Endogenous Viral Etiology of Prion Diseases

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Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of incurable neurodegenerative disorders, including Kuru and Creutzfeldt-Jakob disease in humans, "mad cow" disease in cattle, and scrapie in sheep. This paper presents structural, genetic, and evolutionary evidence supporting an endogenous TSE virus model that integrates the three major traditional views on the nature of TSE pathogens, the conventional virus view, the prion hypothesis, and the virino concept, into a novel conceptual and evolutionary framework. According to this model, the TSE pathogens are symbiotic endogenous viruses that inadvertently produce transmissible viral particles that lack the viral genome and are composed primarily of the viral prion protein (PrP). Production of defective viral particles that contain a partial genome or lack the viral genome entirely is a relatively common event in the life cycle of many viruses. Similar to the normal viral particles, which contain a genome, these defective viral particles can be transmitted to new host cells. Obviously, in the absence of viral genome, these protein-only viral particles cannot establish a productive infection. However, if these viral particles enter a host cell that carries the parental or a related virus and induce the production of similar protein-only particles, then they would appear as self-replicating, protein-only infectious pathogens if mistakenly taken out from the context of the viral life cycle. This misconception, which is rooted into the current dogma of viruses as viral particles, led to the development of the prion theory. The endogenous TSE virus model is consistent with the TSE data and offers solutions to many enigmatic features associated with TSE, including the function of PrP that, despite more than two decades of TSE research conducted primarily within the framework of the prion hypothesis, is still not known. According to the TSE endogenous virus model, PrP is the protein of an endogenous virus that has co-evolved with their vertebrate hosts by providing a protective function against pathogenic viruses. The evidence for the endogenous TSE virus model and for the antiviral protective function of PrP is strong, and they are fully open to additional experimental testing. The endogenous virus model opens the TSE research field to new interpretations and directions, both in basic research and in associated biomedical and public health fields, and could lead to development of new diagnostic and therapeutic approaches.

Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of incurable neurodegenerative disorders, including Kuru and Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy, or "mad cow" disease in cattle, and scrapie in sheep (1;2). Despite considerable progress in understanding the pathology, transmission, and the genetics of TSE, the nature of the TSE pathogens is enigmatic. Based on biological and biochemical properties, such as strain variation and resistance to denaturing agents, heat, and radiation, three rival views about the nature of the TSE pathogens have been proposed [reviewed in (3-17)].

Consistent with the viral etiology of similar diseases, the TSE pathogens were initially regarded as viruses with unique properties and a long incubation period. However, as early as the 1960s, it was speculated that TSE pathogens might lack a nuclei acid-based genome and might be composed entirely of carbohydrates, lipids, proteins, or more intricate biochemical complexes such as glycoproteins or cellular membranes (18-20). One of these early ideas, that of a "replicating protein"(20), was later developed into the prion hypothesis (12;21). This hypothesis claims that TSE are caused by a novel type of infectious pathogens, called prions, which consist of a self-replicating protein identified as proteinase K-resistant structural isoform (commonly referred to as PrP-res, or PrP^{Sc}) of a cellular glycoprotein (PrP) (12;22). The virino

concept was developed in the 1970s based on an earlier "replication site" hypothesis for scrapie agent (15;16;23;24). According to this concept, the TSE pathogens are low molecular weight nucleic acid molecules, probably small RNAs, which replicate and are transmitted in a complex with a host protein (a product of *Sinc* locus in mice) that was later identified as PrP.

It is apparent, however, that neither of these traditional views about the nature of TSE pathogens can fully explain the current TSE data [discussed in (3-17)]. This data is more consistent with an endogenous TSE virus model, in which the TSE pathogens are germ-line or endogenous viruses that inadvertently produce transmissible viral particles (TSE-VPs) composed primarily of the endogenous viral PrP and lacking a viral genome (25). This model is strongly supported by the fact that most if not all viruses produce viral particles that contain a partial viral genome, or lack the viral genome entirely; interestingly, some of these defective particles, referred to as "interfering particles," can co-infect new host cells and interfere with the life cycle of co-infecting parental or related viruses (26;27) [for a general review of this and other facts about viruses see (28)].

Obviously, in the absence of a viral genome, these protein-only defective viral particles cannot establish a productive infection. However, if these viral particles enter a host cell that carries the parental or a related viral genome and induce the production of similar particles, they would appear as self-replicating, protein-only infectious pathogens if mistakenly taken out of the context of the viral life cycle. As previously suggested (25), this misconception, which is rooted in the dogma of viruses as viral particles (see below), led to the development of the prion theory - the leading working hypothesis in the TSE research field for more than two decades.

This paper presents additional evidence and arguments for the endogenous TSE virus model and offers solutions to many enigmatic TSE features, such as the function of PrP. The paper begins with a brief discussion of the misleading dogma of viruses as viral particles, which has played a critical role in the historical development of the views about the nature of TSE pathogens. Next, the paper presents structural, genetic, and evolutionary evidence for the endogenous TSE virus model, and it outlines the implications of this model for TSE research and public health, including development of new diagnostic and therapeutic approaches.

Box 1. Quotations illustrating the critical role played by the dogma of viruses as viral particles in the development of hypotheses about the nature of TSE pathogens, particularly in the development of prion hypothesis:

Quote 1 [from]: "To avoid prejudging the structures of these infectious particles, prions were defined as "small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids" and three hypothetical structures for the prion were proposed: (i) proteins surrounding a nucleic acid that encodes them (a virus) (ii) proteins surrounding a small non-coding polynucleotide, and (iii) a proteinaceous particle devoid of nucleic acid. Data from many laboratories have established that scrapie is not caused by a virus."[a]

Quote 2 [from (12)]: "Although both prions and viruses multiply, their properties, structures, and modes of replication seem to be fundamentally different. Viruses contain nucleic acid genomes that encode progeny viruses; proteins necessary for producing infectious viruses are encoded by the viral genome. In contrast, prions contain little or no nucleic acid, and PrP is encoded by a cellular gene."

[a]As pointed out in this (12) and other publications (11;13;31), initially, the concept of "prion" included viruses. Only later, due to lack of evidence for a TSE associated viral genome did the concept of prion evolved to specifically stand for a protein-only, non-viral pathogen. As described in the following quote from reference (32), the development of prion concept was challenging: "The discovery of prions and their eventual acceptance by the community of scholars represents a triumph of the scientific process over prejudice." It is within the spirit of this process that the endogenous TSE virus model is proposed here as an alternative to prion hypothesis.

The dogma of viruses as viral particles and the nature of TSE pathogens

Since their discovery at the turn of the last century, viruses have been identified with and defined based on the properties of their viral particles [reviewed in (29;30)]. The viral particles are highly specialized viral structures used by many viruses for their transmission to new host cells. However, not all viruses produce viral particles. Some use alternative modes of transmission, such as vertical transmission from mother to daughter cells (28;33-35). Moreover, the defining biological properties of all viruses, whether they do or do not produce viral particles, are expressed during the intracellular stage of their life cycle when they replicate their genomes and synthesize their specific molecules, many of which are not components of the viral particles. As discussed in detail in two other papers of this series on the origin and evolution of cellular and viral domains [reviewed in (30;36)], the dogma of viruses as viral particles misrepresents the nature of viruses and sets them outside the mainstream biological and evolutionary paradigms. Moreover, this dogma has led to misconceptions that have constrained the full progress in some biomedical fields. One of the most pragmatic examples is the TSE field, which is discussed here.

In order to define the nature of the TSE pathogens in context of the dogma of viruses as viral particles, the TSE studies focused on the composition and properties of TSE transmissible units [Box 1; see also (3-17)]. Accordingly, central to the historical debate about the viral or non-viral nature of the TSE pathogens was the presence or absence of

nucleic acids (i.e. viral genome) associated with these pathogens. During the last few decades, hundreds of TSE studies addressed this issue. The vast majority of these studies have shown that the TSE agents do not contain a viral genome and, therefore, according to the dogma of viruses as viral particles they could not be viruses. Based on the results of these studies, the prion theory became the leading working hypothesis in the TSE field (32). According to this hypothesis, prions are non-viral, protein-only infectious agents (see Box 1), which replicate by a mechanism in which the PrP-res molecules induce by a direct template-based mechanism the conversion of cellular PrP into new PrP-res (37).

The endogenous TSE virus model (25), which was inspired by a new view about the nature and evolutionary origin of viruses (29), was based on well-documented facts about the life cycle of many viruses and on additional lines of evidence and reasoning. At that time, it was well known that viruses make defective viral particles that could enter new host cells and interact with the life cycle of co-infecting parental or related viruses (26;27). It was also well documented that humans and other vertebrates host numerous germ-line, or endogenous viruses, some of which produce viral particles [reviewed in (33)]. Based on these facts, and on the rationale that it is highly unlikely that cellular genes would evolve to produce pathogenic, infectious products, I proposed that the TSE pathogens are endogenous viruses, suggestively labeled prionic viruses, that produce transmissible, protein-only viral particles (25). These particles would appear to be self-propagating if, upon entering new host cells, they activate the parental or a related endogenous virus to produce similar particles. This activation process is the key for understanding the mechanism of producing new TSE-VPs and for understanding the TSE phenomenon. As proposed next, this process is analogous to a common mechanism for self-assembly of viral particles found in many virus families.

A virus-like mechanism for the assembly of TSE transmissible units

In viruses that make viral particles, the structure, biochemical composition, and the overall complexity of these particles are highly diverse [see (28)]. Fundamentally, however, the morphogenesis of many types of viral particles is based on the self-assembly of viral protein monomers into polymeric structures (38-41). In some viruses, this process requires the participation of chaperoning or scaffolding viral proteins and of the viral genome (38-41). It is important to emphasize that similar to the assembly of viral proteins into viral particles, the assembly of many cellular proteins into molecular complexes is dictated not only by their primary amino-acid sequence (i.e. by the genetic information), but also by interactions with other molecules, such as other proteins, nucleic acids, or lipids that could act as chaperons, scaffolds, or templates for their correct folding and assembly (42;43).

During the self-assembly of some viral particles, the capsid protein monomers undergo isomeric conformational changes rich in β-sheet domains. These β-sheet domains are a common feature in viral capsid proteins to the extent that, despite little or no primary amino acid sequence homology among capsid proteins of different viruses, this feature is used as critical evidence for inferring evolutionary relationships among viral families across the three cellular kingdoms, Archaea, Bacteria, and Eukarya (44;45). Interestingly, the β-sheet-based protein domains are also the main structural change during PrP isomeric conformational transition to PrP-res (37;46). Additionally, efficient self-assembly of viral particles in many viral species requires a viral genome, or nonspecific nucleic acid molecules (38-41;47), a phenomenon that is also observed during assembly of the transmissible TSE units (48-55) [reviewed in (56;57)]. There is also very strong evidence that PrP has structural and functional properties similar to those of retroviral proteins (50;51;58).

Another piece of evidence supporting the idea that the TSE transmissible structures are analogous to viral particles is the finding that these units contain 14 to 28 isomeric monomers of PrP (17-27 nm particles) and that single monomers or particles containing fewer than five monomers do not induce TSE (59). Taken together, these findings support the hypothesis that the TSE transmissible units are structures analogous to viral particles, and that the mechanism for their assembly mimics that of the viral particle. This might explain the common finding of virus-like particles in TSE tissues and cell culture (60-64).

Although some of these findings are circumstantial, they are consistent with an endogenous TSE virus model in which the TSE transmissible units are the viral particles (TSE-VPs) of an endogenous virus. The TSE-VPs are composed of isomeric conformational monomers of PrP labeled here PrP-vp. The structure of PrP-vp might be similar but likely not identical to that of PrP-res found in the TSE-associated amyloid plaques. In the endogenous TSE virus model, the native PrP molecules use the incoming TSE-VPs and possibly additional molecules, such as non-specific nucleic acid molecules, as scaffolds, templates, or chaperons for self-assembly into new transmissible TSE-VPs.

For reasons that will become more transparent in the following sections, in context of the endogenous TSE virus model, the generation of new TSE transmissible units (i.e. TSE-VPs) is regarded as an intrinsic property and activity of the native PrP molecules rather than of the incoming TSE-VPs, which is a subtle but highly significant departure from the current view. Likely, the PrP-res and their aggregates, the amyloid plaques, are byproducts of the TSE-VPs assembly process; this scenario is consistent with the results of numerous studies showing a poor correlation between the amount of PrP-res and TSE transmission or pathology, a finding that has been used as strong evidence against the prion theory [reviewed in (4;13)]. Interestingly, some human TSEs can be caused by protease sensitive

isoforms of PrP (65), reinforcing the idea that the PrP-res and its amyloid plaques might not be essential for infection and pathogenesis.

Genetic and evolutionary evidence for the endogenous TSE virus model

The gene coding for PrP, the *PRNP*, which was identified in the hamster and mouse genome as a single chromosomal gene, was found in all examined vertebrate species (66-68). The phylogenetic analysis of *PRNP* in mammals, birds, reptiles, and fish showed that the evolution of *PRNP* mirrors the established evolutionary relationships among the vertebrate species, suggesting that this gene was present early in the vertebrate lineage and has been relatively stable during evolution. This stability is reflected also by a similar molecular architecture of PrP protein in vertebrates (69). However, some additional genes similar to *PRNP*, such as the doppel gene in humans and *SPRN* in mammals and fish, have been discovered [reviewed in (68)]. These findings are consistent with the hypothesis that the endogenous TSE virus had entered the germ line of vertebrates very early in their evolution and has diversified along with vertebrate lineages.

One of the most striking features of the *PRNP* and related genes is the lack of introns within the protein coding region of the gene. This is a relatively rare phenomenon among vertebrate genes, but highly predictable for viral genes. The *PRNP* chromosomal region contains numerous endogenous retroviral sequences and transposable elements (68;70), which makes it difficult to uncover other potential clues associated with the organization and expression of *PRNP* that would indicate a viral origin. Interestingly, though, the *PRNP* contains several sequence domains that are similar and collinear to domains in the retroviral reverse transcriptase gene (71). The full significance of this sequence similarity remains to be revealed. Taken together, these genetic features are consistent with *PRNP* being an endogenous viral gene.

The strain repertoire of TSE pathogens, relating to features such as incubation period, pathology, and tissue tropism, is a remarkable feature [reviewed in (3;4;7;14;72-74)]. Although in many cases the strain specificity of newly formed TSE transmissible units appears to be dictated by the incoming TSE units, in some animal models this specificity is apparently a function of the native PrP [reviewed in (3;4;7;14;72-74)]. It is possible, however, that in addition to PrP, nucleic acid molecules, such as small RNAs [discussed in (3;55;75)], might participate as chaperons or scaffolds during the assembly of new TSE transmissible units (i.e. TSE-VPs), contributing to TSE strain specificity even though they might not be included in the transmissible units (Box 2). Similarly, the assembly of viral particles in many viral species requires participation of viral molecules that act as chaperons or scaffolds (38-41). Interestingly, the participation of chaperone protein molecules, which are not included in the transmissible forms, has been demonstrated in yeast prions (76;77).

Box 2. The current experimental data supports the hypothesis that nucleic acid molecules (NAs) participate in TSE [discussed in (3;55;75)]. To evaluate this hypothesis, both conceptually and experimentally, it is critical to define:

(a) the stage in the life cycle of TSE pathogens in which these NAs participate,
(b) whether NAs participate as structural or as informational molecules, or both, and
(c) the source of these NAs.

As I previously emphasized [see comments in (78)], combining these features generates a rather complex set of potential hypothetical models for the participation of NAs such as small RNAs in TSE. Possibly, these TSE-associated RNAs (tse-RNAs) participate as chaperones in facilitating the assembly of new transmissible TSE viral particles (TSE-VPs). Moreover, these tse-RNAs might play an "informational role" by dictating the rate and/or the pattern of the assembly of PrP into new TSE-VPs, thereby conferring at least partially the strain characteristics of TSE pathogens. However, it is likely that the strain specificity of TSE pathogens is dictated primarily by the endogenous viral PrP molecules during their interaction with the incoming TSE-VPs (see text). The sequence of the hypothetical tse-RNAs is probably nonspecific, although their secondary structure might be a factor in dictating their chaperoning efficiency. Some of these chaperoning tse-RNAs might be included in the TSE-VPs as bystanders but, likely, they are not essential for TSE-VPs' transmission to a new host. Moreover, in this hypothetical model, the tse-RNAs do not replicate; they are arbitrary transcripts encoded by the host or by endogenous viral genomic sequences for various reasons.

The TSE strain phenomenon has been used as one of the strongest circumstantial evidence for the presence of a nucleic acid genome in the TSE transmissible units [discussed in (3-17;79)]. Considering the overwhelming evidence that the TSE transmissible units do not contain a viral genome, or that low molecular weight nucleic acids that could confer strain specificity have yet to be identified, this argument remains weak [see Box 2 and my comments in (78)].

However, the TSE strain diversity is still one of the strongest pieces of evidence against the prion hypothesis, but from an entirely different perspective. Based on a highly regarded observation that "Nothing in biology makes sense except in the light of evolution" (80), the prion theory would need to explain the evolution of a cellular protein that is: (a) pathogenic, (b) infectious, (c) able to transfer its structure and properties to new molecules by a template-based mechanism, and (d) able to undergo multiple isomeric conformations that are pathogenic and infectious. Certainly, owing to physiological factors or to mutations

some cellular proteins can form amyloid-like aggregates that are pathogenic [reviewed in (43;81)]. It should be noted, however, that TSE amyloid plaques per se apparently are not neurotoxic, nor infectious; i.e. they are not the TSE pathogens. Therefore, an analogy between the TSE pathogens and other amyloid proteins might not be relevant. Although, evolutionarily, pathogenic genes that are expressed during the reproductive period of a species are usually selected against and eventually eliminated, it could be rationalized that if the PrP gene is an essential cellular gene, then its occasional pathogenic activity would be evolutionarily tolerated. However, the fact that some isomeric forms of PrP are not only pathogenic but also infectious requires yet another evolutionary explanation that is much more difficult to rationalize. Next, the prion hypothesis needs to address a highly intricate and difficult issue, which is explaining the evolution of a mechanism by which the PrP infectious units transfer their structure and properties to the native PrP molecules, often in a strain-specific manner; the evolution of such an extraordinary feature would require strong selection. In summary, in order to remain a leading working hypothesis in the TSE field, the prion hypothesis must offer solutions to all these evolutionary issues.

Unlike the prion hypothesis, which doesn't appear to have evolutionary support, the origin of endogenous TSE viruses is an evolutionary expected phenomenon (25;82) and, as proposed in the next section, the remarkable property of endogenous viral PrP to assume diverse isomeric conformations (which dictates the strain specificity of the TSE pathogens) has been specifically selected to fulfill an antiviral protective function.

A protective, anti-viral function for endogenous viral PrP

Vertebrate species harbor thousands of germ-line retroviruses, some of which are evolutionally related across all vertebrates [reviewed in (83;84)]. Although most endogenous viruses are not active, some express their genes and even produce viral particles; however, little is known about their expression pattern (85-88). Based on well-supported evolutionary principles of host-pathogen co-evolution, it is expected that endogenous viruses that decrease the fitness of their host would be eliminated along with their host by natural selection. On the other hand, endogenous viruses that increase the fitness of their host would be able to thrive evolutionarily (3;89).

The evolutionary history of *PRNP* in vertebrates is evidence for strong selection, implying a significant function for PrP. Surprisingly, after more than two decades of research conducted within the framework of the prion theory, which is based on the idea that *PRNP* is a cellular gene, the function of PrP is still not known (90-93). Remarkably, under experimental conditions, mice lacking *PRNP* have a normal phenotype (94).

As previously emphasized, understanding the function of PrP is critical for understanding the nature of TSE pathogens [discussed in (69;75;95;96)]. Considering that endogenous viruses that are pathogenic cannot survive evolutionally, it is likely that *PRNP* has been selected to fulfill a symbiotic function [see also ref. (3)]. In the endogenous TSE virus model, an endogenous virus that entered the germ-line of vertebrates early in their evolution co-evolved with their hosts by providing protection against other viruses. This protective function was especially beneficial for neurological tissues, particularly the central nervous system, which have a limited potential for regeneration and, therefore, could not be under the normal surveillance of the immune system because of its associated deleterious inflammatory effects. The same rationale is also valid for the function and evolution of the doppel protein, a homolog of PrP, which is expressed primarily in the testes [reviewed in (68)].

The property of PrP molecules to undergo isoform transitions into new structural conformations and their propensity to interact with RNA molecules and assemble into particles (48-54) [reviewed in (56;57)] are clues not only to their viral evolutionary origin but also to potential mechanisms for their anti-viral function. Based on these properties, PrP could interfere with the entry of parasitic viruses, replication of their genome and expression of their genes, or with disassembly or assembly of their viral particles, potentially generating a multifaceted protective barrier against viral infection. Similar anti-viral protective mechanisms are found in the life cycle of many viruses, which protect their host cells, and themselves, from infection with competing viral species (89;97-100). A potential anti-viral mechanism of PrP could be based on its intrinsic properties to recognize and interact with the components of the pathogenic viruses, such as nucleo-capsid monomers, by mimicking their structure. PrP would use these viral components as scaffolds, or chaperons, to adopt diverse isomeric conformations that by mimicking the structure and properties of the incoming viral nucleo-capsid components would be able to disrupt the viral life cycle. To be able to exercise their protective mechanism against a variety of virulent viruses, including endogenous viruses, it is proposed here that the PrP molecules evolved the extraordinary property of adopting diverse isomeric conformations, which fundamentally explains the TSE strain phenomenon.

In the endogenous TSE-virus model, the transmissible units - the TSE-VPs - are recognized by the native endogenous viral PrP molecules as pathogenic viruses. The native PrP molecules use the incoming PrP-vp as chaperons, scaffolds, or templates to change their native structure into an isomeric conformation that mimics that of incoming PrP-vp. However, unlike the interactions of PrP with genuine pathogenic viruses, which would lead to their inactivation, the interaction of native PrP molecules with the incoming TSE-VPs (and probably also with chaperoning tse-RNAs; see Box 2) leads to their self-assembly into new TSE-VPs, which continue the cycle.

Several lines of evidence indicate that neuro-

degeneration and TSE pathogenesis is induced only when the endogenous viral PrP molecules are anchored on the cellular membrane by a glycosyl-phosphatidylinositol (GPI) moiety (101). Presumably, the process by which the membrane-anchored PrP molecules interact with one another and with the incoming TSE-VPs and, possibly, with additional chaperons during their assembly into the new TSE-VPs damages the cellular membranes, causing host cell death. Considering the potential cellular signaling properties of GIP-anchored proteins, it is conceivable that PrP could exercise an additional antiviral protective mechanism by triggering the death of infected host cells through apoptosis [reviewed in (7;72)] which would block the spread of infectious pathogenic viruses.

Although the protective function of the endogenous viral PrP was selected to be exercised primarily in neurological tissues, this molecule is also expressed in other tissues, such as immune cells, which explains the multiplication of TSE agents in the peripheral lymphoid tissue before reaching the central nervous system. Lack of clinical signs associated with multiplication of TSE agents in immune cells is enigmatic (7). However, TSE-associated pathology has been observed in lymphoid tissues (102;103), and evidence for a presumed antiviral PrP function against endogenous retroviruses in mouse spleens has been shown (75). A plausible explanation for the apparent lack of clinical symptoms associated with the assembly of new TSE-VPs in lymphoid tissue is the relatively high turnover of immune cells as compared to the cells in the neurological tissue. It would be expected, however, that TSE-associated cellular toxicity affects the immune memory cells, which is a testable hypothesis. The production of new TSE-VPs in the peripheral tissues seems to be necessary for their migration to the central nervous system, and it is proposed here that the elusive cellular receptor for the TSE-VPs in all cells types is the native PrP. Interestingly, PrP has been previously suggested as the receptor for TSE pathogens but from a different rationale, specifically to explain the essential requirement for PrP in TSE in context of the conventional virus hypothesis (4;13).

Similar to endogenous TSE viruses, other endogenous viruses have co-evolved with their hosts by providing anti-viral protective functions [reviewed in (89;97-99)]. A well documented example is the murine endogenous viral gene *Fv1*, which is evolutionally related to the *gag* gene of the L family of murine endogenous retroviruses. Apparently, the product of *Fv1* inhibits the infection by murine leukemia virus by a post-entry mechanism. Because *Fv1* and other functionally related endogenous retroviral genes in mammals, including humans, have entered their host germ-line more recently than *PRNP*, their homology with endogenous retroviruses is easier to detect.

Another interesting, circumstantial finding that is suggestive of an anti-viral protective function for PrP is the function of one of the yeast prions, the [Het-s], which was shown to protect their fungal host from infection by debilitating fungal viruses [reviewed in (104)]. However, there is strong, direct evidence that PrP interacts with other viruses and confers protection (58;75;105-112). For example, the production of HIV-1 in a human cell line expressing high levels of PrP was reduced eightfold, and HIV infectivity by three- to fourfold (58). Also, it was recently shown that activation of endogenous murine retroviruses in germinal centers of mouse spleens following immune-stimulation leads to up-regulation of PrP expression, which in turn reduces the level of retroviral activity (75).

There is no doubt that viruses have played a major role in the evolution of their hosts. Along with other parasites and pathogens, viruses have shaped the evolution of their hosts by parasitism and disease under the classical Darwinian selective pressures imposed by host-parasite co-evolution. However, because of their ability to insert their genome into the host genome and to be transmitted vertically, endogenous viruses have shaped the evolution of their hosts by direct genomic mechanisms, including the contribution of viral genetic material, modulation of the host's gene expression, and promoting recombination events [reviewed (84;113;114)]. Interestingly, most of the vertebrate genome is composed of non-coding DNA (nc-DNA), or "junk DNA," that in large part still maintains a retroviral signature [reviewed in (84;115;116)]. There is strong evidence that one of the major functions of ncDNA, including introns, is to protect the host genes from insertional mutagenic activity of proviruses and other mobile genetic elements by serving as a "sink" for their integration (117-119). This represents one of the most significant cases of host-virus co-evolution in which the host uses viral genetic material as an anti-viral defense mechanism. However, it is evident that this co-evolution also led to the development of a series of anti-viral protective mechanisms via viral proteins [reviewed in (89;97-99)], such as (a) the anti-viral resistance factors Fv1, Lv1 and Ref1 in mammals, (b) syncytin, a protein encoded by an endogenous virus that is apparently associated with multiple sclerosis (120) (note: similar to other predictable anti-viral proteins, syncytins are expressed primarily in tissues that are not under normal immune system surveillance), (c) possibly some of the interferon- and TNF-families of proteins (121;122), and (d) the PrP and doppel proteins, as hypothesized here.

Summary and Perspective

For more than two decades the prion theory, which outside the TSE field is usually regarded as fact, has been the leading working hypothesis in TSE research. However, numerous researchers in this field have questioned this theory, and many of them have supported the other traditional views about the nature of the TSE pathogens - the conventional virus view and the virino concept [discussed in (3-17;79)]. A legitimate question is why would different groups of highly regarded TSE researchers interpret basically the same experimental data in such disparate ways? As I discussed recently [see comments in

(78)], it is unconceivable that so many scientists have been wrong for such a long period; this is unprecedented in the history of modern science. A sensitive answer is that these traditional views about the nature of the TSE pathogens are partially correct, but the problem is with the reductionist approach imposed by the current dogma of viruses as viral particles in evaluating the TSE phenomenon.

As pointed out here, one of the main objectives of the TSE research and the focus of the developing hypotheses and concepts about the nature of TSE pathogens has been the presence, or the absence, of a nucleic acid genome in the TSE transmissible, infectious units. Although practical from a medical and public health perspective, this focus on the infectious stage in the life cycle of infectious pathogens, particularly in the case of viruses, has obscured the relevance of the other life cycle stages in establishing their nature. This focus on the infectious stage in the life cycle of pathogens has created also the false expectation that the infectious forms should contain all the major structural and informational components of a pathogen, including their genome. However, for endogenous pathogens, which are vertically transmitted and therefore are present in all the host cells, this doesn't necessarily need to be the case.

Failure to recognize these fundamental biological principles when conducting research and interpreting experimental data could lead to conflicting views [see comments in (78)]. For example, based on the overwhelming evidence that TSE infectious units do not contain a viral genome and, therefore, according to the dogma of viruses as viral particles they could not be defined as viruses (see Box 1), the prion hypothesis seemed justified despite the fact that it made little biological and evolutionary sense. Similar concerns about the prion hypothesis have been expressed by many TSE researchers [discussed in (3-17;79)], leading to open contentions in the TSE research field (31;95;96).

The endogenous TSE virus model, which is rooted in a new view about the nature and evolution of viruses (29;82), integrates aspects of all three traditional views about the nature of TSE pathogens into a unifying scenario [see my comments in (78)]. This model might be a more effective working hypothesis in TSE field as compared the current hypotheses. The endogenous TSE virus model opens this field to new interpretations and directions, both in basic research and in associated biomedical and public health fields.

First of all, this model completely changes the perspective on TSE. In the endogenous TSE virus model, the generation of new infectious TSE units is regarded as an intrinsic property and activity of the native PrP molecules, rather than of the incoming TSE transmissible units. This perspective accommodates not only infectious TSE, but also provides the rationale for understanding inherited TSE such as familial Creutzfeldt-Jakob disease, which results from germ-line mutations in *PRNP*, and for understanding spontaneous TSE, which is the most common form of TSE in humans. Likely, spontaneous TSE is caused by somatic mutations in *PRNP* or by epigenetic factors that lead to spontaneous assembly of PrP into TSE-VPs, which induces a PrP antiviral response and consequently the production of new TSE-VPs.

Evidently, TSE strain variation and inter-species transmission patterns are more effectively explained in the context of the endogenous TSE virus model than in that of the other hypotheses. Moreover, this model sets up a conceptual framework for considering a similar endogenous viral etiology for other disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases. Interestingly, it was recently found that the PrP has a protective role against development of Alzheimer's disease (123), suggesting subtle, but significant interactions among endogenous viral elements.

From a medical and public health perspective, the endogenous viral nature of the TSE pathogens and their potential interaction with other viruses, both exogenous and endogenous, is highly relevant as these interactions could lead to altered transmission patterns and to the evolution of new strains. Of particular interest is the possibility that PrP-vp might be included into the viral particles of exogenous or endogenous viruses by mechanisms analogous to viral phenotypic mixing, which could enhance the horizontal transmission of TSE (124). This phenomenon might explain the enigmatic high rate of natural transmission of scrapie in sheep and wasting syndrome in elk (1;2). In addition to phenotyping mixing, genetic recombination of TSE endogenous viruses with other endogenous and exogenous retroviruses, could lead to new pathogenic viral strains (125-128). Certainly, highly pathogenic endogenous viruses would not survive evolutionally, but neither would their host, which might explain why pathogenic endogenous viruses are not commonly found.

An obvious advantage of the endogenous TSE virus model as compared to the traditional hypotheses is that it is consistent with the experimental evidence that previously has been used to specifically support these traditional views, and it integrates them into a unifying scenario about the nature of TSE [see also my comments in (78)]. Therefore, based strictly on the current experimental data, the endogenous TSE virus model might be a superior working hypothesis for TSE research compared with the prion hypothesis or the other views; this is highly significant, because productivity in any research field depends on the quality of the working hypothesis. Additional supporting evidence for the endogenous TSE virus model could be found by searching for other genetic features that would attest for the endogenous viral nature of *PRNP*, or from studying the assembly properties of the PrP molecules into viral particle-like structures and their interaction with other viruses. It is also possible that some extant viral lineages are evolving into TSE-like viruses, which would be the ultimate proof for the endogenous TSE virus model.

The endogenous TSE virus model makes several predictions, which are fully open to experimental testing.

The antiviral protective function of PrP is one of the most significant predictions. The current evidence for this function is strong, and considering the existence of numerous wild-type and *PRNP* knockout animal models, additional studies testing this hypothesis should be relatively straightforward. Additional predictions, such as the hypothesis that PrP serves as the cellular receptor for TSE infectious units or that TSE leads to depletion of immune memory cells, are also fully testable. One of the most significant implications of the endogenous TSE virus model and the presumed property of PrP to assemble into viral particles is that analogous viral processes could be used as model systems for developing new TSE diagnostic reagents and therapeutic approaches.

In conclusion, the endogenous TSE virus model provides a highly plausible conceptual and evolutionary framework for explaining, integrating, and directing TSE research, which could accelerate the progress towards understanding and controlling TSEs and related disorders.

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