chewing insects at bay. It is clear that sap-feeding insects must drain a plant of nutrients. Yet logic suggests that the cost extracted by trophobionts must be less than the potential cost of leaf herbivory that would take place if the ants that tend trophobionts were not on patrol against leaf herbivores (*9*). The evidence provided by Davidson *et al*. that much of the large biomass of rainforest canopy ants is maintained by plant products suggests that the total costs of herbivory to rainforest plants may be much greater than previously believed. Lowman (*10*) has suggested that "sap-suckers" may be important rainforest canopy herbivores, and now this seems likely to be true. By feeding on extrafloral nectar or the exudates of sap-sucking trophobionts (*11*), ants make a substantial contribution to herbivory in the rainforest canopy.

Predacious ants act as agents of natural selection on their prey, whereas scavenging ants are the garbage collectors of the rainforest—ecologically useful but with little evolutionary impact on the species that they scavenge. However, if rainforest canopy ants are herbivores, then, like predators, they would be agents of natural selection on their prey, rainforest canopy plants. The energy and nutrient budgets of affected plants may strongly reflect the impact of ant and ant-mediated herbivory. A possible ramification of considerable consequence is that as global climate change pushes tropical trees toward the limits of their physiological abilities (*12*), plants that pay high costs to herbivory may face constraints on both short-term and adaptive responses to the stress of rising temperatures. Ants as herbivores could be major players in the ecological dynamics of tropical rainforest trees and, thereby, in the carbon balance of Earth. "Little things" really do matter. Rainforests should be preserved.

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BIOMEDICINE

A View from the Top—Prion Diseases from 10,000 Feet

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The causative agent of the transmissible spongiform encephalopathies (TSE) or prion diseases, which include sheep scrapie, mad cow disease, and human variant Creutzfeldt-Jakob disease (vCJD), is still hotly debated. The "protein only" hypothesis postulates that the TSE agent is an infectious, self-replicating prion protein called PrP^{Sc}. This abnormal protein is considered to be an insoluble, partially protease-resistant isoform of a normal cellular protein, PrP^{C}, which is both soluble and protease-sensitive (see the figure). The biochemical differences between these two PrP isoforms are due to a change in conformation whereby the primarily α-helical and loop structure of PrP^{C} is refolded into the predominantly β-sheet structure of PrP^{Sc}. The conformational switch from PrP^{C} to PrP^{Sc} is "seeded" by PrP^{Sc} (most likely in the form of aggregates), which induces PrP^{C} to take on the aberrant form. This conversion process is undoubtedly a key event in TSE pathogenesis. However, questions remain as to how or even whether PrP controls replication of the TSE agent and its strain phenotype, contributes to TSE neurodegeneration, and causes heritable forms of human TSE disease. It is certainly a tall order for any single protein to encode all of these different properties, and researchers in the often combative prion field have differing opinions about whether, in fact, PrP^{Sc} does it all. At a recent meeting (*1*), participants discussed how the normal mammalian protein PrP^{C} and its subverted counterpart PrP^{Sc} contribute to the pathogenesis of TSE diseases. Perhaps it was the spectacular setting, maybe it was the great skiing, or maybe it was oxygen deprivation due to the altitude, but some common threads began to emerge detailing how PrP influences TSE pathogenesis.

Strains of TSE agent are distinguished in part by differences in disease-associated pathology in the brain. These pathological changes include spongiosis, neuronal vacuolation, gliosis, and diffuse versus plaque-like PrP^{Sc} deposits. Several groups investigated the relation between PrP^{Sc} and brain pathology. Formation of PrP^{Sc} takes place at the cell surface and/or at some point in the endocytic pathway that shuttles surface proteins into intracellular lysosomes for recycling or degradation (see the figure). Studies in sheep infected with scrapie or bovine spongiform encephalopathy (BSE or mad cow disease) illustrated not only that different sheep scrapie strains could target different cells in the brain, but also that the deposition and size of PrP^{Sc} in BSE- versus scrapie-infected sheep were different. This suggested that different strains of PrP^{Sc} become localized in different cellular compartments (Martin Jeffrey, Lasswade Veterinary Laboratory, Edinburgh). These differences were especially exciting because they could be used to distinguish BSE-infected sheep from scrapie-infected sheep. Interest in such discriminations was heightened by data demonstrating that many sheep genotypes are susceptible to BSE infection (Fiona Houston, Institute for Animal Health, Compton, UK).

In humans, the strain of the agent causing CJD also appeared to influence the extent of pathological changes in the brain: gliosis, spongiform changes, and the pattern of PrP^{Sc} deposition (Fabrizio Tagliavini, Carlo Besta National Neurological Institute, Milan). In transgenic mice, a single mutation in PrP^{C} changed not only the degree of susceptibility of the mice to infection but also targeting of CNS pathology in vCJD infection. This suggested that host cell PrP^{C} is a potent susceptibility factor capable of distinguishing among different TSE strains (Jean Manson, Institute for Animal Health, Edinburgh). In vitro studies also provided evidence that strain-specific differences in PrP^{Sc} may depend on host cell PrP^{C} as well as on the strain of injected PrP^{Sc} (Suzette Priola, Rocky Mountain Laboratories, Hamilton, MT). Thus, it is beginning to appear that strain-specific phenotypes are not necessarily encoded by the PrP^{Sc} molecule alone but can also be determined by the type of cell expressing PrP^{C} and the cellular location where PrP^{Sc} is formed (see the figure).

Studies of the association of PrP^{C} with membranes revealed that both glycosylphosphatidylinositol (GPI) anchor–dependent and anchor-independent modes of attachment may influence PrP^{Sc} formation and propagation between cells. When GPI-anchored in the plasma

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membrane, PrP^C can be induced to convert to the protease-resistant state by PrP^{Sc} in the same membrane but resists conversion by PrP^{Sc} in separate membranes. This suggests that interactions between PrP^C and PrP^{Sc} occur through molecular surfaces held close to the membrane by the GPI anchor (see the figure) (Gerald Baron, Rocky Mountain Laboratories). When bound to membrane lipid rafts in the GPI-independent mode, PrP^C can be converted by exogenous PrP^{Sc} and can also undergo spontaneous conformational changes and aggregations (Teresa Pinheiro, University of Warwick). Finally, studies of PrP trafficking demonstrated that PrP^C can rapidly exit its raft membrane environment and enter coated pits during endocytosis, further suggesting that different membrane environments might influence the ability of PrP^C to form PrP^{Sc} (Angela Jen, King's College London).

At the meeting, a consensus emerged that truncated forms of PrP^{Sc} and alternatively processed forms of PrP^C may be important in TSE pathogenesis. Truncated forms of PrP^{Sc} derived from full-length PrP^{Sc} found in the human TSE disease, Gerstmann-Straussler-Scheinker syndrome (GSS), may provide clues to how the protease-resistant core of PrP^{Sc} is formed (Pierluigi Gambetti, Case Western Reserve University). These truncated PrP molecules may be the result of strain- and species-specific conformational differences in PrP^{Sc} that are influenced by the amino-terminal portion of PrP^C (Bruce Chesebro, Rocky Mountain Laboratories).

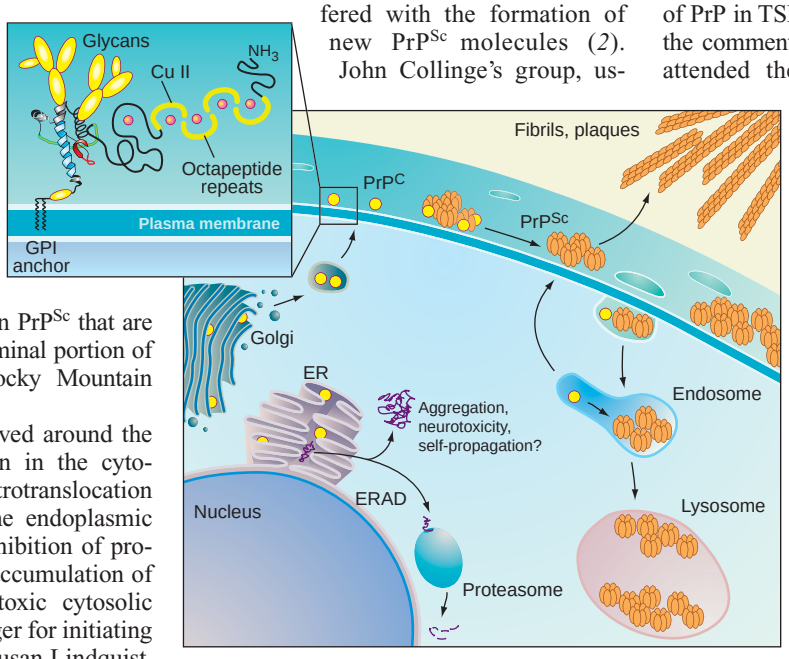
A vigorous debate revolved around the issue of PrP^C accumulation in the cytoplasm (see the figure). Retrotranslocation of misfolded PrP^C from the endoplasmic reticulum to the cytosol, inhibition of proteasomal degradation, and accumulation of a small amount of neurotoxic cytosolic PrP^C was proposed as a trigger for initiating all forms of TSE disease (Susan Lindquist, Whitehead Institute, Cambridge, MA). David Harris (Washington University, St. Louis, MO) disagreed with the mechanism of formation of cytosolic PrP, suggesting that inefficient translocation of PrP^C due to overexpression and not retrotranslocation led to accumulation of cytosolic PrP^C with an uncleaved signal peptide. Both groups derived transgenic mice that accumulated different forms of cytosolic PrP resulting in significant neurotoxicity. Overall, it appears that at the very least, PrP^C located partly or entirely in the cytosol is extremely toxic and may contribute to the neuronal degeneration underpinning TSE disease.

Convincing evidence was presented that TSE infection via the tongue is more efficient than oral infection, suggesting that the tongue may be a route of TSE agent entry following natural oral exposure (Richard Bessen, Creighton University, Omaha, NE). After infection, the junction between follicular dendritic cells and nerves in the spleen is a likely place for the transfer of TSE infectivity from the periphery to the nervous system, an event that is still poorly understood (Adriano Aguzzi, University Hospital of Zürich). This neuroimmune interface was also identified as the point at which polyene antibiotics have their anti-scrapie effect (Dominique Dormont, Commissariat à l'Energie Atomique), suggesting that this interface may be a good target for developing new anti-TSE drugs.

New forms of PrP may also be useful as anti-TSE compounds. Aguzzi reported that transgenic mice expressing PrP^C molecules dimerized via fusion to gamma immunoglobulin Fc were resistant to scrapie infection. The dimerized, soluble PrP^C associated with PrP^{Sc} and apparently interfered with the formation of new PrP^{Sc} molecules (*2*). John Collinge's group, using transgenic mice in which PrP^C was depleted in the neurons of 8-week-old animals, demonstrated that such depletion during the preclinical phases of disease significantly prolonged disease incubation times and reversed early spongiform changes (Giovanna Mallucci, MRC Prion Unit, London). Thus, PrP^C may be a better target for therapeutic intervention than accumulated deposits of PrP^{Sc}.

In terms of TSE diagnostics, a promising new assay may finally provide the TSE field with a quantitative and sensitive in vitro method for measuring mouse scrapie infectivity (Charles Weissmann, University College London). Fluorescent labeling of PrP^{Sc} with a sensitivity in the femtomolar range (Hans Kretzchmar, Ludwig Maximilians University, Munich) and a conformation-dependent immunoassay that detects presymptomatic TSE infection in mice (Jiri Safar, University of California, San Francisco) both offer the potential for highly sensitive, early diagnostic tests.

The conflicting opinions about the role of PrP in TSE diseases were summed up by the comments of two Nobel laureates who attended the meeting. Stanley Prusiner (University of California, San Francisco) strongly advocated PrP^{Sc} as the TSE infectious agent, whereas Kurt Wüthrich (Institut für Molekularbiologie und Biophysik, Zürich) maintained that accumulated "PrP^{Sc} is garbage" and that TSE diseases will probably be best understood by studying the normal cellular PrP^C form. In contrast, the "father" of yeast prions, Reed Wickner (NIH), argued that the definition of prion should be expanded to include any protein responsible for its own activation that is also transmissible from individual to individual (for example, protease B in yeast). As was evident from the lively presentations and debate, and as long as altitude was not a factor, significant progress has been made in dissecting how the PrP protein contributes to the enigmatic group of TSE diseases that includes scrapie, mad cow disease, and vCJD.



Cellular trafficking of PrP^C and PrP^{Sc}. PrP^C (yellow dots) follows the secretory pathway of the cell through the endoplasmic reticulum (ER) and the Golgi. Mature PrP^C is inserted via its GPI anchor into plasma membrane lipid rafts. The conversion of PrP^C to PrP^{Sc} (orange ovals) occurs either on the cell surface or, following endocytosis, in a cellular compartment such as the endosome. PrP^{Sc} formed at the surface and released into the extracellular space may cause the plaques seen in TSE diseases such as human vCJD. The diffuse PrP^{Sc} deposits and neuronal vacuolation common to many sheep scrapie strains may be due to PrP^{Sc} formation in endocytic compartments or to endocytosed surface PrP^{Sc} accumulating inside the cell. Misfolded PrP^C (squiggle) accumulating in the cytosol may also trigger PrP^{Sc} formation. (**Inset**) Structure of PrP^C showing the GPI anchor, the glycan chains, the copper-binding octapeptide repeats, and the regions where the α helices and loop structure of PrP^C (red, blue) may be converted to the β sheets of PrP^{Sc}. ERAD, endoplasmic reticulum associated degradation.

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