syncytium, their *env* genes coding for the proteins syncytin and syncytin 2 respectively, which fuse the trophoblast cells of the placenta into the confluent multinucleated syncytial layer (Ryan, 2004; Mi et al., 2000; Blond et al., 2000; Blaise et al., 2003). Each virus fulfils a specific and different role in placental construction, working in a complementary way with a third virus, ERV-3 (also known as HERV-R), in a complex developmental coordination (Rote et al., 2004).

Retroviruses have also been detected in the placentas of many different species of mammals, including non-human primates, cats, mice and guinea pigs (Harris, 1998; Daniel and Chilten, 1978) and other retroviral genes, known as syncytin-A and syncytin-B, have been found to play a vital role in placentation in mice (Dupressoir et al., 2005), as has the *env* gene of enJSRV in periimplantation and placental growth in sheep (Dunlap et al., 2006). A less developed interaction with ERVs appears to play a part in the pregnancy of marsupials, suggesting viral involvement at a very early stage in placental evolution. For example, the

fat-tailed dunnart is known to be colonized by *env* producing retroviruses. This species also undergoes a transient placentation with trophoblast fusion into multinucleated giant cells, highly suggestive of the expression of retroviral *env*-gene protein (Villarreal, 2005; Roberts and Breed, 1994).

Another important symbiogenetic implication of viral-host union is the conferment of an increased genetic, and thus evolutionary, creativity for the holobiontic genome. In his companion paper, Villarreal (2007) provides an overview of the ways in which viruses have been contributing to and manipulating genomes throughout evolution. This is particularly relevant to symbiotic unions involving retroviruses, where the LTRs and derived Lines and Sines are capable of a wide range of evolutionary innovations.
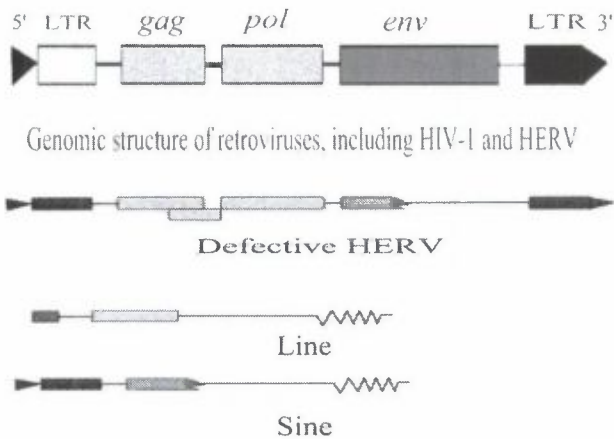

Figure 5. HERV derivatives.

One way in which they do so is through modification of transcriptional control (Britten, 1996). Sverdlov (2000), for example, supports the view that newly integrated HERVs may have influenced hominid evolution by changing the pattern of gene expression. O'Neill and her colleagues (1998; 2001) have shown how retroviruses inhabiting the centromeres of interspecies hybrid rock wallabies are fast-tracking speciation. In humans, increased genomic plasticity through host-viral interaction is responsible for large scale deletions, duplications and chromosome reshuffling (Hughes and Coffin, 2001). This increased genomic plasticity remains active long after original virus-host genomic fusion and is likely to have contributed to the surprisingly high level of genetic variation currently being observed between individual humans (Redon et al., 2006).

A previous generation saw HERVs as a manifestation of selfish DNA and assumed their invasion of the human genome was irreversible. This was contradicted by Medstrand and colleagues (2002), who demonstrated that HERVs can be removed, for example through chromosome deletions and sexual homologous recombination. The excessive accumulation of retroelements on the Y chromosome lends support to this, since, as an unmatched chromosome, it is precluded from homologous recombination. But why then, if not through the selective pressures of selfish DNA, have such vast numbers of HERVs and their fragments been retained? It seems likely that positive selection at holobiontic level is at least part of the answer. The exogenous retrovirus that gave rise to the HERV-W endogenous family is believed to have entered the ancestral genome less than 40 million years ago. When Bonnaud and his colleagues (2004) tracked the action of natural selection on the HERV-W locus ERVWE1 (whose *env* gene codes for syncytin) in chimpanzee, gorilla, orang-utan and gibbon, they found that the genetic signature crucial to the gene's fusogenic action had been conserved by selection over the tens of millions of years of primate divergence.

HERVS in human embryogenesis

Given what we now know about retroviruses and their symbiogenetic potential, it should come as no surprise to learn that HERVs and their products play important roles in human embryological development. HERV LTRs contain regulatory elements that influence the cellular expression of proteins (Dunn et al., 2003). HERV-derived promoters of various potencies are frequently found in human promoter regions, where they play important roles in development. Andersson and colleagues have shown that ERV-3, one of the three viruses involved in placentation, is also highly expressed in many human fetal tissues, including adrenal cortex, kidney tubules, tongue, heart, liver and central nervous system (Andersson et al., 2002). The *env* gene of the same virus is highly expressed in the sebaceous glands of normal skin (Andersson et al., 1996). Mager and colleagues have extensively investigated the role of the ERV-L LTR and its promotional activity relative to the human gene *β3GAL-T5*, demonstrating that this viral promoter is responsible for most of the relevant gene transcripts in the human colon (Dunn et al., 2003; 2005). Although humans and chimpanzees have many retroviral families and viral products in common, there are significant differences in the viral components of the two genomes. For example, the viral families, CERV 1 (PTERV1) and CERV 2, are unique to chimpanzees (Polaverapu et al., 2006), meanwhile eight out of ten full length HERV-K species present in the human genome appear to be unique to humans (Barbulescu et al., 1999; Medstrand and Mager, 1998). It is also interesting that the LTR of this ancient family of human-associated endogenous retroviruses encodes a functional homolog of HIV-1, suggesting an evolutionary link between the two (Yang et al., 1999).

Moreover there is evidence that LTR evolutionary activity related to specifically human-associated HERV-K species is still active in the human population (Hughes and Coffin, 2004). These constitute important genetic and evolutionary differences that are frequently omitted when biologists compare species of primates. Future comparative genomics will need to take the viral components into consideration if we are to derive meaningful evolutionary commonalities and differences.

7. Conclusion

Although I have focused on retroviruses in human evolution, persisting virus-host interactions, often with genomic fusion, extrapolate to all vertebrates, and indeed far beyond, to insects and many other invertebrates, and, when extended to pararetroviruses, to angiosperms. Clearly a major component of evolution has been underestimated. Marilyn Roossinck, inspired by the new thinking about viruses, has conducted a reappraisal of the evolution of plant viruses, concluding that, although both competition and symbiotic interaction applied in different circumstances, evolution by symbiotic association was the most likely model for many evolutionary events leading to rapid changes or the formation of new species (Roossinck, 2005). With her colleagues, she has begun to pioneer this symbiogenetic perspective into new avenues of virus-plant associations, leading to the discovery of a novel species of symbiotic plant virus (Márquez et al., 2007). It is to be hoped that other colleagues will expand this new and exciting division of evolutionary biology. Below I have listed some ways and means in which colleagues might consider some new approaches:

Screening for viral symbiosis

In this final section I have attempted to put together some basic guidance for colleagues who might be interested to explore viral symbiogenesis in nature. In time, perhaps with the encouragement of the ISS, it should be possible to build up a useful methodological database that will assist and encourage future studies.

- Sometimes a chance observation will suggest a hitherto unsuspected viral presence. This was the case with the viruses in *Elysia chlorotica*, where the electron microscopist spotted viruses in tissue sections being examined for an entirely different purpose. The precise nature of the viruses involved is still under investigation. But there is growing evidence of gene transfer from xanthophyte to slug (personal communication, S. Pierce).

- On other occasions a specific prokaryotic or eukaryotic taxon is associated with certain species or families of viruses, but nobody has thought to examine the virus-host relationship from a symbiotic perspective. This inspired the discovery of symbiotic mycoviruses currently being pioneered by Marilyn Roossinck and her colleagues in geothermal plants (Márquez et al., 2007). Curious about mycoviral diversity and about the prevailing assumptions that these viruses lack horizontal transmissibility, she and her colleagues discovered that fungi isolated from geothermal plants always showed the presence of virus while isolates of the same fungus from non geothermal areas did not. Removal of the virus caused the death of the plants. This led her to demonstrate that the virus was playing a hitherto unknown mutualistic role in the survival of grasses in a geothermal ecology.

- It may be worth looking for viruses in any situation where horizontal gene transfer is suspected or known to occur in nature. This will embrace a vast diversity of situations in prokaryotes, where much is known already (Villarreal, 2005; Sonea and Mathieu, 2000). The involvement of phage integrase in the symbiosis island in rhizobia is a good example, where the enzyme is the smoking gun pointing to an earlier phage-bacterial symbiosis. The fusion of prokaryotes implicit in Margulis's SET theory would also suggest a possible viral layer of interaction. Less well-understood are situations involving gene transfer in eukaryotes, whether from prokaryote to eukaryote, or from one eukaryote to another, such as we have seen in *Elysia chlorotica*. The possibility of a viral role should be queried in all such cases.

- It may also be worth looking for viruses in life forms that inhabit extreme environments. Symbiogenesis is already known to promote survival in such circumstances, for example in lichens. The recent discovery of a number of hitherto unknown families of viruses in Yellowstone's hot springs would suggest the possibility of novel symbiogenetic viral roles. Similar considerations would apply to the ecology of the deep-sea thermal vents.

- It may be useful to explore situation in which one finds an intimate and long-standing association between prokaryotes and eukaryotes, even when there is no known horizontal gene transfer. In particular, one might consider screening for viruses in intimate symbiotic associations such as gut flora, and in bacterial symbionts in other internal organs, such as luminescent marine life forms. All associations involving obligate mutualistic bacteria, such as *Buchnera, Wolbachia, Wigglesworthia glossinidia* and *Sodalis glossinidius,* would appear worthy of some preliminary exploration for an additional symbiogenetic viral role.

- Perhaps one of the most obvious arenas worthy of symbiogenetic viral exploration is the exploding field of genomic sequencing. Viral endogenization is being consistently reported in the genomes sequenced to date.

This presents us with an opportunity for new study, and understanding. The objectives and achievements of McClure and her colleagues are worthy of special mention here, with her investigation of motif-detection in whole genome analysis (McClure, 1999; 2000; McClure et al., 2005). The same applies to Suttle's approach in screening the "viriosphere" of the oceans not only for viruses but also for viral genetic exchange (Suttle, 2005; Hambly and Suttle, 2005). Biologists interested in viral symbiogenesis might begin by examining the accumulating library of information emerging from such wholesale screening and then choose a "book", or even a "shelf of books", worthy of further study.

– Once a specific family or species of virus is suspected, a wide choice of genetic sequence screening programs are available for further analysis. Some of these are referenced in the abstracts at the Conference on the Genomic Impact of Eukaryotic Transposable Elements (Batzer et al., 2006). Another approach worthy of specific mention in this context is the growing usefulness of morpholino techniques for precise gene targeting and specific pathways inhibition (Ekker and Larson, 2001). A notable example will be found in the study of ERVs and sheep placentation by Palmarini and his colleagues (Dunlap et al., 2006).

Acknowledgements

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