

# A scientific revolution?

The prion anomaly may challenge the central dogma of molecular biology

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Science, Thomas Kuhn argued in *The Structure of Scientific Revolutions* (1962), proceeds at two different paces. One is what he called “normal science”, which professionals, the general public, the press and politicians generally understand as “research firmly based upon one or more past achievements that some particular scientific community acknowledges for a time as supplying the foundation for its further practice.” This stepwise progression towards a better understanding of Nature, by building on established knowledge, has been described in a myriad of textbooks, dictionaries and scientific papers.

However, Kuhn distinguishes this form of knowledge creation from so-called “puzzle-solving science”. The latter results from anomalies—experimental observations or other evidence—which do not fit into the widely accepted theoretical framework of how Nature functions. Puzzle-solving science, according to Kuhn, can therefore trigger a scientific revolution as scientists struggle to explain these anomalies and develop a novel basic theory to incorporate them into the existing body of knowledge. After an extended period of upheaval, in which followers of the new theory storm the bastions of accepted dogma, the old paradigm is gradually replaced. Perhaps the best example of such a paradigm shift in science is the Copernican revolution in cosmology: the move from a geocentric to the heliocentric view of our solar system. Curiously, although Aristarches had already laid the seeds of heliocentrism in the third century BC, it took another 18 centuries before Nicolaus Copernicus proposed that the Earth moves around the sun and not vice versa. Many anomalies,

such as the orbit of Mars, were already known at that time, but the power of the Aristotelian dogmas, including the geocentric view of the universe, was too strong to be overcome easily. Truly speaking, however, the notion of a paradigm, as defined by Kuhn, does not have exactly the same meaning in cosmology, physics, chemistry, geology and biology.

What I propose here is that biology is heading towards a similar scientific revolution that may shatter one of its most central paradigms. The discovery of a few small proteins with anomalous behaviour is about to overcome a central tenet of molecular biology: that information flows unidirectionally from the gene to the protein to the phenotype. It started with the discovery that prions, a class of small proteins that can exist in different forms, cause a range of highly debilitating diseases. This sparked further research, particularly by Stanley Prusiner at the University of California, San Francisco (USA), who eventually established that prions induce conformational changes in other proteins and thus transmit their conformational information. More recent research by Susan Lindquist at the Whitehead Institute (Cambridge, MA, USA) and Eric Kandel at Columbia University (New York, NY, USA) indicates that this may well be a form of protein-based information flow, which seems

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to be important in various biological processes ranging from the establishment of long-term memory to the adaptation of organisms to new environments.

Scientific revolutions are still rare in biology, given that the field, unlike astronomy or physics, is relatively young. Until the middle of the eighteenth century, biology was essentially a descriptive activity rooted in medicine and observations of living Nature. Early biologists did not practise large generalizations as was the norm in physics or chemistry. During the eighteenth century, biologists started to ask themselves how they could explain the enormous variability of living organisms and their ability to adapt to their environment. Early thinkers, such as Jean-Baptiste Lamarck, Erasmus Darwin or Georges Louis Leclerc de Buffon suspected that environmental factors, over time, trigger physiological changes in the organism, which help it to cope with its surroundings. Lamarck's central example was the long neck of the giraffe, which he thought was the result of the animal stretching its neck to reach leaves high up on trees.

But it was Charles Darwin and his famous theory of evolution that finally provided biology with a sound theory on which to build (Darwin, 1859). The idea that all living beings stem from a primordial cell dating back two billion years is, in my opinion, a true paradigm. It does not have a heuristic value, unlike paradigms in physics such as gravitation or

Einstein's famous equation, but it has a fundamental aspect. The striking success of Darwin's theory of evolution and Alfred Russel Wallace's similar theory resides in the proposed mechanism: through mutations and reproduction, organisms create new variations that are selected for or against by their environment. Although it was widely resisted initially—many people could not accept the idea that humans descended from apes and even lower organisms—the work of pro-darwinist scientists, in particular Gregor Mendel and Francis Galton, solidly established the paradigm of the evolution of species with the discovery of mutations at the end of the nineteenth century. We must therefore speak of Darwinism as the foundation of molecular biology. In fact, Darwin himself was not anti-lamarckian, although the work of his successors eventually proved Lamarck wrong.

Curiously enough, it was not so much experimental evidence or anomalies that helped to establish Darwin's theory but an intellectual movement among biologists: the idea of a goal-directed process was fading away to be replaced by a stochastic mechanism. Whether the evolution of species is a true paradigm is subjective—Karl Popper (1976) claimed that the "survival of the fittest" is simply a pure tautology, although he later distanced himself from this statement.

Nearly everything that followed Darwin was 'normal science'. The search for Mendel's units of hereditary information, termed 'genes' by the Danish botanist Wilhelm Johannsen in 1909, revealed that they comprise nucleic acids, and was first published by Oswald Avery and colleagues in 1944 (Avery *et al*, 1944). The seminal paper on the double-helical structure of DNA by James Watson and Francis Crick (1953) finally provided the structural and chemical explanation of how cells store, use and pass on information to daughter cells. This gave rise to the central paradigm in molecular biology, which rests on four pillars of accepted knowledge: (i) all genetic (hereditary) information is stored in nucleic acids; (ii) the double-helical structure of DNA explains how this information is stored and copied; (iii) the information is stored in a digital code; and (iv) information flows irreversibly from nucleic acids to proteins.

The discovery that hereditary information is stored in DNA as a digital code was a huge advance for biology. This insight by Watson and Crick is as important, epistemologically, as was Darwin's theory of evolution. The whole process from Darwin to Mendel, to Avery, to Watson and Crick was a scientific revolution that gave birth to molecular biology as a new discipline. Molecular biology now rests on an enormous corpus of experimental evidence and I doubt that this will ever just disappear. Its fantastic successes during the past 50 years are due mainly to two things: a fundamental theory to explain how information is stored; and an epistemological turn, that is, an extreme reductionism in heuristic processes that allowed practitioners to generalize their observations. As Jacques Monod said, what is true for *Escherichia coli* is true for the elephant.

However, a small group of biologists have discovered disquieting anomalies that could threaten this central theory. The question then is this: Is the central paradigm of molecular biology—that all genetic information is stored and transferred digitally through DNA—the only possible explanation of how life evolved, or are there other mechanisms of heredity in living organisms? Indeed, it seems that we see growing evidence that information can be transferred genetically in an analogous way through the prion.

At the 1967 Cold Spring Harbor Symposium on Quantitative Biology devoted to antibodies, I discussed with Francis Crick the problem of irreversibility of information transfer from nucleic acids to proteins. Although he was ready to accept some kind of reversibility between DNA and RNA through retroviruses, he was adamant about the absolute irreversibility of the RNA-protein road: "Nature could not proceed in another way." Similarly, Niels Jerne, in his concluding summary at this Symposium, asked "Does the specificity of an antibody molecule reside in its primary structure or can different combining sites arise by different folding of identical polypeptide chains?...The answer in the former is yes" (Jerne, 1967). This statement further cemented the view that proteins are simply the active incarnation of information stored in the DNA, and rang the death bell for the template theory of antibody formation (Haurowitz, 1950).

But the idea that a protein could transmit information did not disappear. Carleton Gajdusek had already proposed that proteins could be infectious, based on his discovery that *kuru* disease was possibly caused by a protein from the brain (Gajdusek, 1977). It took a long time before this idea was accepted. Only after an extended uphill battle did the biomedical community finally accept Prusiner's theory that proteins are the only pathogen to cause scrapie in sheep, bovine spongiform encephalopathy in cattle



**Fig 1** | The sea hare *Aplysia*. Eric Kandel used this model organism to show that prion elements control long-term memory formation. Image reproduced with permission from Columbia University, New York, NY, USA

and Creutzfeldt–Jakob disease in humans (Prusiner, 1982). Prusiner's work, which earned him the Nobel Prize, was a minor revolution against an established dogma as the biomedical research community believed that only viruses and bacteria—organisms that carry nucleic acids—could be infectious. I will not discuss the enormous scientific work that followed the establishment of prions as pathogens, but will instead concentrate on the more recent discovery of prions as genetic elements that store and transmit information in various organisms, mainly yeast, the fungi *Podospora* and the sea hare *Aplysia* (Fig 1; Shorter & Lindquist, 2005).

In science, the introduction of a new technical tool commonly opens new avenues of knowledge. Yeast quickly became a model organism for molecular biologists because it is a very simple eukaryote with a growth cycle of 80 minutes, which allows a large amount of material for biochemical analysis to be collected in a few hours. It was in yeast that researchers found the first evidence of non-mendelian transmission of phenotypic traits (Cox, 1965; Lacroute, 1971). These phenomena baffled scientists for more than 40 years before they could be attributed to prions: [PS1+] suppresses nonsense codons in translation, and [URE3] inhibits nitrogen catabolite repression (Wickner, 1994). Both are caused by self-replicating conformational changes—to the translation suppressor sup35 in the case of [PS1+], and the Ure2 protein, an antagonist of the transcriptional activators Gln3 and Gat1, in the case of [URE3]. Another prion, [Het-s] in *Podospora anserina*, was found to be involved in programmed cell death when two fungal strains with different genotypes fuse (Coustou *et al*, 1997; Maddelein *et al*, 2002). In addition, work by Kandel showed that a prion has an important role in the formation and maintenance of long-term memory (Si *et al*, 2003).

Much of this evidence relies on Lindquist's work on yeast prions. Not only did she show that prion domains in some proteins act as molecular switches that activate or deactivate the protein (True & Lindquist, 2000), she also showed that prions are non-mendelian genetic elements that have an important evolutionary role by producing new phenotypes, which are often beneficial. Her work on sup35

revealed that the protein switches to its prion state [PS1+] when the environmental conditions for yeast deteriorate, which decreases translation fidelity and causes the ribosome to read beyond nonsense codons (Liebman & Sherman, 1979). This in turn enables the expression of formerly silent genes and gene variants, and creates new phenotypes. [PS1+] is passed on to daughter cells in which it self-replicates by imposing its conformation on normal sup35 proteins, until a new phenotype eventually emerges that is better adapted to the new environment (True & Lindquist, 2000). In another elegant experiment, Li and Lindquist showed the generality of this mechanism for controlling protein activity by fusing a yeast prion domain to a rat protein (Li & Lindquist, 2000).

Another physiological role for prions emerged through work by Kandel on the molecular basis of long-term memory in *Aplysia*. Kandel's group investigated how neurons maintain learning-related synaptic growth and stability over long periods as biological molecules have a very short life of hours to days compared with the permanently altered molecular composition of a memory synapse. They found that the cytoplasmic polyadenylation element protein (CPEB), which activates dormant mRNAs through the elongation of their poly(A) tail, seems to enhance long-term synaptic stability. Surprisingly, the neuronal isoform of CPEB shares properties with prion-like proteins (Si *et al*, 2003). It seems to exist in two functionally distinct and stable forms with the ability to self-perpetuate in a dominant epigenetic fashion when in the prion state. CPEB is active in this state and is capable of activating dormant mRNAs (Bailey *et al*, 2004).

The fascinating aspect of this discovery is that by proposing a prion-based mechanism to regulate long-term memory, we have entered the domain of neurology. Apart from the importance of this new concept in neurophysiology, the discovery establishes the universality of the prion system in life. A recent summary in *Nature* (Krishnan & Lindquist, 2005) on the structure of the yeast prion and its existence as

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a 'protein-only' inheritable element further supports a non-mendelian hereditary mechanism, which obviously will have a major impact on biology. The system used by the authors allows a biochemical analysis of protein folding during nucleation and assembly of the molecule, and provides a major step forwards for understanding the molecular changes involved in prion synthesis. To highlight the growing interest in and importance of prions, the same issue of *Nature* published two more papers on the molecular structure of amyloid-like fibrils (Nelson *et al*, 2005; Ritter *et al*, 2005), which will help scientists who study the amyloid fibrils in the brains of patients with Alzheimer's.

The famous motto "one gene, one enzyme" has been dead and buried for some time. Now we may have to abandon another concept, spelled out at the above-mentioned Cold Spring Harbor Symposium by Jerne (1967), namely that the primary structure of a protein determines its tertiary structure. For all of my working life in molecular biology, this was an 'act of faith'. It will be interesting to see if the prion anomaly can shatter some basic concepts in immunology and revive, at least in part, the template theory—obviously the tertiary structure and function of a protein is not determined solely by its amino-acid sequence (Bussard, 2003).

Similarly, Lindquist's work on the role of [PS1+] in adapting to new environmental conditions poses some important questions on the nature of evolution. As inherited variance is apparently not restricted to DNA but could also be caused by protein-based genetic elements, some now wonder about reviving Lamarck's idea that the environment triggers adaptive structural and physiological changes in the organism. "This suggests a possible mechanism for the inheritance of acquired traits, postulated in the Lamarckian theory of evolution. The prion model also puts in doubt the notion that cloned animals are genetically identical to their genome donors, and suggests that genome sequence would not provide a complete information about the genetic makeup of an organism"

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(Chernoff, 2001). It reminds me again of the 1967 Cold Spring Harbor Symposium, during which my friend Stephen Fazekas de St Groth sent me a little scribbled note saying "a Lamarckian always sleeps in the heart of a Frenchman."

In his last book, *The Road Since Structure* (2000), Kuhn devotes a long chapter to what he calls "commensurability, comparability and communicability". Scientific revolutions concern the replacement of an old paradigm by a new incommensurable one. However, the conflict of paradigms often does not necessarily end with the death of the old one. Rather, the new theory is incorporated into the older framework so as to make it more universal. Physicists have become quite used to this and have been quick to adapt their common views to new theories. Take, for example, Newton's laws of mechanics, Einstein's general theory of relativity, and quantum physics, first proposed by Niels Bohr and Werner Heisenberg. At relatively low speeds, such as a satellite orbiting Earth, Newton's classic laws are sufficient for a technician to calculate how to launch a rocket that will send the satellite into the correct orbit. However, close to the speed of light, Newton's mechanics no longer apply and Einstein's law takes over. Similarly, Newtonian mechanics aptly describe the behaviour of objects larger than a molecule. Only when we reach the level of atoms, electrons or quarks do the laws of quantum mechanics apply. It seems to me that we are in a similar situation with regard to the anomalies presented by prions. The large corpus of existing knowledge will continue to hold true, but may have to be expanded into a more general theory to incorporate the increasing evidence that inheritance is not solely governed by nucleic acids.

During the recent euphoria surrounding molecular biology, it was common to find scientists who believed that the "secret of life" had been deciphered. This was the view expressed by Gunther Stent (1969) in his book *The Coming of the Golden Age*, and his naive thought was

shared by many biologists. A few years ago I was amused to see the same idea used in general history, in Francis Fukuyama's *The End of History* (1992). Biologists need to get used to the idea that there is no end in sight when it comes to new insights and scientific breakthroughs; this idea has long been abandoned by physicists who are subject to regular scientific revolutions. I wonder if knowledge is, like the Universe, basically endless and in constant expansion, just as the complexity of life itself is also expanding infinitely.

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