Comment to "Endogenous Viral Etiology of Prion Diseases"

Claudiu Bandea (23 December, 2009)

This note is intended as a comment to a paper in Nature Precedings entitled "Endogenous viral etiology of prion diseases" (http://precedings.nature.com/documents/3887/version/1). Because it contains illustrations that could not be displayed in the comments section of the paper, I'm posting this comment here as a PDF document.

In his comment to my paper, Jesus Requena states that I'm citing "some circumstantial evidence" supporting the endogenous TSE virus model. I also received an e-mail from another researcher in the TSE field pointing to the obvious absence of "figures or tables of data" in the paper, apparently indicating lack of hard evidence for the endogenous TSE virus model; in fact, this researcher spiced up his comment with a humorous inference that I probably posted the wrong document in Nature Precedings!

Here, I would like to briefly address "the quality" of the evidence supporting the endogenous TSE virus model. Also, I will specifically address the concern of the other TSE researcher regarding the absence of figures (i.e. "hard data") by presenting several electro-micrographs of assembled PrP, which I think is strong evidence for its viral nature. In fact, I'm even going to show a gel (the epitome of "hard data" in molecular biology), which might be the first evidence supporting the hypothesis that nucleic acids, such as small RNA molecules, might be required for efficient in vivo assembly of PrP into TSE transmissible units. Incidentally, these pictures are from published studies coming from that researcher's own laboratory.

I have emphasized previously that the endogenous TSE virus model is consistent with the current TSE data, including the data that has been specifically used to support the prion hypothesis [1]. As a matter of fact, I have yet to find any published data that doesn't conform to the endogenous TSE virus model. And, I have yet to receive any indication of such data from the many researchers who reviewed this paper; however, if such data exist I encourage my colleagues to present it.

The point I'm trying to make is that if the current TSE data is "circumstantial" when I use it in context of the endogenous TSE virus model as stated by Jesus Requena, then it should also be qualified as circumstantial when it is used in support of the prion hypothesis. After all, that might indeed be the case, because despite the fact that outside of the TSE field the prion view is considered a proven reality, and reworded and rewarded accordingly, the majority of the researchers in the TSE field consider it a hypothesis (i.e. most TSE researchers still call it "the prion hypothesis," and the prion proponents are still trying to gather data and try to convince others that the "prion hypothesis" is correct). Therefore, it might be that, true to science, the evidence should always be considered circumstantial in context of any hypothesis, until that hypothesis becomes a fact.

I have already discussed that some of the current TSE data, as circumstantial as it might be, is more consistent with the endogenous TSE virus model than with the prion hypothesis. For instance, the protein coding region of PrP gene contains no introns. This

feature is more consistent with PrP gene being a viral gene rather than a eukaryal gene as asserted in the prion hypothesis. Indeed, this is one of the few significant TSE findings that have not been used as evidence for prion hypothesis. Interestingly, though, a recently published study on the evolutionary origin of the PrP gene reports that this gene has descended from the family of metal-ion transporters [2]. However, the members of Zip family of genes are relatively rich in introns, and PrP has only weak, if any, sequence homology with this family of transporters. Therefore, the conclusion of this study is highly questionable.

Another line of evidence that favors the endogenous TSE virus model but is not addressed in the context of the prion hypothesis concerns the property of PrP to undergo isomeric conformational changes and assemble into structures that are characteristic of viral proteins. As shown in **Figure 1** (from reference [3]), during the formation of TSE transmissible units, the PrP molecules assemble into classical hexagonal virus-like structures. Artistic interpretations, which shows the similarity between the assembled PrP hexagonal lattice (Fig.1F; from [3]) and a viral capsid-protein hexagonal structure (Fig.1G; from [4]), are also presented.

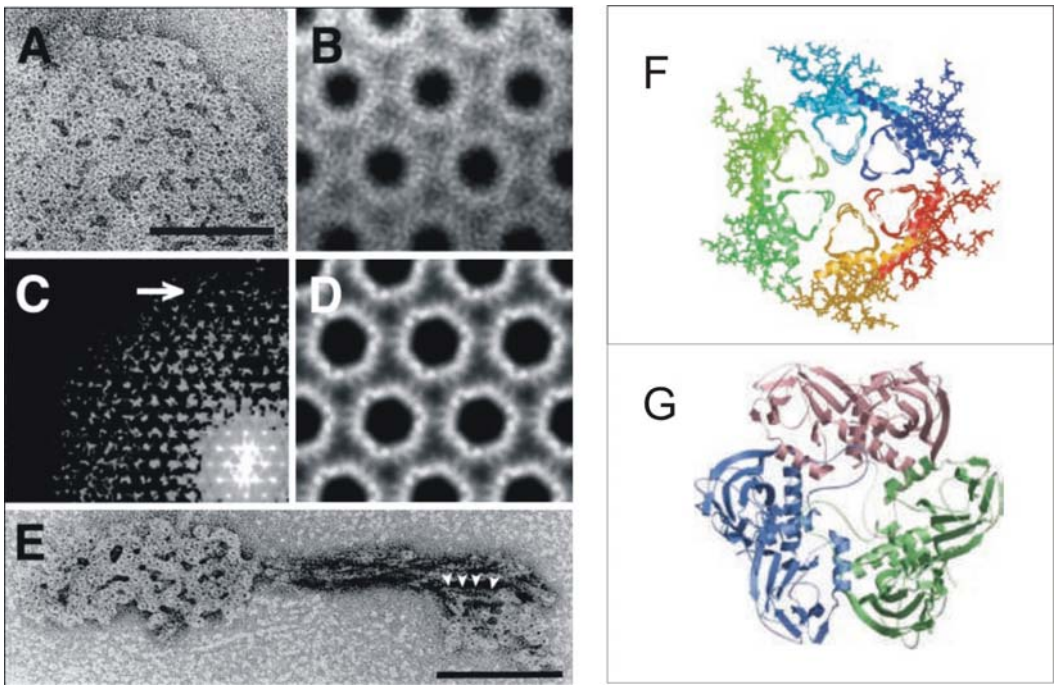

Figure 1. 2D crystals of PrP 27-30. (*A*) A 2D crystal of PrP 27-30 stained with 2% uranyl acetate showing an apparent hexagonal lattice. (*B*) High power view of a crystal after CTF correction and several rounds of correlation-mapping and averaging. (*C*) Section of a power spectrum after averaging showing spots out to the 11th order, corresponding to _7 Å (arrow). (*D*) Crystallographic averaging further improved the amount of detail visible. A p3 plane group was used. (*E*) Typical prion rod with an aggregate of ‘‘crystal’’ subunits at each end. Some protofilaments reveal rows of dense stain accumulations, suggesting stacked subunits (arrowheads). (Bars = 100 nm.). (Note: The text for Figure 1 A-E, and the text for Figure 2, below, is from the original publications [3] and [4], respectively; the readers should refer to the original sources, where they can find additional pictures as well as more information and discussion). (*F*) and (*G*) represent artistic interpretations of assembled PrP hexagonal lattice (see [3]) and of viral capsid-protein hexagonal structure (see [4]).

Rather than elaborating here on the significance of these findings, I would let readers "qualify" this evidence and decide for themselves if this evidence supports the endogenous TSE virus model, or the prion hypothesis. However, I do want to mention here that these specific PrP virus-like hexagonal lattice structures are strongly associated with "TSE infectivity."

The gel in **Figure 2** (from reference [5]) shows the effect of bis-acridines on the accumulation of scrapie-associated PrP in cultured cells. As it can be seen in this gel, the accumulation of scrapie-associated PrP is inhibited by a bis-acridine compound in a dose-dependent manner. This study [5] was concluded with the following statement: "Although we currently do not understand the mechanism by which acridine compounds affect PrP-Sc formation, these compounds offer unique tools to study the mechanism of prion replication." (As discussed in the paper and in my previous comments, obviously prions do not replicate, but that is of secondary relevance in context of this comment, which is all about "evidence"). Interestingly, in an earlier, original study [6], it was shown that the accumulation of scrapie-associated PrP was not a result of direct interaction of the acridine compounds with the PrP molecules. Based on these findings, I would like to predict here that the mechanism by which acridine compounds inhibit the accumulation of scrapie-associated PrP is based on their well known property of binding to nucleic acid molecules. By binding to nucleic acids molecules such as small RNA molecules that are required for efficient in vivo assembly of PrP into TSE transmissible units, these compounds inhibit the accumulation of scrapie-associated PrP. Although "circumstantial," this might be the first evidence that nucleic acids are involved in the formation of TSE transmissible units in vivo.

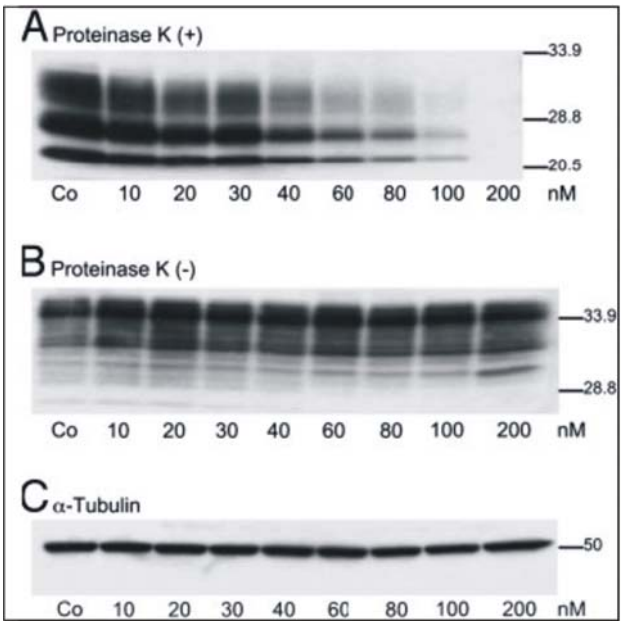

Figure 2. Piperazine-based bis-acridine, **11**, reduces PrP^{Sc} concentration from ScN2a cells in a dose-dependent manner. ScN2a cells were incubated with compound **11** at the concentrations indicated (10–200 nM) for 7 d. Control cells (Co) were untreated. Cell lysates were harvested and either PK-digested (*A*) or undigested (*B*) before immunoblotting with anti-PrP Fab D13. (*A*) Dose-dependent reduction of PrP^{Sc} concentration in ScN2a after incubation with compound **11**. (*B*) PrP^{C} levels remained unchanged by treatment. (*C*) Immunoblot of undigested cell lysate probed with antitubulin.

As I previously pointed out, in the context of the endogenous TSE virus model the data generated in the TSE studies remains valid. But generating data is only part of the research process. The other part, which some scientists consider to be the most significant aspect of this process, is how we interpret this data. And, that's where I think the prion hypothesis has failed!

Reference List

(1) Bandea CI: Endogenous viral etiology of prion diseases. Nature Precedings 2009; http://hdl.handle.net/10101/npre.2009.3887.1.

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(6) Doh-Ura K, Iwaki T, Caughey B: Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol 2000; 74(10):4894-4897.