likely to make profound changes to how we think about topology, space-time and quantum field theory. ■

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1. http://www.claymath.org/Millennium_Prize_Problems
2. Perelman, G. Preprint math.DG/0211159 at <http://arXiv.org> (2002).
3. Perelman, G. Preprint math.DG/0303109 at <http://arXiv.org> (2003).
4. Stewart, I. *Flatterland* (Macmillan, London, 2001).
5. Hamilton, R. S. *J. Diff. Geom.* **17,** 255–306 (1982).

Prion diseases

Cannibals and garbage piles

Adriano Aguzzi and Mathias Heikenwalder

There's a lot of disagreement among prion scientists, as a recent conference made very clear. Even the revered 'prion hypothesis' came under attack.

After more than 280,000 mad cows and two Nobel prizes for research on transmissible spongiform encephalopathies, we must know all there is to know about these 'prion' diseases. Or do we? This question was asked at a recent symposium*, which looked at topics such as the cell biology of the normal prion protein and of its misshapen, disease-associated form; how the brain becomes damaged; and diagnosis and treatment. It was clear that, in all of these areas and more, there's still a long way to go.

Prion scientists have a reputation for being a contentious bunch — a fact that was amply confirmed on this occasion. Diametrically divergent opinions emerged on central questions such as the physiological function of the normal prion protein (PrP^{C}) and the role of its aberrant form (PrP^{Sc}) in disease.

Even the prion hypothesis, which nowadays is often regarded as dogma, was challenged. This hypothesis states that PrP^{Sc} is the infectious agent in transmissible spongiform encephalopathies (TSEs), and that it replicates by imparting its misshapen conformation onto PrP^{C}. In a spirited lecture, however, new Nobel laureate Kurt Wüthrich (ETH, Zurich) pointed out the continued failures to create infectivity *in vitro* by modifying bacterially expressed prion protein — a crucial prediction of the prion hypothesis. Another important experiment involves abolishing the structure (and infectivity) of the disease-associated prion protein with specific salts, and then attempting to restore infectivity by reinstating the original structure. This, too, has so far failed. Wüthrich referred to PrP^{Sc} as simply a build-up of "garbage", and submitted that we must understand the function of the normal prion protein before we can understand prion diseases.

The TSEs are characterized by the death of nerve cells, and another point of controversy concerned the mechanisms by which this occurs. A strong case was presented that the accumulation of PrP^{Sc} within the cytosol of neurons is to blame (S. Lindquist, Whitehead Inst., Cambridge, Massachusetts)[1,2]. PrP^{C} is usually located in the plasma membrane, and travels there by way of a network of internal membranes, the endoplasmic reticulum (ER). Lindquist proposed that a certain proportion of PrP^{C} never reaches the plasma membrane, but instead re-enters the cytosol by 'retrotranslocation' from the ER — a standard means by which other misfolded proteins are directed to the cell's waste-disposal unit, the proteasome. Lindquist found that a form of the prion protein that was specifically targeted to the cytosol caused rapidly lethal neurodegeneration in mice. This protein did not acquire resistance to protein-digesting enzymes (proteases), which has long been thought to be a key characteristic of PrP^{Sc}. But proteasome inhibition led to the accumulation of a slightly protease-resistant prion protein in cultured cells. Lindquist speculated that the diverse mutations in the prion protein that are associated with familial Creutzfeldt–Jakob disease (CJD) — a human TSE — might all lead to enhanced retrotranslocation, which, upon impaired proteasome function, could trigger disease. The big surprise here is that the cytosol might be the place where PrP-mediated neuronal death begins.

However, this model was contested by D. Harris (Washington Univ., St Louis, Missouri), who found that cytosolic prion protein retains its 'signal peptide' — normally removed after proteins enter the ER — and does not contain the glycosyl phosphatidylinositol 'anchor' needed for attachment to membranes[3]. This suggests that the protein never entered the ER, and so could not have undergone retrotranslocation. Harris also pointed out that proteasome inhibitors have powerful effects on the levels of prion messenger RNA; these effects might have contributed to previous results.

Even within a single population of inbred animals, prions come in distinct varieties, which — upon transmission to further

Keystone Symposium on Molecular Aspects of Transmissible Spongiform Encephalopathies (Prion Diseases). Breckenridge, Colorado, 2–6 April 2003.

100 YEARS AGO

Further particulars of the work and position of the National Antarctic Expedition have been brought by the New Zealand mail… The chief scientific work accomplished by the expedition is summarised as follows:— (1) The discovery of an extensive land at the east extremity of the great ice barrier. (2) The discovery that MacMurdo Bay is not a "bay," but a strait, and that Mounts Erebus and Terror form part of a comparatively small island. (3) The discovery of good winter quarters in a high latitude — viz. 77° 50′ S., 166° 42′ E. — with land close by suitable for the erection of the magnetic observatories, &c. The lowest temperature experienced was 92° of frost Fahrenheit. (4) An immense amount of scientific work over twelve months in winter quarters, principally physical and biological. (5) Numerous and extensive sledge journeys in the spring and summer, covering a good many thousand miles, of which the principal is Captain Scott's journey, upon which a latitude of 82° 17′ south was attained, and an immense tract of new land discovered and charted as far as 83° 30′ south, with peaks and ranges of mountains as high as 14,000 feet… As the *Discovery* has not returned to Lyttelton, there is little doubt that the expedition has been forced to spend a third winter in the Antarctic.

From *Nature* 7 May 1903.

50 YEARS AGO

In June 1952 the crew of a fisheries patrol boat cruising in Miramichi Bay, New Brunswick, observed what they took for a sand-bar in an unexpected situation. On closer inspection they found that there was no such bar, but that the surface of the sea over a wide area was in a state of violent turmoil ("a sort of boiling") due to the presence of enormous numbers of polychaetes. The area covered was some 150 yards in radius and was broken up into several minor patches… The polychaetes involved were the heteronereid forms (both sexes) of *Nereis succinea* (Leuckart). This is the first record of that species from eastern Canadian waters, though it is known from the eastern coast of the United States, usually under the name *Nereis limbata* Ehlers… We know of no other case of the swarming of a nereid with such activity and in such concentrated masses as occurred in this instance.

From *Nature* 9 May 1953.

animals — produce characteristic, heritable incubation times and patterns of brain damage. This phenomenon of prion 'strains' continues to produce surprises. When hamster prions were inoculated into mice, the animals lived a long, TSE-free life, and mostly did not accumulate PrP^{Sc} in their brains (B. Chesebro, Rocky Mountain Labs, Hamilton, Montana). The injection of PrP^{Sc}-negative brain extracts from these mice into further mice again resulted in no clinical disease, over a study period of more than 650 days. But when brain extracts from the latter mice were injected into hamsters, the animals died rapidly. So the infectious agent had silently replicated for several years in mice but maintained full virulence towards hamsters. Given that mouse prions are generally harmless to hamsters, it is hard to understand why — in this instance — they retained their infectivity. It would seem that prion strain characteristics dominate over the amino-acid sequence of the prions from the infected host. It is challenging, but maybe not impossible, to reconcile these data with the protein-only prion hypothesis: maybe the strain-specific properties are really encoded in the tertiary or quaternary structure of PrP^{Sc} rather than in its amino-acid (primary) sequence.

At the genetic level, variations in the human prion gene that protect against the development of CJD have disseminated much more efficiently than non-protective variations throughout human populations worldwide (J. Collinge, Inst. Neurology, London)[4]. This provides a compelling case that these protective changes were 'selected for' during the course of evolution — but why would they have been necessary? Collinge suggested that they protected against cannibalism-transmitted prion diseases. He derived the disturbing conclusion that cannibalism was once commonplace among our ancestors, and that prion diseases such as kuru — once a prime cause of death in New Guinea tribes that practised cannibalism — ravaged human populations in the distant past.

On a different note, it was suggested that the concept of prions as proteins that exist in two or more conformations, and replicate by causing other such proteins to change shape, applies not just to those proteins implicated in TSEs (R. Wickner, NIH, Bethesda, Maryland)[5]. Thus, in yeast, heritable traits can be attributable to prions under the following conditions: if they can disappear and later reappear (in the same or a later generation); if their propagation depends on the presence of the gene encoding the protein in question; and if their spontaneous frequency increases upon overexpression of that protein. If one accepts this definition, self-activating enzyme precursors (zymogens) might fall into this category. For instance, yeast proprotease B will self-activate by limited proteolysis (T. Roberts, NIH, Bethesda, Maryland, and R. Wickner). As the activated state is stable and transmissible by transferring the cytosol from one cell to another, it could be regarded as a prion.

Moving into the clinic, we find improvements in the diagnosis of prion diseases. The conformation-dependent immunoassay allows sensitive diagnosis by exploiting the fact that parts of the prion protein become inaccessible to antibodies during conversion to PrP^{Sc} (S. Prusiner, Univ. California, San Francisco). This led to the discovery that, in certain circumstances, the disease-associated prion protein is conformationally changed but is not protease-resistant (J. Safar, Univ. California, San Francisco)[6]. So, conformational assays might be inherently more sensitive than the venerable protease-based tests used currently.

But the most startling diagnostic development is the advent of a quantitative, sensitive assay that measures the ability of samples to infect prion-susceptible cells (C. Weissmann, Imperial College, London). The test is inexpensive and can be performed in days, as opposed to conventional infectivity assays in

Animal behaviour

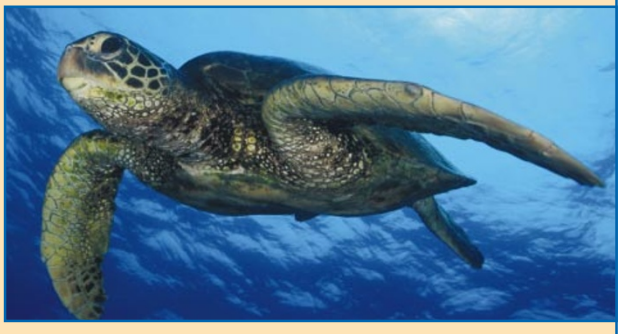
Homing is a breeze for sea turtles

Charles Darwin was one of the first biologists to wonder how migrating green turtles find Ascension Island, a 10-kilometre-long speck of land in the vast mid-Atlantic Ocean (*Nature* **7,** 360; 1873). These turtles arrive at Ascension Island between December and March, having travelled a daunting 2,200 kilometres or so eastwards from their feeding grounds off Brazil. Graeme C. Hays and colleagues now suggest a role for wind-borne information in this remarkable island-finding ability (*Proc. R. Soc. Lond. B* doi:10.1098/rspb.2002.2308; 2003).

The navigational feats of marine turtles make them good subjects for researchers interested in migratory behaviour. Early theories proposed that green turtles (*Chelonia mydas*; pictured) pinpoint Ascension Island by using water-borne odours, carried towards Brazil by the South Atlantic Equatorial Current. More recent laboratory studies have shown that hatchling loggerhead turtles can detect the inclination and intensity of the Earth's magnetic field, perhaps using these features as 'magnetic markers'. However, these promising ideas have yet to be tested on sea turtles in their natural environment.

Hays *et al.* have now performed just such a test of another theory: that green turtles use wind-borne cues to find their way. The authors found six female turtles that had just nested on Ascension Island, and then 'displaced' them. During the nesting period, persistent trade-winds blow from the southeast. So three of the turtles were transported 50 kilometres upwind of the island, and the other three 50 kilometres downwind. The animals' movements were then tracked by satellite. Female *C. mydas* come ashore to lay eggs several times in a season, so they had a strong urge to return to lay their remaining eggs.

Incredibly, Hays *et al.* found that the three downwind-displaced turtles returned to the island within 1, 2 and 4 days. Two of those moved upwind eventually returned after 10 and 27 days — but only after passing downwind of the island. A third upwind-displaced turtle was unable to locate Ascension Island after 59 days, and was heading back to Brazil when satellite transmissions ceased.



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It seems, then, that wind-borne cues emanating from the island — perhaps odours or sounds — might direct turtles. The authors concede that such cues will not work accurately over very long distances, so might not on their own account for island finding. But perhaps a combination of mechanisms is at work, with a geomagnetic sense aiding navigation over large scales (hundreds to thousands of kilometres), and smell or hearing operating on scales of tens of kilometres. Other long-distance movers might also use such a scale-dependent battery of sensory 'channels' for migration. Identifying the role of particular channels, and when they kick in, will be a challenge, but one that will surely benefit from comparisons of turtles with other vertebrates. **David W. Sims**

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which scores of animals must be observed for months or even years. For now, however, only mouse prionologists will profit, as the test has not been adapted to other species.

Little progress was reported on the therapeutic side. The antimalarial drug quinacrine can cure prion-infected cells[7], but hard evidence for any antiprion effects *in vivo* is still lacking[8]. Instead, quinacrine-treated CJD patients suffer severe liver damage (N. Streichenberger, Univ. Hospital, Lyons)[9]. Stimulation of a specific type of signalling pathway in the innate immune system delays prion diseases, perhaps by eliciting the production of anti-PrP antibodies (H. Kretzschmar, Univ. Munich)[10], or perhaps because long-lasting stimulation might lead to immune disruption (M. Heikenwalder), which has been documented to slow the progression of prion diseases. Finally, the antiprion properties of soluble PrP derivatives might merit further study (A. Aguzzi)[11].

It is clear that exciting times lie ahead in this field. Prion diseases are far from understood, and there are many bones of contention. We could not escape the exhilarating feeling that, more than in other areas of biology, fundamental discoveries are yet to be made. ■

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1. Ma, J. & Lindquist, S. *Science* **298,** 1785–1788 (2002).
2. Ma, J., Wollmann, R. & Lindquist, S. *Science* **298,** 1781–1785 (2002).
3. Drisaldi, B. *et al. J. Biol. Chem.* doi:10.1074/jbc.M213247200 (26 March 2003).
4. Mead, S. *et al. Science* **300,** 640–643 (2003).
5. Wickner, R. B. *Science* **264,** 566–569 (1994).
6. Safar, J. *et al. Nature Med.* **4,** 1157–1165 (1998).
7. Korth, C., May, B. C., Cohen, F. E. & Prusiner, S. B. *Proc. Natl Acad. Sci. USA* **98,** 9836–9841 (2001).
8. Collins, S. J. *et al. Ann. Neurol.* **52,** 503–506 (2002).
9. Scoazec, J. Y. *et al. Ann. Neurol.* **53,** 546–547 (2003).
10. Sethi, S., Lipford, G., Wagner, H. & Kretzschmar, H. *Lancet* **360,** 229–230 (2002).
11. Meier, P. *et al. Cell* **113,** 49–60 (2003).

Cell biology

Patches for wounded muscle

Juliet A. Ellis

The stresses and strains imposed on certain cells mean that their membranes require constant repair. Study of the damage that affects muscle membranes reveals a new component of the repair process.

Cell membranes in tissues such as skin, gut and muscle are routinely exposed to mechanical damage, which can produce holes in them. When that damage is not repaired, the consequences can be severe — often resulting in cell death — and may contribute to the development of the muscle degenerative diseases termed muscular dystrophies. From a combination of observations on human muscular dystrophy patients and experiments with mice, Bansal *et al.*[1] (page 168 of this issue) now report that a protein called dysferlin is a component of the mechanism for resealing the holes, and thus healing the muscle membrane.

Membrane resealing is generally carried out by a mechanism that resembles the calcium-regulated release of vesicles from a cell (exocytosis). The repair pathway is initiated by an influx of calcium through a wound, resulting in an increase in calcium levels at the site of injury. This, in turn, triggers the accumulation of vesicles, which fuse with one another and then with the plasma membrane, within the injury. A 'patch' is thereby added across the wounded area, resealing the plasma membrane[2]. The entire process — which remains largely mysterious — takes between ten and thirty seconds.

Specific participants include members of the SNARE and SNAP family of proteins, which are associated with vesicle fusion in nerve transmission. Among them is a protein called synaptotagmin, which is thought to act as a calcium sensor through its possession of two C2 domains. This feature means that it can bind phospholipids — which are the main components of membranes — in a calcium-dependent manner. The protein investigated by Bansal *et al.*, dysferlin, is found in the muscle plasma membrane (sarcolemma) and in cytoplasmic vesicles, and its participation in membrane repair is all the more thought-provoking given its association with muscle degeneration.

Mutations in dysferlin cause two conditions — limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Both are muscular dystrophies, which are all characterized by skeletal-muscle wasting and weakness (see Box 1). Many of the genes implicated in this group of diseases encode proteins that make up the 'dystrophin–glycoprotein complex', which straddles the sarcolemma and crosslinks structural components inside the cell with the extracellular environment[3]. The complex stabilizes muscle fibres by acting as a shock absorber for the forces of contraction and relaxation to which they are continually subjected. Mutations in any component of the dystrophin–glycoprotein complex lead to a secondary loss of other components, and to the eventual breakdown of the sarcolemma.

Muscle fibres isolated from LGMD2B/MM patients exhibit disruptions to the sarcolemma that are characterized by the appearance of clusters of vesicles beneath the damage[4]. Bansal and colleagues generated mice that don't produce dysferlin (dysferlin-null mice), and these animals developed symptoms of progressive muscular dystrophy similar to those seen in human patients. But neither the dysferlin-null mice nor the LGMD2B patients showed any evidence of malfunction of the dystrophin–glycoprotein complex, or any sign of sarcolemma instability. So, given that dysferlin and several other proteins implicated in muscular dystrophy

Box 1 Types of muscular dystrophy

The muscular dystrophies include about 50 diseases that affect the muscles, particularly those of the arms and legs. In these diseases, the muscles weaken with age: affected people find it increasingly difficult to walk, many becoming wheelchair-bound in their mid-teens. Some dystrophies are also associated with heart problems, which can be life-threatening. The defects are all due to a breakdown in the integrity of the cells that make up muscle fibres, which gradually wither away. The most common and severe muscular dystrophies are Duchenne, which mainly affects young boys, and a form of myotonic dystrophy that develops in adults. The Muscular Dystrophy Campaign, UK, estimates that, taken as a group, worldwide these conditions may affect as many as 1 in 2,000 people born (most sufferers die young, and so the incidence in the adult population is less).

The problem in Duchenne muscular dystrophy is due to a fault in a protein, dystrophin, that lies just under the cell surface. Its prime function is to strengthen the muscle cell by acting like a kind of scaffolding which, in a complex with various glycoproteins, connects the structural components inside the cell with the extracellular environment. Other dystrophies are associated with proteins, such as laminin-α2 and the sarcoglycans, that link to dystrophin, assisting it in its scaffolding role.

Another form of the disease arises from defects in proteins in other parts of the cell that also contribute to the cell's integrity. Examples are the nuclear proteins emerin and lamin A/C, and the cell-surface proteins caveolin-3 and dysferlin. At present, there is no cure for the muscular dystrophies. Most research is centred on developing animal and cell models that mimic the dystrophy symptoms observed in patients, as well as on the design of gene-therapy trials. **J.A.E.**