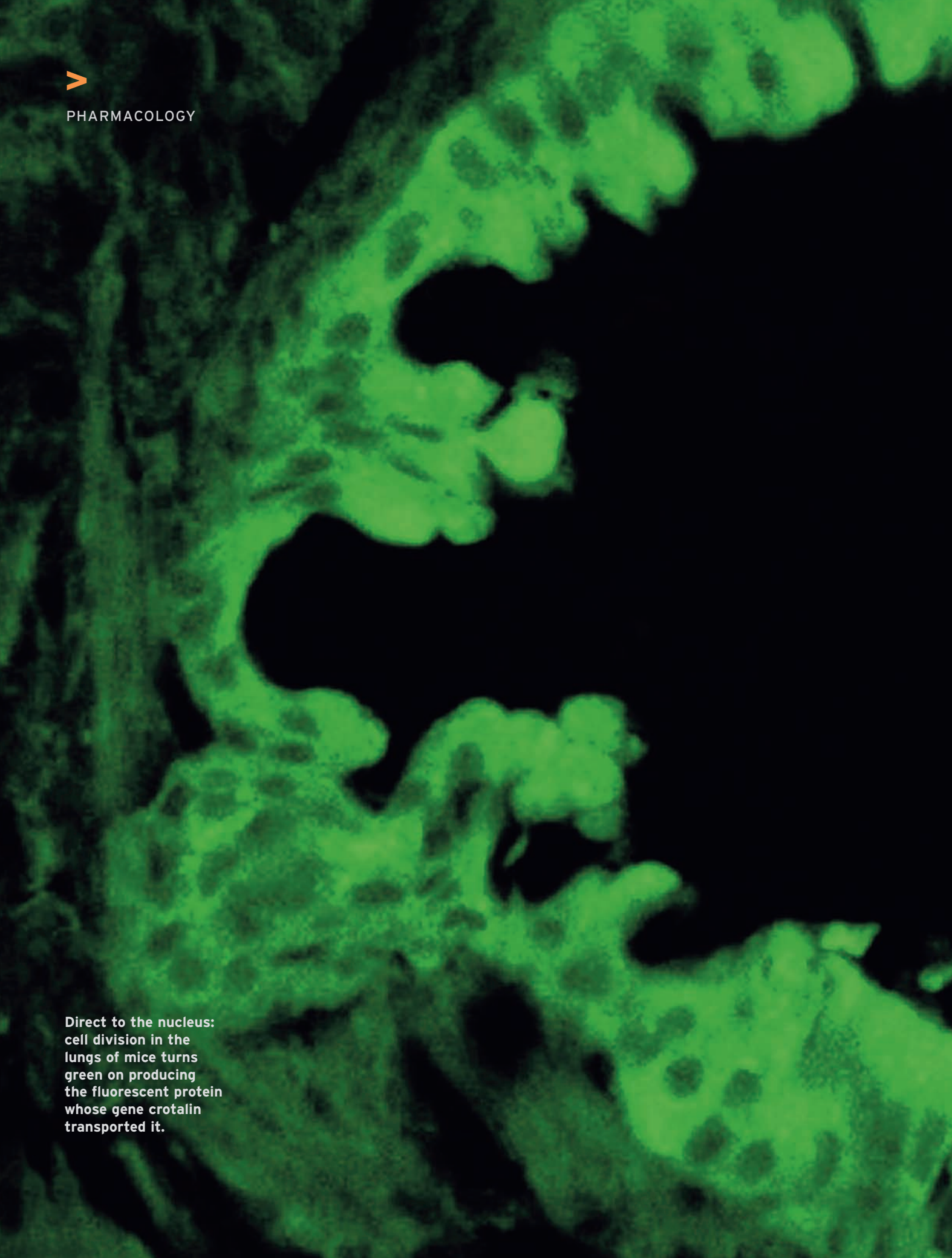

Direct to the nucleus: cell division in the lungs of mice turns green on producing the fluorescent protein whose gene crotalin transported it.

The cellular postman

Protein from the rattlesnake's poison penetrates cells in the process of dividing and shows potential as a conveyor of medication and as an anti-tumoral agent

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Skilled in finding talented people who he allows to work as they please, chemist Tetsuo Yamane formed a research team in Brazil, with ramifications in other countries that in just a couple of years has led to the identification of very rare properties in the protein of a snake typical of the Brazilian *Cerrado* and *Caatinga*, the rattlesnake. Crotalin as this protein is called, traverses the cellular membrane and transports genes and other molecules to the interior and even into the nucleus of the cells – not just any cell, but those that are multiplying. For this reason, this protein may be used in the diagnostics of diseases, in conveying medication and, as the latest experiments seem to indicate, in destroying tumors.

Yamane, currently 76, as head of the biotechnological laboratory at the Institute of Energy and Nuclear Research Institute (Ipen) at the Biotechnology Center in the Amazon Region (CBA), began to create new sources for the study of crotalin some time in 1993, when he was considering the idea of returning to Brazil, after 40 years in the United States. Isolated in the 1950s by the biochemist José Moura Gonçalves, crotalin had already been thoroughly studied, due to its capacity of paralyzing the muscles of rodents. Forty years later, it did not seem to pose major enigmas – except for a chemist, of Japanese origin, whose boldness had been fed on day-to-day basis by his association with scientists at the level of Richard Feyman and Linus Pauling, during his graduation and post-graduation years at California's Institute of Technology (Caltech). His propensity to ask new questions perfected itself even more during his ten years working with physicists at the Bell Laboratories, where the transistor, the laser, the integrated circuit and communications by satellite were invented. On becoming acquainted with crotalin, Yamane became intrigued about the mechanisms, at that time still undeciphered, by which the protein acts on the system and by the possible interactions of this molecule whose structure reminds one of a woollen dragon.

Could crotalin interfere with the division of cells? It was this question by Ymane, active since 1994 in the Butantan Institute and at that time working only with the biochemists Gandhi Rádis-Baptista and Álvaro Prieto da Silva, that drew the attention of the cellular biologist Alexander Kerkis and his wife, biologist. Both are Russian. They had been active in one of the most important Russian research centers in Siberia, before perestroika fragmented the scientific knowledge structure there. After spending some time at the State University of Northern Rio de Janeiro, the Kerkis came to São Paulo in 1999. They took up the study of mice stem cells at the Insti-

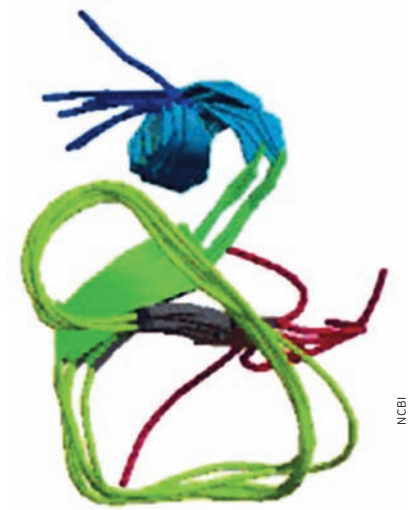
ALEXANDER AND IRINA KERKIS/ BUTANTAN INSTITUTE

tute of Biomedical Sciences (ICB) at the University of São Paulo USP. It was then that they met Yamane and, jointly, discovered new properties in a toxin that seemed not to have anything further to yield. In very small doses, they verified, that crotalin very quickly reached – in only five minutes – the nucleus not only the embryonic stem-cells of mice, but also other types of cells.

For the first time it was demonstrated that a protein until then considered only as a toxin also acted as a cellular postman: it traversed the membrane of cells in the splitting up process and reached the nucleus, where the chromosomes are situated. Once there, this little protein adheres to the centromeres, the agent whereby the chromosomes are duplicated and remain united during the cellular division. Thereupon, the chromosomes separate themselves into independent cells; the crotalin leaves the cells remaining in the intercellular space, as if waiting for another moment to become active once more. The results, published by the *FASEB* journal in July 2004, opened new prospects for research and for the use of this protein. "We began to regard the toxin from a new perspective", states pharmacologist Mirian Hayashi, who worked with Yamane at the Butantan Institute for three years on this research project, subsequent to another three in the development of pharmaceutical products in Japan.

Against pain and parasites - It was also at Butantan that another team found a substance in the rattlesnake with an analgesic power 600 times that of morphine and, apparently, without relevant side effects.. In an experiment carried out at USP, the poison of the *Crotalus durissus terrificus*, which inhabits the south and west of Brazil, was the one to show itself the most effective against the parasite causing da leishmaniasis, in comparison with two other subspecies, one typical of the *Caatinga* and the other of the south western and central western regions. The fractions most active in tests within cells and mice were gyrotoxin and crotalin

Crotalin, the major component of a rattlesnake's poison, is a small protein. It contains 42 amino acids, almost as many as insulin, the hormone of 51



NCBI

The structure of crotalin, the major component of the rattlesnakes poison

amino acids that controls the sugar level in the blood. However, it is minuscule when compared, for example, with hemoglobin, the portentous molecule of four chains of 140 amino acids each, which conveys oxygen to all of the body's cells. Given that it is so small, it becomes understandable that it would easily traverse the membranes of the cells. But how?

Here is the answer: by coupling itself to the molecules of the cellular surface known as proteoglycans of heparin-sulfates that circulate within the cell's interior. This coupling is not fortuitous. "The moment in which the cells most produce heparin-sulfates is during their reproductive cycle", states biochemist Ivarne Tersariol, professor of the Federal University of the State of São Paulo (Unifesp) and of the University of Mogi das Cruzes (UMC). As this group demonstrated in a paper by the *Journal of Biological Chemistry*, the affinity between crotalin and proteoglycan of heparin-sulfate molecules is due, essentially, to electrostatic forces: crotalin is a molecule with a positive electrical charge, whereas the proteoglycan of heparin-sulfate molecules are negatively charged.

It so happens that crotalin is not entirely positive: one side is electrically neutral. This peculiarity serves the purpose of connecting other molecules both to the positive and neutral sides. However, this was still only theory, as was the hypothesis of crotalin, given its positive electrical charge, connecting itself to the negative DNA. There were indications that this might be possible; however, crotalin might couple itself, not directly to, but to some other protein associated with the DNA.

To clear up the doubt, Mirian spoke with Vitor Oliveira. A chemist at the Federal University of São Carlos (UFSCar), he was working on projects relative to the analysis of protein structures in a private university. It was he who operated equipment at Unifesp that analyses how molecules absorb components of a special type of light. The answer became available within five minutes, by means of a graph: yes, crotalin coupled itself directly to the DNA. Soon after, an experiment by the group showed that crotalin might in fact convey a form of circular DNA known as plasmid to the nucleus of liver, lung and bone marrow cells, which find themselves under continuous multiplication. The alien DNA functioned normally, as if natural, within one of each four cells: a noteworthy result.

There is also growing evidence that crotalin might be connected to the system's defense – and not just because it is part of a poison. Its three-dimensional structure is similar to that of *beta-defensine*, a protein found in the saliva and in the mucous (of the nose, for example) of human beings and of other animals. "Molecules, such as these, are part of the first defense line of organisms", states Irina. In a manner similar to the *beta-defensine*s, crotalin might integrate the innate immune system, which functions intensely during pregnancy and

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the first months after birth when the organism does not yet produce antibodies against microorganisms. Therefore, snakes also use something of themselves, albeit in a larger volume, to defend themselves: the poison gland is a modified salivary gland, states Mirian. However, not all *Crotalus durissus terrificus* produce crotalin. Gandhi Rádis-Baptista verified that all carry the gene responsible for the production of crotalin; some of the representatives of this species – measuring up to 1.5 meters in length and easily identified by their tail rattles – produce a protein with a similar structure, called crotasin, the effect of which is not yet known.

Rádis-Baptista is now with the Federal University of Pernambuco, complying with what seems to be the fate of the participants of this story; not to put down roots or, at least, to go after even more challenging projects. Mirian left Butantan a year ago, soon after Yamane, and is now at Unifesp as a professor of pharmacology; Vitor Oliveira left the private university and returned to Unifesp as a professor of biophysics. Irina Kerkis left USP and is now at Butantan, whereas Alexander Kerkis now works in the lab of a medical clinic. Fábio Nascimento, the biochemist who carried out the heparin-sulfate experiments, is currently working in a biotechnological company in Switzerland.

EDUARDO CESAR

***Crotalus*: hope also against parasites and leishmaniasis**

Uncertainties ahead - Even if at a distance, apparently they did not lose enthusiasm to work together on problems they might not solve alone. Extracted and purified with dexterity by Eduardo Oliveira at USP in Ribeirão Preto, crotalin forms aggregates of two or three units that reduce its capacity to convey molecules. To avoid the formation of these aggregates is, perhaps, more difficult than to produce a synthetic version of crotalin, which would avoid dependence on the purification of snake poison.

Although they have a lot of work ahead of them, the researchers are already negotiating with companies interested in employing crotalin sections as gene conveyers. In 2004, they requested the patent for the potential uses of this molecule, given that they do not wish to repeat the story of other molecules discovered by Brazilians, which, because they had not been patented nor relied on corporate support, ended up being taken over by other research groups. They are aware that being the owner of a patent is only one of the requisites in the long road to the development of a pharmaceutical product.

Further ahead, should the next laboratory tests confirm the crotalin's potential, the group will be faced with another challenge: to produce the molecule in greater quantities – on a trial scale and, thereafter, on an industrial scale – so as to facilitate negotiations with companies or institutions interested in carrying out the final tests, prior to the molecule becoming a medication or a marker in cancer diagnoses. "Those with good ideas are welcome", states Yamane. "We are an open group." Little by little, Yamane has already attracted other groups – from Germany, Poland, the United States and Japan – to work with crotalin. "Each one may contribute; only in this manner does science advance", he adds, without giving up the daring that has marked his scientific career: "Linus Pauling always reminded one that, on beginning a project, one should think of how to contribute in an original manner". ■

CARLOS FIORAVANTI