Prions in plants – potential importance?
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Prions are 'malformed' proteins that have unique properties that resemble 'living' organisms in their infectivity, self replication, genetic/epigenetic transfer and protein composition, but lack DNA or RNA components. Although the emphasis has been placed on their neurodegenerative effects on animals (encephalies), they also occur in fungi and plants, and can be transferred as infectious entities from plants to animals. They may also be contributing factors increasing the severity of various plant pathogens (Scorch type diseases, Goss' wilt, SDS, etc.). There is much we don't know about these seemingly aberrant natural protein entities, but also much that we do know. Plant Pathologists involved in understanding threat pathogens need to be aware of the implications of their apparent infectious nature in plants and association with various new, emerging, and reemerging plant diseases.

Background (extracted heavily from "Prion from Wikipedia", 2014):

A prion (conjugate of protein and infectious unit = prion) is described as an infectious agent composed of a natural protein in a misfolded or aberrant form; however, this concept and its function are still debatable since it is not fully understood. The normal proteins (PrP) that prions are made of are structurally well defined and found on the membranes of normal cells in animals, people, plants, yeasts and other microbes. They can't be separated by centrifugation and are strong Cu chelaters with a high affinity for Cu which is thought to provide both a stability for cell-to-cell adhesion and functional cell signaling role in the brain of mammals. The normal PrP protein forms are readily digested by proteinase K and separated from the cell surface by phosphoinositide phospholipase C.

The normal PrP form can be converted to the polymorphic, infectious isoform (PRP^i) by changing its conformation or shape and its resistance to proteinase K. The end of each protein 'fiber' acts as a template to which free protein molecules attach to permit growth. *In vivo*, the PRP^i formed are morphologically similar for some prions, but may also be highly pleomorphic as they often are *in vitro*. Prions are not considered 'living' organisms because they do not contain DNA or RNA, but propagate by transmitting a misfolded protein state that is thought to provide a template that catalyzes the conversion of normal proteins to the misfolded amyloid prion form.

This triggers a chain reaction to produce large quantities of the highly stable prion form. The misfolded protein structure of a prion is extremely stable, multiplies and accumulates in "infected" tissue to cause tissue damage and cell death. Prion proteins are resistant to chemical and physical agents so disposal and containment of them is difficult. Each disease is thought to be caused by a specific prion strain that 'breeds' true except that epimutations are thought to produce slight differences in structure and account for prion variances in host specificity (Li et al., 2010). With the identification of infectous CWD prions in plants, the specific host or animal-plant barrier may not be as absolute as originally thought.

There are several concepts of PRP^i growth (Wikipedia, 2014). One model assumes that the PRP^i fiber ends bind to similar PrP proteins and catalyze their conversion to PRP^i, the prion form, with the two forms then coming apart to go on and convert more PrP; however, infectious monomeric PRP^i has never been isolated. An alternative model assumes that PRP^i fiber ends bind PrP to produce longer fibrils that eventually break to form more PRP^i. If this were the case, PRP^i prions would increase linearly rather than exponentially as observed with prion diseases. A third concept (Purdey, 1998, 2005) involves the conversion of normal PrP functioning proteins to PRP^i through external catalytic factors (strong chelators such as phosmet insecticide, glyphosate, etc.) that alter the Cu chelating characteristics or environment of the normal PrP with replacement of the Cu by another strong transition element such as Mn that brings about the aberrant protein folding characteristic and the infectious nature of the prion (Brown, et al., 2000; Leoning, 2014; Purdey, 1998, 2005).

There are ten known prion mediated diseases in a variety of animals that are referred to as transmissible spongiform encephalopathies (TSE) because they affect the structure of the brain or other neural tissue (Wikipedia, 2014). Various neurodegenerative diseases (Alzheimer, Parkinson, ALS, tauopathies, etc.) involving protein misfolding disorders may also involve prions (Grad and Cashman, 2014). All of these diseases are currently considered untreatable and universally fatal. These include:

1. Bovine spongiform encephalopathy (BSE, mad cow disease) in cattle
2. Scrapie in sheep
3. Chronic wasting disease (CWD) in deer and elk (also sheep)
4. Creutzfeldt-Jacob Disease (CJD) in humans
    - 4.a. Variant Creutzfeldt-Jacob Disease (vCJD) in humans
    - 4.b. Latrogenic Creutzfeldt-Jacob Disease (lCJD) in humans
    - 4.c. Familial Creutzfeldt-Jacob Disease (fCJD) in humans
    - 4.d. Sporadic Creutzfeldt-Jacob Disease (sCJD) in humans
5. Gerstmann-Straeussler-Scheinkr syndrome in humans
6. Fatal Familial Insomnia (FFI) in humans
7. Kuru in humans.
8. Transmissible mink encephalopathy (TME) in mink
9. Feline spongiform encephalopathy (FSE) in cats
10. Exotic ungulate encephalopathy (EUE) in nyala, oryx, greater kudu

An eleventh tentative prion-like protein (RFP) that causes reproductive failure (infertility, miscarriage, pseudopregnancies, abortion) has recently been identified in a variety of animals and humans with an apparent source being plants (Leoning, 2014). This is similar to the recent identification of the CWD prion in alfalfa and corn as the potential source for infection of animals (deer, elk, mice) with this TSE (Johnson, 2014).

Current thinking is that the primary method of infection of animals is through ingestion of contaminated materials (remains of dead animals, urine, saliva, fecal materials or clay and soil minerals to which the prions have become bound). Prions are quite resistant to most proteases, heat (autoclaving at 134 C /274 F for 18 min may not deactivate PrP^i), UV radiation, and formalin treatment, but can be denatured by bleach, caustic soda, and strongly acidic detergents. A lichen serine protease has been shown to degrade the CWD prion PrP^i (Johnson et al, 2011). Polyanionic molecules (surfactants) can be selectively incorporated with PrP in the formation of infectious PrP^i.

These latent diseases are difficult to detect because of their long (years) incubation periods and chronic nature before manifesting visible symptoms. The introduction of one BSE cow from Canada into the US cost the US over $5 billion dollars in investigative services and management changes. The growing incidence of CWD has changed hunting and recreational activities in many areas. The recent identification of agricultural crops (alfalfa, corn) as a source for infectious CWD [and the abortigenic agent] could have a profound impact on our agricultural economy. Prion diseases have not previously been considered highly infectious diseases although a 2013 study estimates that 1 in 2,000 people in the UK might harbor the infectious protein that causes vCJD (http://www.nature.com/news/one-in-2-000-uk-people-might-carry-vcjd-proteins-1.13962).

Much of our knowledge of prions has been gained by studying prions in fungi and responses to cases of CJD, the epidemics of BSE in Europe and CWD in North America. The presence of these diseases has substantial effects on regulatory and consumer behavior perhaps more from what isn't known than what is known about them. Prion-like proteins have now been identified in a wide variety of fungi and plants.

The role of plants:
Although wheat roots did not pick up the infectious CWD prion protein extracted from homogenized brains in earlier studies (Rasmussen et al, 2014), with the identification of the infectious CWD prion in alfalfa and corn bringing into question the assumed molecular species barrier to transmission, the epidemiology and impact of prion-induced diseases should be monitored closely. The infective prion-like protein causing reproductive failure, like the CWD prion, has been found in soybean plants and meal; corn plants, grain and silage; and pasture plants fertilized with animal manure; and has been shown to reproduce symptoms of reproductive failure on ingestion.

Corn infected with Goss' wilt and soybeans infected with SDS contain especially high concentrations of prion-like protein, with 'extended' symptomatic areas void of the pathogens of these diseases having the highest prion-like protein concentrations. The prion-like protein has been identified in *Fusarium glycines*, the cause of SDS. Protein-like prions also are common in scorch diseases (*Xyllela fastidiosa*) and are rumored to be found in citrus greening (HLB). The intransigent nature of the prion protein poses special challenges to disease management.

The role of pesticides:
The BSE epidemics, Goss' wilt, and SDS may have common factors predisposing to these diseases since they are all increased with the wide-spread application of certain pesticides. Many pesticides, especially the organic phosphate/phosphonate group, are strong mineral chelaters. Purdy (1998, 2005) proposed that phosmet, a strong Cu chelater, was a predisposing factor for BSE in the UK and resulted in replacement of the stabilizing Cu with the protein folding Mn. CWD occurrence has traditionally been associated with areas of low Cu soils and forage (Eastern Colorado, Wyoming and Western Canada – Alberta was the origin of the BSE infected cow that initiated a quarantine and shut down imports of Canadian meat products to the US for several years). The CWD disease has been 'spreading' from its original sites to other areas so it is now found in 23 states and is expected to continue its spread (CDC, 2013). The wide-spread indiscriminant use of glyphosate, another strong Cu chelater, has greatly extended the area of micronutrient deficiency in soybeans, corn and other crops. Glyphosate is a known predisposing factor for Parkinson disease, a neurodegenerative disease associated with prion activity (Grad and Cashman, 2014). Could the glyphosate-induced micronutrient deficiency and its polyvalent surfactant account for the greatly extended occurrence of CWD in Wisconsin and other states (Supattapone, 2013) ?

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