Series

What history tells us
VIII.
The progressive construction of a mechanism for prion diseases

Michel Morange

Centre Cavaillès, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, 33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

In a joint publication in the 2006 issue of *Annual Review of Genetics* (Prusiner and McCarty 2006), Stanley Prusiner (the researcher who provided the strongest arguments in favour of the hypothesis that the agents of scrapie and mad cow disease are formed solely of proteins) and Maclyn McCarty (one of the discoverers of DNA as hereditary material) proposed an interesting parallel between two discoveries. The first discovery, made in 1944, was that genes are made of DNA (Avery *et al* 1944); the second, made in 1982, was that the agent responsible for scrapie is a proteinaceous infectious particle, or prion, devoid of nucleic acid (Prusiner 1982). Both affirmations had challenged the dominant opinion of the time. Avery, Macleod and McCarty had to show that genes were made of DNA, not of proteins. Prusiner had to show that his infectious agents were solely protein and did not contain a nucleic acid moiety. Thus, the authors of these major advances were in the difficult position of proving *the absence* of a protein in the transforming material, or of a nucleic acid in prions. These bold propositions had been made possible by the dramatic progress recently made by the authors and their collaborators. The decisive steps were, respectively, a bioassay to monitor the purification of the transforming principle of *Pneumococcus* with accuracy and sensitivity, and, in the case of prions, the adoption of the hamster animal model and of a bioassay based on measurements of incubation times. Both publications had huge consequences.

The parallel between the discovery that genes are made of DNA, and that the scrapie agent is a protein, is not new. It was the motivation behind the simultaneous award of the Lasker prize to Maclyn McCarty and Stanley Prusiner in 1994. It had already been proposed by Tikvah Alper in 1987 (Alper 1987). It will be the focus of what I have to say. The existence of a good historical study (Keyes 1999), and recent developments in the field – the physico-chemical characterization of different prion strains in yeast (Tanaka *et al* 2004; Krishnan and Lindquist 2005), and the progress made in the *in vitro* conversion of the prion protein into a pathogenic form (Legname *et al* 2004) – put us in a good position to re-examine the complex story of the prion hypothesis. We will see that the parallel made by McCarty and Prusiner raises interesting issues, but does more to obscure the complex history of prions than to illuminate it.

2. Strengths and weaknesses of the comparison

Let us recall the historical background (Aguzzi and Polymenidou 2004). Scrapie had been known since the 18th century, but was only shown to be an infectious disease in 1936, when the French veterinarians Cuillé and Chelle succeeded in transmitting it between sheep by intra-ocular injection of the spinal cord of an affected animal (Cuillé and Chelle 1936). The operation was repeated between sheep and goat (Cuillé and Chelle 1939), and the agent was shown to be virus-like by filtration (Cuillé and Chelle 1938).

Another neurodegenerative disease, Kuru, affecting human populations living in isolated parts of New Guinea, was extensively studied by Carleton Gajdusek (Gajdusek 1967, 1977). He reached the conclusion that the disease was due to an infectious agent of a particular nature present in the brain, and most probably transmitted through cannibalism. The parallel between Kuru and scrapie had been proposed by Hadlow in 1959 (Hadlow 1959). Scrapie was transmitted to mice two years later, opening the door to an easier experimental study of these diseases. The number of publications rapidly increased in the mid-1960s.

Keywords. **Central dogma; mechanism; prions**

The difficulty of excluding the possible presence of a contaminant – protein in the case of the transforming factor, a nucleic acid in the case of prions –, and the richness of the *ad hoc* hypotheses that could be proposed to explain why such a component was not visible, or had resisted the different treatments used to eliminate it, are obviously important points raised by this comparison. In particular, this explains why the possibility of a nucleic acid tightly associated with the agent of scrapie, or its virus-like nature, was regularly proposed, despite the progress made in validating the protein-only hypothesis (Marsh *et al* 1978; Rohwer 1984; Manuelidis *et al* 1995).

There is another interesting parallel between the difficulties encountered by Avery and Prusiner: the absence of a mechanism able to explain the observations. How could DNA control the formation of the capsule of polysaccharide surrounding *Pneumococcus*? How could a protein alone stimulate its own replication? This parallel is probably the most significant part of the comparison, but it is not made by the authors of the *Annual Review of Genetics* paper.

But there are huge differences between the roles that the 1944 and 1982 publications played in the two stories. The 1944 article was the first serious modern proposal that DNA was the genetic material. The 1982 article had been preceded by many contributions in the mid-sixties in which the agent of scrapie had been proposed not to be a nucleic acid, but a protein, for diverse reasons (Pattison 1965; Alper *et al* 1967; Griffith 1967). Besides, the attitudes of the two authors were dramatically different. Avery was extremely cautious in extending the scope of the result obtained on *Pneumococcus*, although he was personally convinced of its general value. In contrast, it was not so much the results obtained by Prusiner – the absence of nucleic acids in the active fraction of scrapie – but his insistence on the heterodoxy of the model he proposed that raised the controversy.

However, the most dramatic difference between the two events is to be found in their consequences. Whereas the discovery of 1944 was a milestone in the development of a molecular vision of life, the existence of prions has so far provided an explanation only for the characteristics of a limited group of diseases, much more limited than was imagined in the 1980s.

3. What was new in 1982? What was the true nature of the discovery made?

In contrast to what was said by a commentator in *Nature* after the award of the Nobel prize to Prusiner in 1997 (Coles 1997), the 1982 *Science* article was not a description of the prion model as we know it today. The current model states that the prion protein, encoded by the *prp* gene, comes in two conformations; one of them is infectious. The infectious form can induce the normal form of this protein to adopt its own pathological conformation, leading to the apparent "reproduction" of the infectious agent. Other mechanisms were proposed by Prusiner in 1982: the direct replication of the pathological protein mediated (or not mediated) by RNA; the possibility that the infectious protein acts as an inducer of a normally silent gene coding for the same protein; the possibility was not excluded that a small nucleic acid tightly associated with the prion could be responsible for the inductive effect.

A review published in *Scientific American* in 1984 (Prusiner 1984) did not discuss what is now considered to be the right model either. In one figure, different mechanisms were proposed. One of them can retrospectively be considered to be very close to the present model, although alternative interpretations can be provided for this enigmatic picture. The right model – that the normal and pathological forms differ only in their conformation – clearly emerged only after two things became clear over the following years. First, it was shown that the prion is encoded by the genome of the host, but that the pathogenic and normal forms of the protein have different characteristics – in particular a different sensitivity to proteases (Oesch *et al* 1985; Chesebro *et al* 1985; Basler *et al* 1986). Second, investigations of the possibility that the different forms represented different post-translational modifications were not successful. Given these facts, it would be inappropriate to equate the successful introduction of the word 'prion' with the proposal of the conformation-alone model. Indeed, to do so constitutes an example of the process, which is well described by the historian Larry Holmes, whereby a series of distinct discoveries get condensed into a single event (Holmes 2004).

The right hypothesis to explain the formation of the infectious scrapie agent was actually proposed by J S Griffith as early as 1967, in an article published in *Nature* (Griffith 1967; not to be confused with Frederick Griffith – as it happens, his uncle – who had discovered the phenomenon of transformation in *Pneumococcus* forty years earlier). The impact of J S Griffith's paper was limited, probably because of the highly abstract thermodynamic description of the model. Therefore, 1982 was neither the first, nor the last, occasion on which it was shown to be plausible that the agents of scrapie and related diseases were proteins. The atypical characteristics of the disease, as well as of Kuru in humans – the long incubation time, the absence of inflammation in the affected tissues, and the absence of an immune response –, very early suggested that the infectious agent was of a particular nature: a slow, unconventional virus according to Gajdusek (Gajdusek 1967, 1977). Its resistance to inactivating agents, such as formaldehyde, was observed very early (Pattison 1965). To these early observations, an abundance of new observations on the small size of the infectious agent (Alper *et al* 1966), its unusual sensitivity

to UV, detergents, nucleases and proteases, led in 1967 to a series of publications in which the absence of large nucleic acids, and the probable protein nature of this agent, were proposed (Pattison and Jones 1967; Alper 1967; Griffith 1967). Nevertheless, the example of the tobacco mosaic virus proposed by Wendell Stanley in 1935 to be a pure protein (Stanley 1935), and later shown to contain an essential RNA component, served as a caution to the different authors not to totally exclude the presence of a small nucleic acid with peculiar physico-chemical properties.

If the 1982 article was not the proposal of a new model, nor the first demonstration of the protein nature of the scrapie agent, where does its impact come from? Obviously, the experimental arguments in favour of an infectious protein were more abundant than before, and were made with a partially purified fraction. The introduction of a new word, prion, was important. But the most crucial novelty was not described in the article, although it was probably already known to Prusiner, and was described in another publication of the same year (Bolton *et al* 1982): the infectious agent was identified with a particular 27-30 kDa protein. This was the decisive advance that underpinned the major breakthroughs made in the following years. The situation created by the description of a possible candidate protein for the prion was radically new.

4. The consequences of the 1944 and 1982 experiments and the attitudes of their authors were radically different

The publications of 1944 and 1982 did not have the same position, and did not play the same role in further developments. Were their consequences of equal importance, as suggested by McCarty and Prusiner? The answer would be 'yes' if we accept the proposal of Uptain and Lindquist (2002) regarding the evidence for prion-like phenomena in many different organisms, including yeasts (Lindquist 1997). Also, the possibility that the conversion of a neuronal protein to a prion-like state might be involved in long-term synaptic changes associated with memory (Si *et al* 2003). Both results support the existence and functional significance of 'protein-based' genetic elements. But the importance, and for that matter even the existence, of some of the previously described phenomena is not generally admitted. In fact the opposite is true: the number of pathological phenomena associated with prions has declined since the 1960s. In 1977, Gajdusek still proposed that slow viruses, similar to those he had described for Kuru, were responsible for other neurodegenerative diseases such as Alzheimer's, auto-immune diseases, and cancer (Gajdusek 1977). In 1982, Prusiner drew up the same list of diseases (Prusiner 1982), which progressively and silently shrunk when the nature of the prion was better known.

That being said, the strongest contrast between the 1944 and 1982 articles was in the attitude of their authors. The result of Avery was revolutionary, but such a word was never used by him. He restricted himself to discussing the chemical nature of the transforming factor; the extension of the result to genes in general was, so to speak, subliminal (though it is well known that he was less cautious in private, as shown by the famous phrase "Sounds like a virus – may be a gene", in a letter to his brother quoted in Judson, 1979). He desperately tried to insert his observations into the long tradition of descriptions made before him (Avery *et al* 1944): Instead of looking for a new mechanism of action for DNA, he proposed experiments in order to demonstrate that DNA could be a bearer of specificity, as proteins 'were'. The attitude of Prusiner was dramatically different: he insisted on the heretical nature of the hypothesis he proposed, on the fact that it contradicted the central dogma of molecular biology. This attitude raised strong objections – the strength of which Prusiner complained about – but also a lot of attention. To raise the debate in such terms was not necessary: in 1967, Griffith had argued that the observation that the agent of scrapie was a protein could easily be explained by at least three different mechanisms compatible with the central dogma of molecular biology – including a mechanism very similar to the one that would eventually be accepted (Griffith 1967).

5. Conclusions

I do not claim here to give a full historical account of the prion hypothesis – the duration and richness of the debates cannot be summarized in a few pages – but rather to examine the value of the parallel drawn between the publications of 1944 and 1982. This comparison shows interesting similarities, but also major differences between these two episodes.

In both cases, the controversial hypotheses made – the transforming factor (a gene) is DNA, the agent of scrapie is a protein – did not receive full recognition because of the absence of a mechanism likely to explain them. How can DNA control the synthesis of the *Pneumococcus* capsule? How can a protein replicate? In the case of prions, many considered that the existence of strains, well demonstrated in the 1970s, was not explained by the model of protein transconformation proposed at the end of the 1980s. It seemed reasonable to admit that a protein can adopt two different conformations – it was already well established for allosteric proteins –, but not ten or more as required by the progressive increase in the number of well-described strains. Another case where the absence of a mechanism delayed a discovery was that of introns, which had been "seen" by many researches before 1977, but put aside (Morange 1998). For an observation to be accepted, there is a need

for some sort of mechanism, even if crude, to account for it. As the philosophers of science Lindley Darden and Carl Craver have strongly argued, this testifies to the importance of mechanisms in scientific explanations (Machamer *et al* 2000; Darden 2006).

The absolute necessity to provide a mechanism pushes scientists to exploit any new discovery as a possible source of mechanisms. This tendency is particularly evident in the case of prions, for which the controversy about the protein nature of the infectious agent has remained lively for nearly forty years. In the 1960s, the model of the operon was used. In the 1980s, the observations made on oncogenes (Weinberg 1983), the rearrangement of genes, the discovery of viroids and virinos (Kimberlin 1982), were all incorporated as possible explanations of the "prion phenomenon". At the beginning of the 1990s, the freshly discovered chaperones were introduced into the picture, and more recently the small RNAs found their place.

In a research field as difficult as prions, it is also striking how scientists use history to reinforce their arguments. The comparison between the 1944 and 1982 experiments is one example; a similar parallel had already been drawn in the 1980s between the discovery of oncogenes and the experiments of Avery (Weinberg 1983). Historical rewriting also accords past experiments a different role and weight. Consider, for instance, in the same review (Prusiner and McCarty 2006), the limited reference to the 1967 experiments proposing that the agent of scrapie was a protein. These are only mentioned at the end, to suggest that such an alternative hypothesis was much easier to propose at a time when the paradigm of molecular biology was not yet fully established, than in the 1980s, when the role of nucleic acids had become fully dominant. Such a presentation ends up strengthening the importance of the 1982 contribution.

Perhaps the most important conclusion that emerges from this story – paradoxically – is the absence of a decisive event in the acceptance of the prion hypothesis. Neither the first observations of 1967, nor the complementary ones of 1982, were decisive. The progressive acceptance of the protein-only and transconformation model was made possible by the convergence, the coalescence, of observations that were all in agreement with the model, whereas no single observation was convincing *per se*: the use of transgenic animals expressing different forms of prions to alter the sensitivity of the host to the disease (Scott *et al* 1989; Prusiner *et al* 1990); the resistance to the disease of animals in which the gene encoding the prion protein has been knocked out (Büeler *et al* 1993); the identification of the prion gene as a gene controlling the incubation time of the disease (Carlson *et al* 1986; Westaway *et al* 1987); the occurrence of mutations in the *prp* gene in a neurodegenerative genetic disease called Gerstmann-Sträussler syndrome (Hsiao *et al* 1989), etc. The elaboration of a model explaining neurodegenerative diseases as scrapie, mad cow disease and Kuru by the self-activated transconformation of a particular protein is one of the best illustrations of the progressive construction of scientific knowledge. And Prusiner continues to play a major role in this ongoing construction.

References

Aguzzi A and Polymenidou M 2004 Mammalian prion biology: one century of evolving concepts; *Cell* **116** 313–327

Alper T, Haig D A and Clarke M C 1966 The exceptionally small size of the scrapie agent; *Biochem. Biophys. Res. Commun.* **22** 278–284

Alper T, Cramp W A, Haig D A and Clarke M C 1967 Does the agent of scrapie replicate without nucleic acid?; *Nature (London)* **214** 764–766

Alper T 1987 Radio- and photobiological techniques in the investigation of prions in *Prions: Novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease* (eds) S B Prusiner and M P McKinley (New York: Academic Press Inc.) pp 113–148

Avery O T, MacLeod C M and McCarty M 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types; *J. Exp. Med.* **79** 137–158

Basler K, Oesch B, Scott M *et al* 1986 Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene; *Cell* **46** 417–428

Bolton D C, McKinley M P and Prusiner S B 1982 Identification of a protein that purifies with the scrapie prion; *Science* **218** 1309–1311

Büeler H, Aguzzi A, Sailer A *et al* 1993 Mice devoid of PrP are resistant to scrapie; *Cell* **73** 1339–1347

Carlson G A, Kingsbury D T, Goodman P A *et al* 1986 Linkage of prion protein and scrapie incubation time genes; *Cell* **46** 503–511

Chesebro B, Race R, Wehrly K *et al* 1985 Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain; *Nature (London)* **315** 331–333

Coles H 1997 Nobel panel rewards prion theory after years of heated debate; *Nature (London)* **389** 529

Cuillé J and Chelle P L 1936 La maladie dite *tremblante du mouton* est-elle inoculable?; *C. R. Acad. Sci. Paris* **203** 1552–1554

Cuillé J and Chelle P L 1938 La tremblante du mouton est-elle déterminée par un virus filtrable?; *C. R. Acad. Sci. Paris* **206** 1687–1688

Cuillé J and Chelle P L 1939 Transmission expérimentale de la *tremblante* à la chèvre; *C. R. Acad. Sci. Paris* **208** 1058–1060

Darden L 2006 *Reasoning in biological discoveries: essays on mechanisms, interfield relations, and anomaly resolution* (Cambridge: Cambridge University Press)

Gajdusek D C 1967 Slow-virus infections of the nervous system; *New Engl. J. Med.* **276** 392–400

Gajdusek D C 1977 Unconventional viruses and the origin and disappearance of Kuru; *Science* **197** 943–960

Griffith J S 1967 Self-replication and scrapie; *Nature (London)* **215** 1043-1044

Hadlow W J 1959 Scrapie and Kuru; *Lancet* **2** 289–290

Holmes F L 2004 *Investigative pathways: Patterns and stages in the careers of experimental scientists* (New Haven: Yale University Press)

Hsiao K, Baker H F, Crow T J *et al* 1989 Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome; *Nature (London)* **338** 342–345

Judson H F 1979 *The Eighth Day of Creation*: *The makers of the revolution in biology* (New York: Simon and Schuster)

Keyes M E 1999 The prion challenge to the 'central dogma' of molecular biology, 1965–1991; *Stud. Hist. Philos. Biol. Biomed. Sci.* **30** 1–19, 181–218

Kimberlin R H 1982 Scrapie agent: prions or virinos?; *Nature (London)* **297** 107–108

Krishnan R and Lindquist S L 2005 Structural insights into a yeast prion illuminate nucleation and strain diversity; *Nature (London)* **435** 765–772

Legname G, Baskakov I V, Nguyen H B *et al* 2004 Synthetic mammalian prions; *Science* **305** 673–676

Lindquist S 1997 Mad cows meet Psi-chotic yeast: the expansion of the prion hypothesis; *Cell* **89** 495–498

Machamer P, Darden L and Craver C 2000 Thinking about mechanisms; *Philos. Sci.* **67** 1–25

Manuelidis L, Sklaviadis T, Akowitz A and Fritch W 1995 Viral particles are required for infection in neurodegenerative Creutzfeldt-Jakob disease; *Proc. Natl. Acad. Sci. USA* **92** 5124–5128

Marsh R F, Malone T G, Sermancik J S *et al* 1978 Evidence for an essential DNA component in the scrapie agent; *Nature (London)* **275** 146–147

Morange M 1998 *A history of molecular biology* (Cambridge: Harvard University Press) 206

Oesch B, Westaway D, Wälchli M *et al* 1985 A cellular gene encodes scrapie PrP 27-30 protein; *Cell* **40** 735–746

Pattison I H 1965 Resistance of the scrapie agent to formalin; *J. Comp. Pathol*. **75** 159–164

Pattison I H and Jones K M 1967 The possible nature of the transmissible agent of scrapie; *Vet. Rec*. **80** 2–9

Prusiner S B 1982 Novel proteinaceous infectious particles cause scrapie; *Science* **216** 136–143

Prusiner S B 1984 Prions; *Sci. Am.* **251** 48–57

Prusiner S B, Scott M, Foster D *et al* 1990 Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication; *Cell* **63** 673–686

Prusiner S B and McCarty M 2006 Discovering DNA encodes heredity and prions are infectious proteins; *Annu. Rev. Genet.* **40** 25–45

Rohwer R G 1984 Scrapie infectious agent is virus-like in size and susceptibility to inactivation; *Nature (London)* **308** 658–662

Scott M, Foster D, Mirenda C *et al* 1989 Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques; *Cell* **59** 847–857

Si K, Lindquist S and Kandel E R 2003 A neuronal isoform of the *Aplysia* CPEB has prion-like properties; *Cell* **115** 879–891

Stanley W M 1935 Isolation of a crystalline protein possessing the properties of Tobacco Mosaic Virus; *Science* **81** 644–645

Tanaka M, Chien P, Naber N *et al* 2004 Conformational variations in an infectious protein determine prion strain differences; *Nature* **428** 323–328

Uptain S M and Lindquist S 2002 Prions as protein-based genetic elements; *Annu. Rev. Microbiol.* **56** 703–741

Weinberg R A 1983 A molecular basis of cancer; *Sci. Am.* **249** 102–116

Westaway D, Goodman P A, Mirenda C A *et al* 1987 Distinct prion proteins in short and long scrapie incubation period mice; *Cell* **51** 651–662

*e*Publication: 9 February 2007