

NEUROSCIENCE

An essential protein

Brazilian team explains how the functioning of the healthy form of prion is essential for the protection of the brain cells

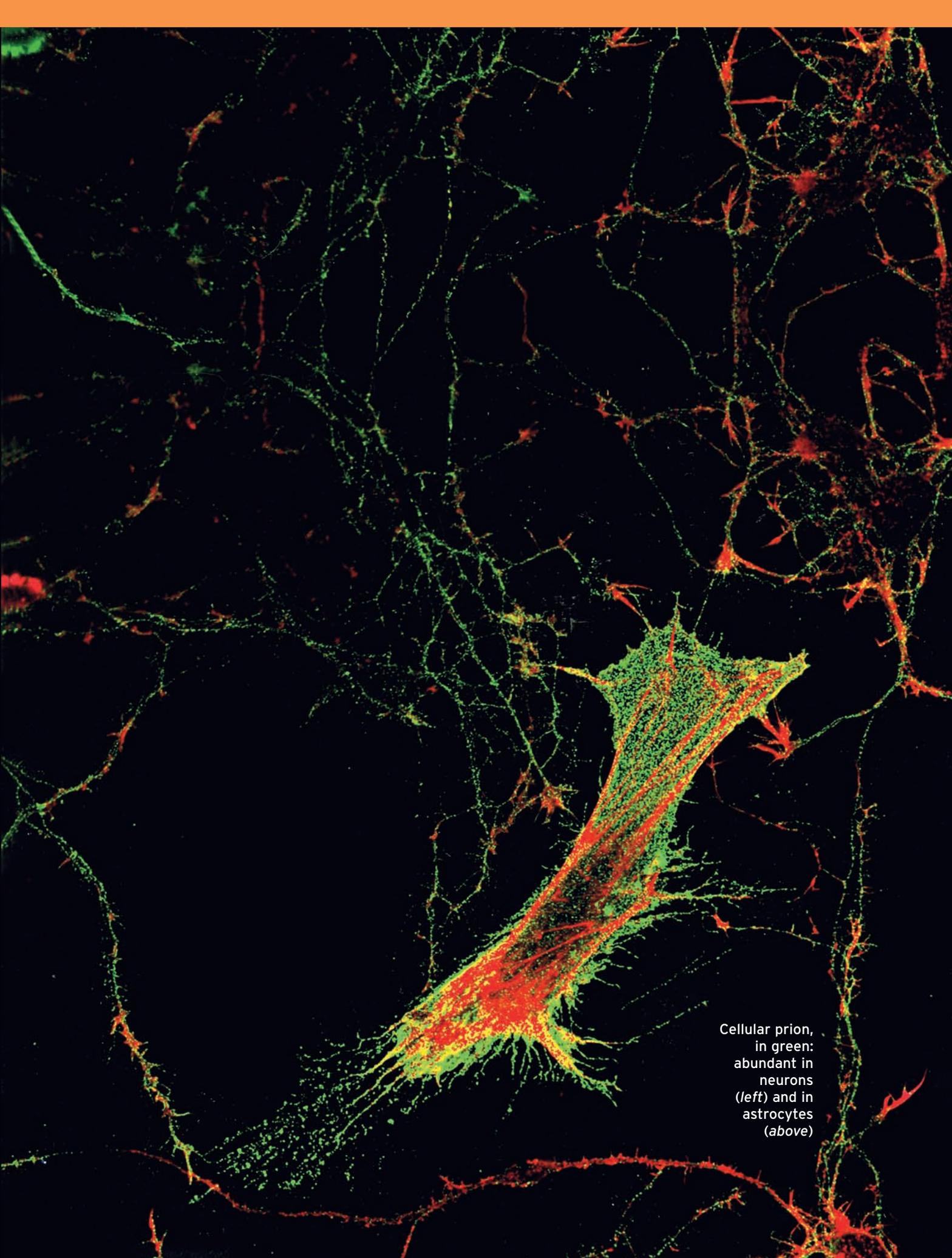
RICARDO ZORZETTO

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In May 1990, the English Minister of Agriculture, John Gummer, was the protagonist in a disastrous public appearance. He posed for photographers and cameramen while savoring a juicy hamburger together with his four-year-old daughter. His idea was to show Britons and the rest of the world that beef consumption was still safe even amidst the most severe crisis his country's cattle raising industry had faced in recent years: contamination of part of the herd with mad-cow disease, or bovine spongiform encephalopathy, which spread throughout Europe, the United States and Canada, and since 1987 has resulted in the slaughtering of 180 thousand infected animals.

Six years after that hamburger, Englishmen would remember Gummer and feel betrayed when the first human cases of the disease came about, probably contracted from the consumption of infected meat. The human version of mad-cow disease was a new form – the fourth known one – of a rare incurable illness: Creutzfeldt-Jakob disease, which kills the nervous system cells (neurons) and leaves the brain full of holes, much like a sponge.

The disease, which reduces the brain to half of its original size, was first described in the 1920s in Germany by neurologists Hans Gerhard Creutzfeldt and Alfons Maria Jakob. There is now a new explanation based on recent studies carried out in Brazil and abroad. In an article published in April in *Physiological Reviews*, the São Paulo, Rio de Janeiro, Minas Gerais and Rio Grande do Sul research group coordinated by oncologist Ricardo Renzo Bretani, from the A. C. Camargo Hospital, in São Paulo, presents the broadest review on the infectious agents



Cellular prion,
in green:
abundant in
neurons
(left) and in
astrocytes
(above)

of this disease, with information that may influence its treatment. The illness develops furtively for two or three decades and evolves at an astonishing speed, finally leading to a tragic death.

The first signs are quite subtle, such as fatigue or depression. Then, balance when walking or handling objects deteriorates progressively; movements become slow and vision, blurred. This is followed by speech impediment and short term memory loss ; it becomes increasingly difficult to find one's way through streets or objects in the house. "In less than one year, nine out of ten infected people become weak, to the extent of being unable to get out of bed, and eventually die," says neurologist Ricardo Nitrini, from the University of São Paulo (USP), who identified the first Brazilian case of a new form of the disease caused by genetic change eleven years ago.

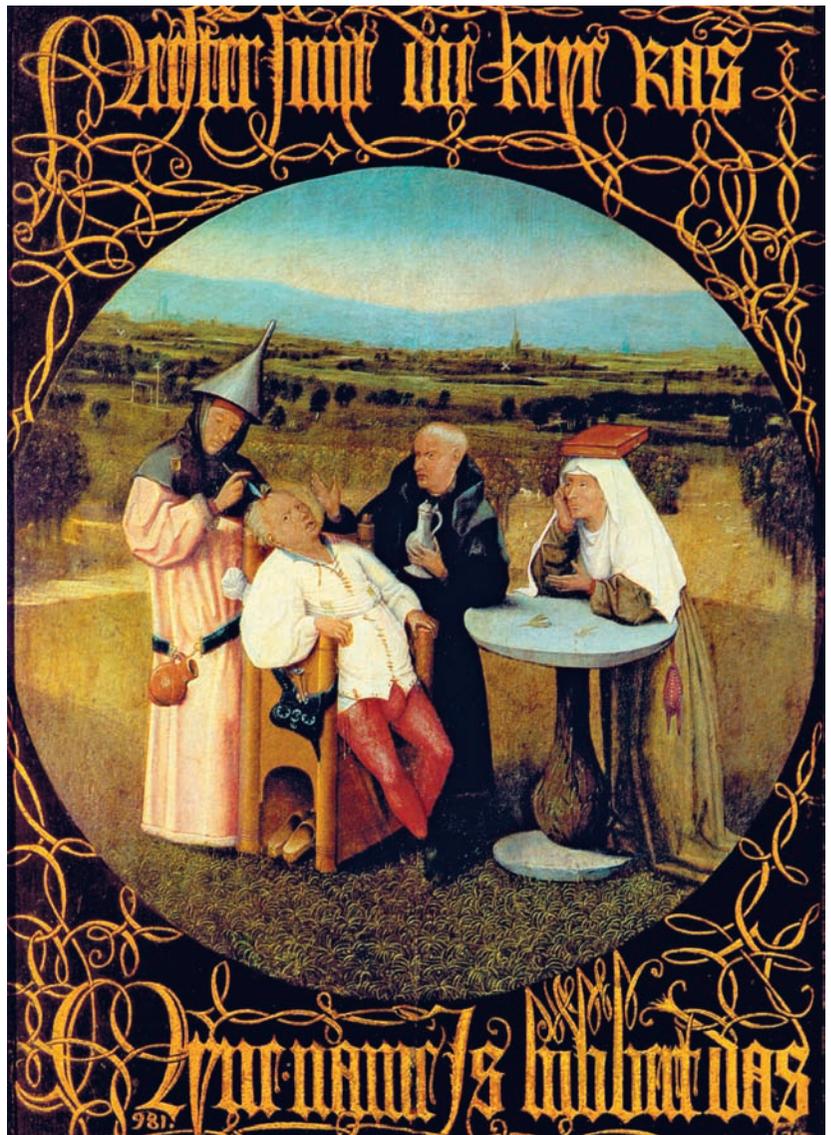
Besides the form caught from contaminated meat – the new variant of Creutzfeldt-Jakob – and the genetic variation, passed on from parents to their offspring, there are still two other types of this disease that corrodes the central nervous system. The most common (and spontaneous) type appears randomly for unknown reasons and affects one out of every one million people. The fourth type is transmitted by infected surgical equipment, blood transfusions and, until a couple of years ago, by the application of growth hormone produced from the brain cells of cadavers, now replaced by synthetic hormones, to treat growth disorders.

The spread of the mad-cow disease in the European and American countryside and the appearance of the new form of the disease in humans – since 1996, the new Creutzfeldt-Jakob variant has killed 160 people in England and in neighboring countries, including the daughter of a friend of former minister John Gummer – helped intensify the search for the cause of this illness. The main suspect for this group of diseases has challenged biologists and physicians for decades. Unlike other infectious diseases, the cause of Creutzfeldt-Jakob is not a virus, a belief held for a long time. Nor is it a bacterium or a protozoan, microorganisms that multiply on their own account and

that are easily passed on. Today, it is believed that a defective protein called a prion (acronym for infectious proteinaceous) causes the disease. The mere contact of the prion with a healthy protein found abundantly on the surface of the neurons seems to induce the protein to assume a changed form, somewhat like the domino that falls and hits the other pieces in the row, and there is not much one can do about it. Because they are more stable than healthy protein, the deformed molecules adhere to each other, generating long fibers that are toxic for neurons.

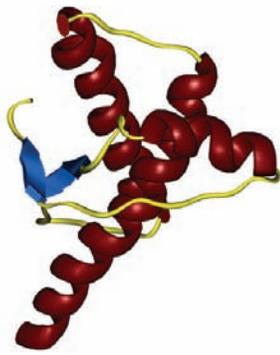
The identification of prion in ewe brains with a certain type of spongi-

form encephalopathy referred to as s and the explanation of how it could deform normal proteins led United States researcher Stanley Pruiser to win the Nobel Prize for Medicine in 1997, and helped scientists worldwide to investigate the defective protein and its effects on the organism. While all the attention focused on the prion, another basic and perhaps more important question remained in the background: what was the normal protein – the cellular prion found on the surface of all body cells, and in larger amounts in the central nervous system – doing? No one knew and no one seemed very concerned about it.



The Cure of Folly (Extraction of the Madness Stone), by Hieronymus Bosch (1475-1480)

REPRODUCTION THE LIBRARY OF GREAT PAINTERS/BOSCH



There was actually a reason for the lack of concern about cellular prion. In 1990, molecular biologist Charles Weissmann created a lineage of rats that did not produce this protein. The animals did not develop the spongiform disease and apparently survived with no harm to their health. For this reason, it was assumed that cellular prion did not play an important role in the organism. “It was a limited point of view,” says Brentani.

Brentani suspected that nature would not waste time and energy to create a protein with no biological activity, so he followed his intuition and decided to go against the trend. “It was an opportunity to take part in a hot area of study in which no one had been interested,” he says. A letter published in 1991 in *Nature* encouraged him to keep going. Three years before, Brentani proposed a theory according to which both helices of the DNA molecule contained the recipe for protein production – and not only one of them, as had been previously believed. He also stated that the proteins codified by complementary parts of the DNA helices had complementary roles: they could chemically interact and fit into each other as a key into its hole. From the evolutionary perspective, it made sense that the parts of the DNA that codify a protein and that connect to the protein be close, since there is a greater possibility of them migrating together to another area of the genetic material, in the case of repositioning. However, according to Brentani, no one believed in this hypothesis – other than him, of course.

Then came the *Nature* letter, in which researcher Dmitry Goldgaber,

from the State University of New York in the United States described how the cellular prion should interact with water – one of the chemical features of the proteins – and stated that if Brentani were right, the complementary DNA portion of the cellular prion gene would contain information about the protein that possibly activated it. In sum, an important clue.

Brentani studied proteins associated with cancer – and he decided to analyze prion and the molecule that worked as its on-off switch. Brentani, along with biochemist Vilma Martins, from the Ludwig Institute for Cancer Research (LICR) and biochemist Vivaldo Moura Neto, from the Federal University of Rio de Janeiro (UFRJ), deciphered the structure of this other protein and described it in 1997 in *Nature Medicine*.

The protein they presented – later identified as STI-1, an acronym for stress inducible protein 1 – was comprised of 543 amino acids (the building blocks of proteins) and was almost twice as large as cellular prion. It was necessary to find out what both of them did. “We had two hypotheses: either they were useless or they were essential for important phenomena in the neurons, such as the neuritogenesis process [*the forming of the ramifications that connect the neurons among themselves*],” says Brentani.

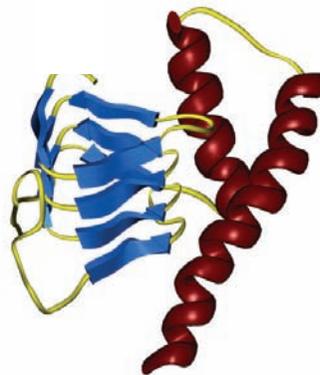
Since the team was not specialized in neurons, Brentani and Vilma invited neuroscientist Rafael Linden, from the

Biophysics Institute of UFRJ, to collaborate with the other tests. The complex formed by the cellular prion and STI-1 was shown to be essential both for the development and the forming of the neuron prolongations and to protect them from apoptosis, or programmed cellular death.

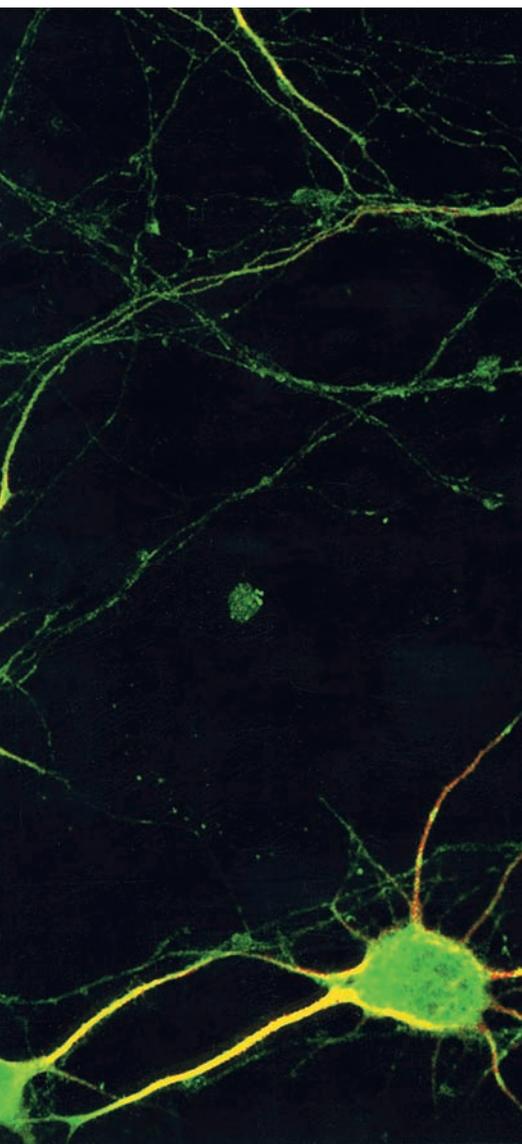
However, these were not the only parts played by the pair. Experiments with lab rats, conducted jointly with Iván Izquierdo, one of the best-known memory researchers worldwide and currently a professor at the Catholic University of Rio Grande do Sul (PUCRS), showed that cellular prion and STI-1 are essential for memory formation. Without them, animals have a hard time remembering something they learnt hours before (short-term memory) and also days before (long-term memory). Tests carried out with genetically modified rats, such as those created by Charles Weissmann, in order not to produce cellular prion, showed that these animals only seemed to be normal. As they aged, they suffered from greater memory-related difficulties than cellular prion producing rats.

The Brazilian group also saw that the healthy form of prion has different effects on different tissues. At UFRJ, Linden’s team found that this protein modulates the immune system’s response to inflammation, at times increasing or reducing defense cell activity. Cellular prion stimulates neutrophils, the most abundant defense cells in the organism. 100 billion neutrophils are produced every day within the long bones and they are the first cells to arrive at the inflammation, where they rapidly involve and destroy invading microorganisms such as bacteria. When Linden caused an inflammation in the rats, he found that the genetically modified animals that did not produce cellular prion had fewer neutrophils, which were also slower than in normal rodents, an undesirable effect in the case of infection.

The opposite effect was found concerning macrophages, the defense system cells that act as ‘cleaners’, eliminating dead cells. Rats with no cellular prion had more active macrophages than animals that produced the protein, a result which is not always advan-



One protein, two forms:
cellular prion (above)
and its infectious form



tageous for the genetically altered animals, since excess macrophage activity can damage to healthy tissues. “The response to inflammation and to dead cells depends upon a delicate balance,” explains Linden. “It is undesirable for them to be either absent or too abundant. The body doesn’t resist infection without an inflammatory response, but excessive inflammation can also kill.”

There is also evidence that cellular prion protects heart cells from chemical aggression. At the A.C. Camargo Hospital, Vilma and physician Beatriz de Camargo analyzed the presence of a slightly altered form (variant) of the cellular prion proteins in 160 patients treated with adriamycin during childhood, a drug that can cause heart dam-

age. Preliminary data suggests that those that carried the cellular prion variation were more susceptible to the drug than those with the protein’s normal version.

As the lab results showed, it was obvious that cellular prion was essential to keep the body healthy; not bad for a molecule that until recently was considered to be devoid of biological importance. However, researchers still did not understand why it protected tissues in certain circumstances, but damaged them in others. An important step was to figure out how this balloon-shaped protein that stays on the outside surface of cells communicated with the inside.

Vilma, Brentani and Linden resorted to the help of cellular biologist Marco Antonio Prado, from the Federal University of Minas Gerais (UFMG), who researches molecule transport within cells. Together with Vilma and Kil Sun Lee, from the Ludwig Institute, Prado and Ana Maria Magalhães marked the cellular prion of the neurons with a fluorescent green coloring to follow its path and analyzed the cells under a confocal microscope, which enables live observation. Then, with the help of Byron Caughey, of the United States National Health Institute, they marked the infectious prions and observed them enter the neurons (*see Pesquisa FAPESP no. 115*).

Anchored in the thick areas of the cell surface by long sugar and lipid molecules in a rope-like form, the cellular prion slides towards smaller areas of the neuron membrane. There it is sucked into the inside of the acid-containing vesicles, where it connects to other pro-

teins and sends commands to the nucleus or to other regions. From the beginning of its plunge until emerging on the surface, the cellular prion takes no longer than one hour and a half.

This displacement is not random, as the Brazilian group was able to show. Cellular prion only moves on the neuron surface after specific proteins attach to it, thus activating it. As a host welcoming guests to a party, the healthy prion guides other proteins to the inside of the neurons. Once inside the cell, the complex formed by the prion and its activating protein sends chemical signals that order the emission of extensions or the production of compounds that protect the neuron from death, as the researchers explained in an article soon to be published in the *Journal of Neuroscience*. “Communication mediated by the cellular prion remains truncated without this plunge inside the cell,” says Linden.

These discoveries about the cellular prion gave rise to more doubts. At the end of 2006, Linden, Vilma, Prado, Izquierdo and Brentani started reviewing all literature on the healthy and the defective prion in order to arrive at a clearer general framework. The assessment of 597 articles resulted in the broadest review of the subject, published in April in *Physiological Reviews*, with a common understanding on the functioning of cellular prion and a new interpretation on how diseases such as Creutzfeldt-Jakob and mad-cow come about.

In their paper “Physiology of the prion protein,” the São Paulo, Rio, Minas Gerais and Rio Grande do Sul teams suggest that cellular prion works as a selective magnet to which only certain molecules in the body adhere. STI-1 is obviously not the only one. Studies carried out in Brazil and abroad identified another 30 proteins connected to cellular prion, activating different chain chemical reactions that represent different cellular commands. “We believe that cellular prion helps to organize the outside signals before they are sent into the cells,” says Prado.

According to Linden, this role of selective magnet or assembly platform of signaling complexes enables the explanation of results previously considered contradictory, such as protection

> THE PROJECT

Cellular prion role in physiological and pathological processes II

TYPE

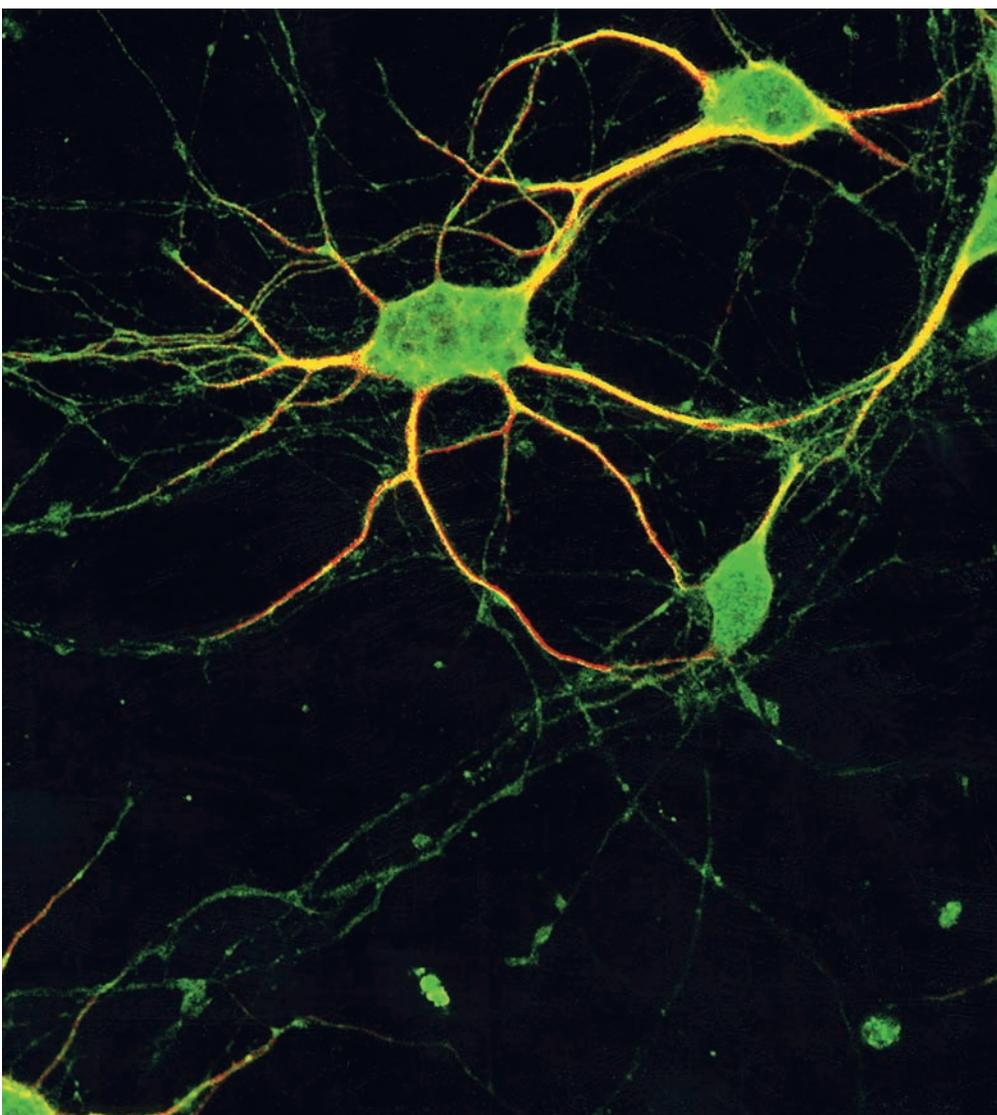
Theme Project

COORDINATOR

VILMA REGINA MARTINS -
Ludwig Institute

INVESTMENT

R\$ 2,053,618.66



IMAGES GLAUCIA HAJJ/LICR

Neuron network: cellular prion (green) helps the connection between the cells

against cellular death in certain circumstances and/or toxic effect in others. “This assembly platform activity of chemical signs is so essential to life that even other proteins possibly carry out the same role in the organism,” says Brentani. “Therefore, rats genetically modified not to produce cellular prion seemingly survive without any harm,” he explains.

This new role changes the understanding on how prion-caused diseases appear. According to this new interpretation, where the Creutzfeldt-Jakob disease is concerned, it is not only due to the adhesion of infectious prions generating toxic attachments that neurons die. The Brazilian group believes that cellular death also takes place due

to the loss of healthy prion molecules, which presumably leaves the neurons unprotected from chemical aggression. According to Prado, the toxic effect of infectious prion could be intensified with the loss of cellular prion. “We will only know if we are right when the ideas presented in this work are tested,” says Linden.

Researchers expect that the understanding on how cellular prions work will lead to treatment alternatives for diseases caused by prions and neurodegenerative diseases such as Alzheimer’s, connected with the attachment of a protein whose production is controlled by the healthy prion. “Therapeutic approaches based solely on what is known about the defective

prion haven’t yielded good results,” says Linden. Quinacrine, a malaria drug used in the 1930s, was capable of hindering the attachment of the infectious prion in *in vitro* experiments with neurons. However, it did not prevent the development of the disease when tested in human beings. “There is still no effective treatment,” says Ricardo Nitrini, from USP.

Together with Hélio Gomes and Sérgio Rosemberg, from USP, and Leila Chimelli, from UFRJ, Nitrini and Vilma are part of the team responsible for the diagnosis of prion-caused diseases in Brazil, which require, since 2005, that the authorities be notified. This is an essential measure to generate an awareness of which regions are the most affected and the populations most susceptible to the four forms of Creutzfeldt-Jakob disease. From 2005 to 2007, the group analyzed 35 suspected cases, of which 26 were deemed probable – confirmation is carried out through brain tissue analysis after death. These people developed the disease spontaneously. No case was related to the consumption of infected meat. “Many cases are not reported in Brazil. We believe there are up to 200 cases a year,” says Vilma.

In the meantime, Vilma’s team at the Ludwig Institute continues to study the action of STI-1. In the last few years, the group found that a fragment of this molecule, a 16-amino acid peptide, has the same role as the full protein and aids memory formation in rats. Initial tests with cells on a glass plate also suggest that the peptide holds back the development of glioblastoma, an aggressive brain tumor that kills in six months, which is why this part of the molecule was patented by the Ludwig Institute in 2007 in the United States. “This is promising information,” says Vilma. For the time being, no further data can be provided until tests are carried out on lab animals and, if all goes well, on human beings. ■

► Scientific article

Linden, R. et al. Physiology of the prion protein. *Physiological Reviews*. v. 88, p. 673-728, 2008