Fatal Flaws by Jay Ingram, the Canadian author, writer and broadcaster, is the latest popular science book on transmissible spongiform encephalopathies. Unlike previous books—such as Deadly feasts by Richard Rhodes, The pathological protein by Philip Yam, Comment les vaches sont devenus folles by Maxime Schwartz, The family that couldn’t sleep by Daniel Max and The collectors of lost souls by Warwick Anderson—Fatal Flaws is able to draw on the emerging mechanistic links between transmissible spongiform encephalopathies and common human neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis and chronic traumatic encephalopathy.

The diseases

Kuru came to the attention of western medicine in 1957, when Carleton Gajdusek and Vincent Zigas described a progressive cerebellar disorder with ataxia and tremor in a population of Fore natives of the Eastern Highlands of Papua New Guinea. The patients did not develop fever and there were no signs of inflammation or antibody production. The duration of disease was 7–9 months, ending in death. Zigas, who had been a medical officer in Papua New Guinea since 1949, brought kuru to the attention of the outside world. Gajdusek, an American national who was born in 1923 and died in 2008, was in search of an important scientific problem. At the time, he was a visiting scientist with the immunologist Frank Macfarlane Burnet at the Walter and Eliza Hall Institute of Medical Research in Melbourne, where he had worked since 1954. As mentioned by Ingram, in a draft letter of April 1957, Macfarlane Burnet described Gajdusek thus:

‘My own summing up was that he had an intelligence quotient up in the 180s and the emotional immaturity of a 15-year-old. He is quite maniacally energetic when his enthusiasm is roused, and can inspire enthusiasm in his technical assistants. He is completely self-centred, thick-skinned, and inconsiderate but equally won’t let danger, physical difficulty, or other people’s feelings interfere in the least with what he wants to do’.

From the published correspondence, it would appear that Macfarlane Burnet resented the fact that Gajdusek was studying kuru in the Australian-administered territory.

Scrappie is the longest-known of the transmissible spongiform encephalopathies. It derives its name from the itch that leads sheep and goats to scrape the wool off their flanks and rub themselves against solid surfaces for relief. By the beginning of the 20th century, it was endemic in Britain, affecting ~1% of adult sheep. Since people have been eating meat from sheep infected with scrapie for hundreds of years, it does not appear to spread to humans. In 1936, the French veterinarians Jean Cuillé and Paul-Louis Chelle reported that scrapie could be transmitted between sheep and in 1939 they showed that it could be transmitted from sheep to goats. Ingram highlights the under-appreciated work of David Wilson, who established during the 1940s that the scrapie agent was small and resisted conventional means to inactivate viruses.

As told before, and recounted by Ingram, the similarities between kuru and scrapie were first noticed by William Hadlow, when he visited an exhibition at the Wellcome Medical Museum in London in July 1959 that described the clinical features of kuru and illustrated its neuropathological changes. Some of the exhibits were based on the work of Igor Klatzo, a neuropathologist at the US National Institute of Neurological Diseases and Blindness in Bethesda, who had noticed similarities with Creutzfeldt-Jakob disease in the brains of patients with kuru that he had received from Gajdusek. Hadlow was an American research veterinarian, Gajdusek’s senior by 2 years, at the Agricultural Research
Council field station in Compton, near London. He concluded his article by saying: ‘It might be profitable, in view of veterinary experience with scrapie, to examine the possibility of the experimental induction of kuru in a laboratory primate’.

In 1966, Gajdusek, Joe Gibbs and Michael Alpers reported the experimental transmission of kuru to chimpanzees that had been inoculated intracerebrally with brain tissues of patients who had died with the disease. The time that elapsed between the inoculation and the appearance of the first disease symptoms was 1.5 to 3 years, suggesting the presence of a new type of infectious agent. In 1968, Gibbs, Gajdusek and colleagues reported the transmission of Creutzfeldt-Jakob disease to chimpanzees. Around 5% of cases with Creutzfeldt-Jakob disease are dominantly inherited and brain extracts from these cases were also found to be infectious. The identification of an infectious hereditary disease was unprecedented. Human transmissible spongiform encephalopathies had come of age and it appeared possible that they represented the tip of an iceberg that also included Alzheimer’s disease and Parkinson’s disease.

In 1976, Gajdusek was awarded the Nobel Prize in Physiology or Medicine (with Baruch Blumberg) ‘for their discoveries concerning new mechanisms for the origin and dissemination of infectious diseases’. Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia and variant Creutzfeldt-Jakob disease were subsequently recognized as additional human transmissible spongiform encephalopathies. Besides scrapie, transmissible encephalopathy in mink, chronic wasting disease in deer, elk and moose, and bovine spongiform encephalopathy (BSE) in cattle are major animal transmissible spongiform encephalopathies. Ingram provides an overview of animal transmissible spongiform encephalopathies in relation to human health. In many ways, human transmissible spongiform encephalopathies are like scrapie. Their causative agent has been variously referred to as ‘unconventional virus’, ‘slow virus’, ‘virino’ or ‘viroid’. However, the challenge of identifying the offending agent remained.

The prion

The causative agent of transmissible spongiform encephalopathies is composed solely of protein. In 1982, the American neurologist Stanley Prusiner, then 40 years old, proposed to call this agent ‘prion’ (for proteinaceous infectious particle, with the vowels reversed). Prions were defined as ‘small proteinaceous infectious particles which are resistant to inactivation by most procedures that modify nucleic acids’. In an interesting aside, Ingram draws attention to other prions, the so-named small Antarctic seabirds of the genus Pachyptila.

The proposal that the scrapie agent is a protein devoid of nucleic acid was initially greeted with scepticism. The prevailing view was that it contained nucleic acid and was an unusual virus, because of its long incubation time and resistance to sterilization. This view was widely held at the time, despite the fact that Tikvah Alper, John Griffith and Raymond Latarjet had previously suggested that the scrapie agent was only made of protein. But no infectious protein had been identified. Later in 1982, Prusiner and colleagues identified a partially protease-resistant protein with a molecular mass of 27–30 kDa (named prion protein (PrP) 27–30) in purified fractions with high levels of scrapie infectivity. No scrapie-specific nucleic acid could be identified in these fractions. Prion protein was expressed at similar levels in healthy and scrapie-infected brains, but only PrP from scrapie-infected tissues was partially protease-resistant, implying a conformational change, and giving rise to the terminology of PrPc (the cellular form of the prion protein) and PrPSc (the scrapie form of the prion protein). In more recent work, Pierluigi Gambetti and colleagues described a protease-sensitive prionopathy.

PrPc has a high α-helical content and little β-sheet, whereas PrPSc has less α-helical structure and a higher amount of β-sheet. PrPSc is believed to act as the template that induces the conversion of PrPc to PrPSc through a conformational change. No chemical difference has been identified between PrPc and PrPSc. The conversion of PrPSc to PrPc does not appear to occur. Understanding prion propagation will require detailed knowledge of the 3D structures of PrPSc and PrPc and the mechanisms that convert them (template-directed refolding versus seeded nucleation). In recent studies, prion infectivity was generated in vitro through the misfolding of PrPc. However, the levels of infectivity were low. The addition of cofactors, such as lipids and nucleic acids, gave rise to high-titre prions. It is unknown if cofactors are integral components of infectious prions or if they help to catalyse the formation of prions consisting only of PrPSc.

Human PRNP, the prion protein gene, is located on the short arm of chromosome 20 and encodes a protein of 253 amino acids. It is post-translationally processed to remove an N-terminal signal sequence of 22 amino acids and a C-terminal peptide of 23 amino acids, which leads to the addition of glycosphatidylinositol (GPI) to S231, tethering mature PrPc to the cell membrane. It comprises 209 amino acids with two glycosylation sites, and consists of an unstructured N-terminal part and a structured C-terminal region, each ~100 amino acids long (Fig. 1). Roland Riek and Kurt Wüthrich first showed that recombinant murine PrP contains three α-helices and two small anti-parallel β-strands in its C-terminal half. Over the past 17 years, the structures of recombinant PrP from many different species have been determined by nuclear MRI, providing a good estimate of the structure of PrPc. The 3D structure of PrPSc remains unknown.

Charles Weissmann and colleagues showed that mice with inactivated PRNP remained well when infected with scrapie, whereas scrapie-infected mice with PRNP died. It appears that any organism lacking PrPc will be resistant to prion infection. Sebastian Brandner and Adriano Aguzzi helped to establish this point by introducing grafts of brain tissues expressing PrPc into the brains of mice lacking PrPc. Following infection with scrapie, the grafted tissues became diseased, whereas brain areas lacking PrPc resisted infection. In recent years, PrPc-deficient cattle and goats have been produced.

Additional work, much of it by Prusiner and colleagues, provided further evidence that PrPSc is the major, if not only, component of the transmissible spongiform encephalopathies agent. This included the demonstration that the species barrier of prion infectivity could be overcome through the introduction...
of PRNP from the prion donor into the recipient. Prion diseases are most infectious when the amino acid sequences of PrPSc in the inoculum and PrPC in the infected organism are identical. Conversely, PrPSc from one species fails to or only inefficiently transmits disease to an organism expressing heterologous PrPC. Humans are apparently resistant to scrapie, but susceptible (at least to some extent) to BSE. Robert Will and James Ironside identified variant Creutzfeldt-Jakob disease in 1996 as the likely human form of BSE. To date, fewer than 200 cases of probable or definite variant Creutzfeldt-Jakob disease have been detected in the UK. During the BSE epidemic, nearly one million cattle are believed to have been infected with prions and to have entered the food chain. A more complete understanding of the species barrier might have been able to prevent variant Creutzfeldt-Jakob disease. Differences in incubation time and neuropathology can also occur when PrPSc in the inoculum and host PrPC have identical amino acid sequences. They result from the existence of distinct prion strains, which represent structurally different PrPSc seeds that recruit host PrPC.

Human transmissible spongiform encephalopathies are sporadic, hereditary or acquired. The most common form, sporadic Creutzfeldt-Jakob disease, occurs worldwide and causes 1–2 deaths per million people per year. Hereditary transmissible spongiform encephalopathies, which account for <10% of the total, are caused by dominant mutations in PRNP, as first shown for Gerstmann-Sträussler-Scheinker disease by Karen Hsiao and Prusiner in 1989. This work predated findings in inherited forms of other human neurodegenerative diseases, including Alzheimer’s disease and Parkinson’s disease, which showed that disease-causing mutations are often located in the genes encoding the proteins that misfold. Some dominant mutations in PRNP cause resistance to prion diseases. A similar phenomenon has been described for APP, the gene encoding the amyloid precursor protein, mutations in which cause either Alzheimer’s disease or protect against it.

Tissues from sporadic, hereditary and acquired cases of disease have been transmitted experimentally, indicating that most cases of transmissible spongiform encephalopathies are infectious. Germline PRNP mutations are not present in sporadic or acquired prion diseases. However, as first shown by John Collinge and colleagues, a common polymorphism at amino acid 129 of PrP (M or V) is a determinant of genetic susceptibility and incubation period in some populations. In Caucasians, the distribution is 37% MM, 51% MV and 12% VV. All the confirmed cases of variant Creutzfeldt-Jakob disease have so far belonged to the MM subgroup. It is therefore possible that additional cases of variant Creutzfeldt-Jakob disease with longer incubation periods will develop in the future. Genome-wide association studies have shown that the PRNP locus is associated with an increased risk of Creutzfeldt-Jakob disease.

Elio Lugaresi and Gambetti collaborated to identify fatal familial insomnia as a novel transmissible spongiform encephalopathy and showed that it constitutes a particularly compelling case in favour of the centrality of PrP. Mutation D178N, in conjunction with V129, gives rise to fatal familial insomnia, whereas D178N, in conjunction with M129, causes a subtype of familial Creutzfeldt-Jakob disease. Fatal familial insomnia is characterized by preferential thalamic degeneration, whereas the subtype of familial Creutzfeldt-Jakob disease shows severe spongiform degeneration of the cerebral cortex. Distinct prion strains may underlie these diseases.

Gerstmann-Sträussler-Scheinker disease is caused by dominant mutations in PRNP and is characterized by ataxia and/or dementia. PrP-positive extracellular plaques and a variable degree of spongiform change are present. Bernardino Ghetti and colleagues showed that filamentous tau inclusions in nerve cells are typical of Gerstmann-Sträussler-Scheinker disease. Tau inclusions are particularly numerous in cases with truncating mutations, which remove most of the globular domain of PrP. This may illuminate the mechanisms underlying the pathologies of Alzheimer’s disease, as well as familial British and Danish dementias, where extracellular amyloid-β or amyloid-bri deposits and intraneuronal tau inclusions are present.

In 1997, Prusiner became the sole recipient of the Nobel Prize in Physiology or Medicine ‘for his discovery of Prions—a new biological principle of infection’. He had previously discussed the possibility that Alzheimer’s disease might also be caused by prions. However, during the 1980s, there was no strong evidence linking Alzheimer’s disease to prion diseases.

The identification of yeast prions by Reed Wickner widened the concept. Fungal prions do not cause disease and are not released into the culture medium; they are instead transmitted directly from mother to daughter cells. Not all animal prions cause disease either. Some prions, including cytoplasmic polyadenylation element-binding protein, mitochondrial antiviral-signalling protein and T cell-restricted intracellular antigen 1 are believed to perform important cellular functions.
Prion-like propagation of protein inclusions

Common neurodegenerative diseases, including Alzheimer’s disease and Parkinson’s disease, amyotrophic lateral sclerosis and chronic traumatic encephalopathy are all protein misfolding diseases. Most cases of disease are idiopathic, with some being inherited in a dominant manner. Disease-causing mutations are located in the genes encoding the proteins that misfold or in genes that increase the levels of these proteins. The proteins in question include amyloid-β, tau, α-synuclein, TAR DNA-binding protein 43 and fused in sarcoma. Cases of disease with inclusions made of amyloid-bri, superoxide dismutase 1 (SOD1), C9orf72 peptides or huntingtin and other trinucleotide diseases are always inherited.

This is reminiscent of prion diseases, but there are also differences. Based on existing knowledge, Alzheimer’s disease and Parkinson’s disease are not infectious. Moreover, they do not occur spontaneously in animals. In contrast with transmissible spongiform encephalopathies, animal models of common human neurodegenerative diseases often require the over-expression of multiple mutant transgenes to incompletely mirror certain features of pathogenesis.

Recent work has established the existence of common mechanisms underlying the propagation of protein inclusions in prionoses and other neurodegenerative diseases. Proteins may aggregate in a small number of nerve cells (because of causative mutations, environmental influences or stochastic events) and, given enough time, spread in a prion-like fashion to other cells, giving rise to clinical disease. This requires that nerve cells release protein aggregates. Once in the extracellular space, aggregates are taken up by adjacent neurons, where they seed the aggregation of soluble protein, followed by the release of protein aggregates. Anne Bertolotti and colleagues showed that macropinocytosis mediates the uptake of mutant SOD1 aggregates in cultured cells. A direct relationship between the propagation of protein aggregates and neurotoxicity has not been shown. In prion diseases, Giovanna Mallucci and colleagues have suggested that the toxic species may cause persistent translational repression of protein synthesis and concomitant synaptic failure and nerve cell loss.

This work continues to be informed by earlier studies on peripheral amyloidoses, in particular those of Gunilla Westermark, Per Westermark and Keiichi Higuchi. Murine forms of amyloidosis have been shown to be transmissible and cross-seeding has been documented. Moreover, the likely transmission of AA amyloidosis has been described in the captive cheetah.

Unsuccessful attempts at transmitting Alzheimer’s disease and Parkinson’s disease to primates were carried out by Gajdusek and colleagues before amyloid-β, tau and α-synuclein were known as major components of the diagnostic pathologies. In 1993, Harry Baker and Rosalind Ridley showed that 6–7 years after the injection of Alzheimer’s disease brain homogenates into wild-type marmosets, amyloid-β deposits, but no tau inclusions, formed. In more recent work, Mathias Jucker and Larry Walker showed in collaboration that brain homogenates from Alzheimer’s disease and mice with numerous amyloid-β plaques accelerated the deposition of amyloid-β in the brains of mice transgenic for human mutant amyloid precursor protein. The intraperitoneal injection of such brain homogenates also resulted in the cerebral deposition of amyloid-β, indicating that seeds acquired in the periphery were able to induce pathology in the brain. Aggregated synthetic amyloid-β had a similar effect. Amyloid-β deposition could also be induced following the injection of brain extracts from Alzheimer’s disease into transgenic mice that did not normally develop such deposits.

Evidence in support of the prion-like properties of α-synuclein in humans came from the work of Patrik Brundin and Warren Olano on patients with Parkinson’s disease with embryonic neural tissue grafts to replace lost dopaminergic neurons. At autopsy, patients who had received transplants more than a decade earlier exhibited α-synuclein-positive Lewy pathology in the grafts. A likely explanation was that α-synuclein aggregates had transferred from the host brain to the grafted cells. This was consistent with the staging scheme of α-synuclein pathology put forward by Heiko Braak and Kelly del Tredici, according to which Lewy pathology spreads through the brain from regions that are connected to the peripheral nervous system. In cultured cells, α-synuclein inclusions are endocytosed and nucleate the aggregation of soluble α-synuclein in the cytoplasm, followed by the release of these aggregates. In wild-type mice, Virginia Lee and Masato Hasegawa showed independently that the intracerebral injection of synthetic α-synuclein filaments seeded the aggregation of mouse α-synuclein. Additional findings by Lee and colleagues demonstrated the existence of two distinct conformers of assembled α-synuclein (“strains”), one of which was able to cross-seed tau aggregation.

Several lines of evidence support the intercellular transmission of tau aggregates, consistent with the staging scheme put forward by Eva Braak, del Tredici and Heiko Braak. The first tau aggregates form in the locus coeruleus, followed by the entorhinal cortex, the hippocampus and large parts of the neocortex. Florence Clavaguera and colleagues showed that the injection of brain extracts from human mutant tau-expressing transgenic mice with silver-positive inclusions into the brains of mice transgenic for human wild-type tau without silver-positive inclusions induced the formation of silver-positive inclusions made of wild-type human tau. Aggregate formation increased over time and spread to anatomically connected brain regions. The same was the case, when brain extracts of human tauopathy cases were injected. Hallmark lesions of the four-repeat tauopathies argyrophilic grain disease (silver-positive grains), progressive supranuclear palsy (tufted astrocytes) and corticobasal degeneration (astrocytic plaques) were recapitulated following the injection of the corresponding brain extracts, consistent with the existence of distinct conformers of aggregated four-repeat tau. Recombinant tau filaments also promoted the aggregation of tau in transgenic mouse brain.

In parallel, Marc Diamond and colleagues showed that tau aggregates can be internalized by neurons, where they seeded the assembly of soluble tau in the cytoplasm. Tau aggregates were subsequently released and endocytosed by neighbouring cells. These findings were confirmed and extended by reports of the propagation of tau pathology from the entorhinal cortex to synaptically connected brain regions in transgenic mice. In bigenic
mice that apparently only expressed human mutant P301L tau in parts of the entorhinal cortex and the subiculum, tau inclusions propagated in an anterograde fashion to the dentate gyrus and layers CA1 and CA3 of the hippocampus, consistent with their trans-synaptic spread.

These findings demonstrate the existence of non cell-autonomous mechanisms of pathology propagation. Instead of being acquired through infection, the first seeds of misfolded proteins may be produced inside some brain cells. In the absence of prion-like spreading, these seeds would probably not cause disease. For dominantly inherited cases of disease, where a given protein is mutant wherever it is expressed, prion-like mechanisms may be less important. Nonetheless, misfolded huntingtin and SOD1 have been shown to exhibit prion-like properties.

Fatal Flaws provides a very superficial and at times ill-informed picture of the history of transmissible spongiform encephalopathies, hence the need for this overview. The discussion of the emerging links between prionoses and other neurodegenerative diseases stands out and future books will no doubt focus more on these links. The proteins that are central to the pathologies of Alzheimer’s disease and Parkinson’s disease have all been shown to exhibit prion-like properties. It appears that protein aggregation, propagation and the ensuing gain of function are what unite this disparate group of diseases.

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