# Viruses, vaccines and billions

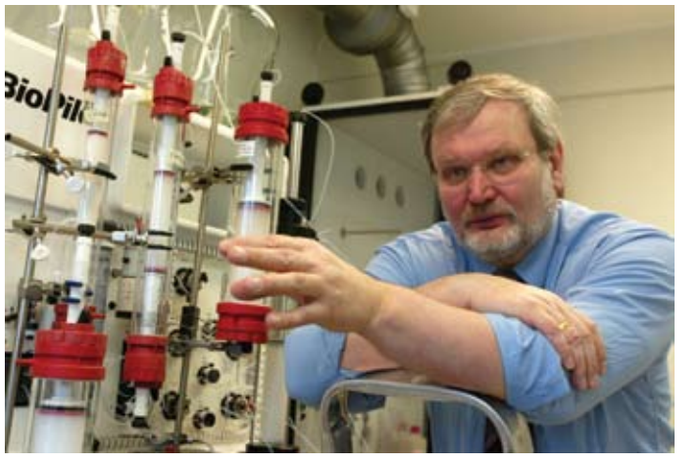
It doesn't often happen that, instead of the science pages of daily newspapers, it is the business columns of financial papers which begin to publicize the work of a scientist. "Estonian scientists' work worth billions" – such were the headlines which drew attention to the work of Estonian biochemist Mart Ustav and his colleagues this year. Mart Ustav himself does not keep track of the billions or, if he does, such figures mainly indicate the number of the DNA base pairs in humans or viruses.

"In order to secure its existence, the university has always had to pay duties – those had to be paid to God or ideology and to the king or government. But what was important was that which was left for the academic community: the opportunity to learn, to teach, to carry out research and create something important which nobody else could do." Those were the words of Professor Mart Ustav at the celebration of the 375th anniversary of the University of Tartu.

## The big contribution of a small research group

Professor Ustav was not speaking in abstract terms. He was talking about himself and about his generation. When the 1960s gave way to the 1970s, at the time when Ustav was a first-year undergraduate, there were strong organic chemistry, physical chemistry and bio-organic chemistry schools born in Tartu. The same happened in physics, biology and other natural sciences. Ustav became part of the working group of the active and intelligent scientist Artur Lind, which began to research ribonucleic acid. "The Estonian Bio-Centre, the Molecular- and Cell Biology Institute and the Technology Institute of the University of Tartu, not to mention several biotechnology companies, grew out of this small research group," says Ustav. He doesn't mention it, but Ustav himself played a significant role in the creation of each new research organisation. The newest one founded on his initiative is the Technology Institute of the University of Tartu, where this year he exchanged the Director's post for the role of professor, which enables him to do more research.

Ustav's activities in the organizing of science have always stemmed from the need to do top level research. "We have a unique historical opportunity to take the University of Tartu to a level in science where our predecessors simply could not take it. This is due to the fact that Estonia is part of the free



world," said Ustav at the university's anniversary celebration. Festive speeches are nice, but daily routine is often dull and tedious, requiring persistence and more persistence. Throughout his life as a scientist, Mart Ustav has concentrated on viruses. He has been interested in how these formations were born and changed during evolution, and how they ensure their infectiousness, multiplication and persistence in a human organism, which sometimes bring misery and unhappiness to mankind. "Viruses are the best objects to investigate the basic rules of nature – they multiply and adapt, and then the existence of this life form is possible," he explains.

## Virus waiting for its time

Viruses are strange formations. They go about their activity, or rather exist, on the borderline of life and lifelessness. "Outside the cell, a virus is an inert crystalline complex of polymerous molecules, inside the cell a genetic element busily multiplying its genetic material," Ustav explains. "All cellular organisms have their own viruses and all organisms have, on various levels, beginning with the cellular level, natural defence mechanisms against viruses. As viruses still exist, they are at least partially capable of breaking through this defence. It is a paradox that viruses need a host organism where they can multiply – hence viruses need to co-exist with, rather than destroy, the host organism."

Everyone has had some trouble with viruses, whether it be flu or just a runny nose. But the last time flu caused life-threatening epidemics was half a century ago. Today's killer viruses create more complex and devastating illnesses. A fifth of cancerous growths are caused by viral infections. And, of course, one of the biggest concerns of humankind, AIDS, is created by a virus – the HIV virus.

If you discover warts on your hand or your doctor says, "Madam, you have condyloma," then you should know that the cause is a virus. The knowledge by itself is not helpful. Not even if you know that the virus is called the papilloma virus. But Ustav and his colleagues have found out how this unpleasant virus multiplies in cells.

Warts are non-malignant tumours, in the sense that they do not cause death. But the papilloma virus can be fatal. Every year, there are 300 new cancers of the neck of the uterus discovered in Estonia, which can be blamed on the tiny troublemaker. "Papilloma is a virus that we know causes tumours," Ustav says, explaining why his research group is interested in it.

When it comes to viruses, a person may be infected with one for a lifetime, but nine times out of ten nothing happens. But if the body gets weaker, a virus uses the situation and starts to multiply. Viruses are inter-cellular parasites and have the ability to multiply only in human cells by using the metabolism of the cell. One virus in infected cells becomes a hundred new viruses. "We have researched the multiplication of viruses, or replication in cells," Ustav explains, "how it happens and which genes of the virus lead the replication." Tartu scientists have characterised the different stages in the replication of the papilloma virus. They have found out how the virus secures its replication inside cells. The genome of the virus binds itself to the host cell's chromosomes which carry the heredity material and one of the virus' proteins takes care of this binding. When the cell splits, the chromosomes are divided between subsidiary cells, and this is how the virus transmits itself. In this way the virus is able to utilise the host's information and strength.

If we found out why the cell obeys the virus, how it surrenders its metabolism to the virus, then it would be possible to develop a medication which would protect us from the virus. "Perhaps it already exists among the tens of thousands of existing compounds, but we are unaware of it," notes Ustav.

Instead of experimenting on animals, the Tartu scientists do tests on cells. This enables them to investigate the details of a virus' life activity. The aim is to find out how the virus subjugates the cell.

## Pursuing vaccines

As the Tartu scientists know, there are two sides to every story. If the papilloma virus is so good at invading the cell and living there, why can't it be used for good purposes? "The idea is to take away the harmful genes of the virus which can cause cancer and use the virus as a vehicle in order to transport necessary genes into the cell," Ustav explains. This idea, using the papilloma virus as a resource of gene therapy, is worthy of a patent, and it has been patented. The virus vehicle is one of the few gene technology patents to be applied for from Estonia.

There are more than a hundred different types of the papilloma virus. Some are considered low-risk and others high-risk viruses. In further researching the papilloma virus, Ustav's research group has determined that the uterus cancer caused by the human high-risk papilloma virus is a gradual process, during which the virus randomly integrates into the genome of the host cell. Once inside the cell, the virus starts to take over, reducing the cell to its slave, and causing the cell to produce specific proteins which benefit the virus. The researchers have demonstrated that the replication mechanism of the papilloma virus is able to cause genetic changes inside the host cell.

"Most important is the ongoing work in the field of the replication of the human papilloma, where we've understood the mechanism through which the virus generates genome changes inside the infected cell, which leads to the development of a tumour," explains Ustav. The first article on this discovery was published this April in the EMBO Journal, the magazine of the European Molecular Biology Organisation.
Also, the dreaded HIV virus has no single line in humans. But, to a large extent, it does in Estonia. Mart Ustav and his colleagues have shown that the Estonian AIDS epidemic was caused by complex recombinant HIV1 subtypes called CRF 06 and CRF 32.
This line started to spread after August 2000, when HIV1 swept through injecting drug addicts. They ranked and typified two areas of the virus' DNA. HIV1 has spread widely in Ukraine, Russia and Byelorussia, and this epidemic began in 1996 in southern Ukraine. It is important to know the exact genetic structure of this virus and this has been identified by Estonian scientists.
The structure of the virus is one thing, while beating the illness caused by the virus is quite another. To date, the right medicine has not been found: although medications have been developed to alleviate the viral infection, and those are in use, this medication cannot get rid of HIV1 in the body; it just slows down its replication. Therefore, after infection, patients have to take medication for the rest of their lives, and this costs about 100,000 kroons a year. However, unlike in the case of a new flu virus strain, there is no vaccine to date. The HIV virus sneaks into the cell very slyly and uses its machinery in its own interest. In this sense, it is similar to the human high-risk papilloma virus.

Learning about the inner life of viruses has taken Ustav and his colleagues to the brink of a new HIV vaccine. In cooperation with the Finnish biotechnology company FIT Biotech, they have created a genetic vaccine which, if all works out, will be safer, more effective and cheaper than the AIDS medication available today. "The idea of this vaccine is to use the genetic elements of the papilloma virus, as a result of which the genetic vaccine molecule uses the papilloma virus' characteristics to function effectively," Ustav explains. It is as if it has been copied from the papilloma virus.
Directed by those artificial genes, a human cell starts to produce proteins indicating the virus and, in turn, these proteins activate the immune system. It has been tested in test-tubes and on laboratory animals. Animal testing has demonstrated that the vaccine works very well.
"No other research group can show results as good as those," says Ustav. "Tests on apes showed that our vaccine created a long-lasting and very strong immunity, which effectively removes cells infected with HIV1 from the organism."
The vaccine has also passed four first-stage clinical trials on voluntary Finnish HIV-positive patients and on non-infected volunteers. The trials showed that the vaccine is safe for humans. At the moment, second-stage clinical trials are underway on sixty patients from South-Africa, which should determine the effectiveness of the drug and any short-term side-effects.
"We have included HIV1 sequences in the vaccine which the organism's immune system should be able to recognise in more than 95% of the cases. But theoretical calculations on the basis of bio-informatic analysis aren't enough; we need to prove it with clinical trials," says Ustav.

Therefore, in order to release the vaccine into general use, there is a need for more widespread trials, with thousands of people, to determine the vaccine's effectiveness and safety. But this is no longer the concern of Ustav and his colleagues. They are working on finding new vaccines which will prevent cancer. If these vaccines are found, Ustav and his colleagues will be true billionaires – winning the gratitude of billions.