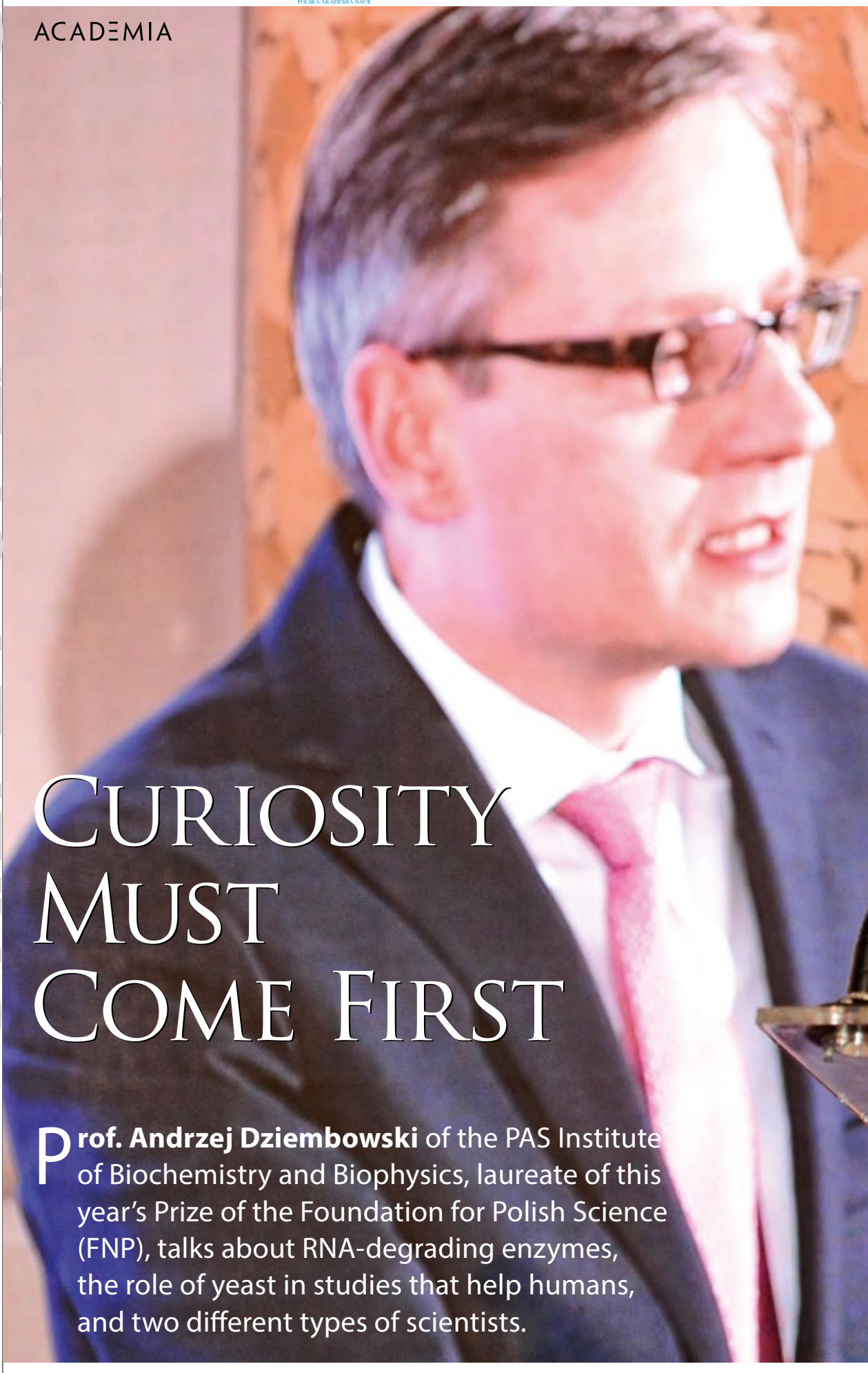
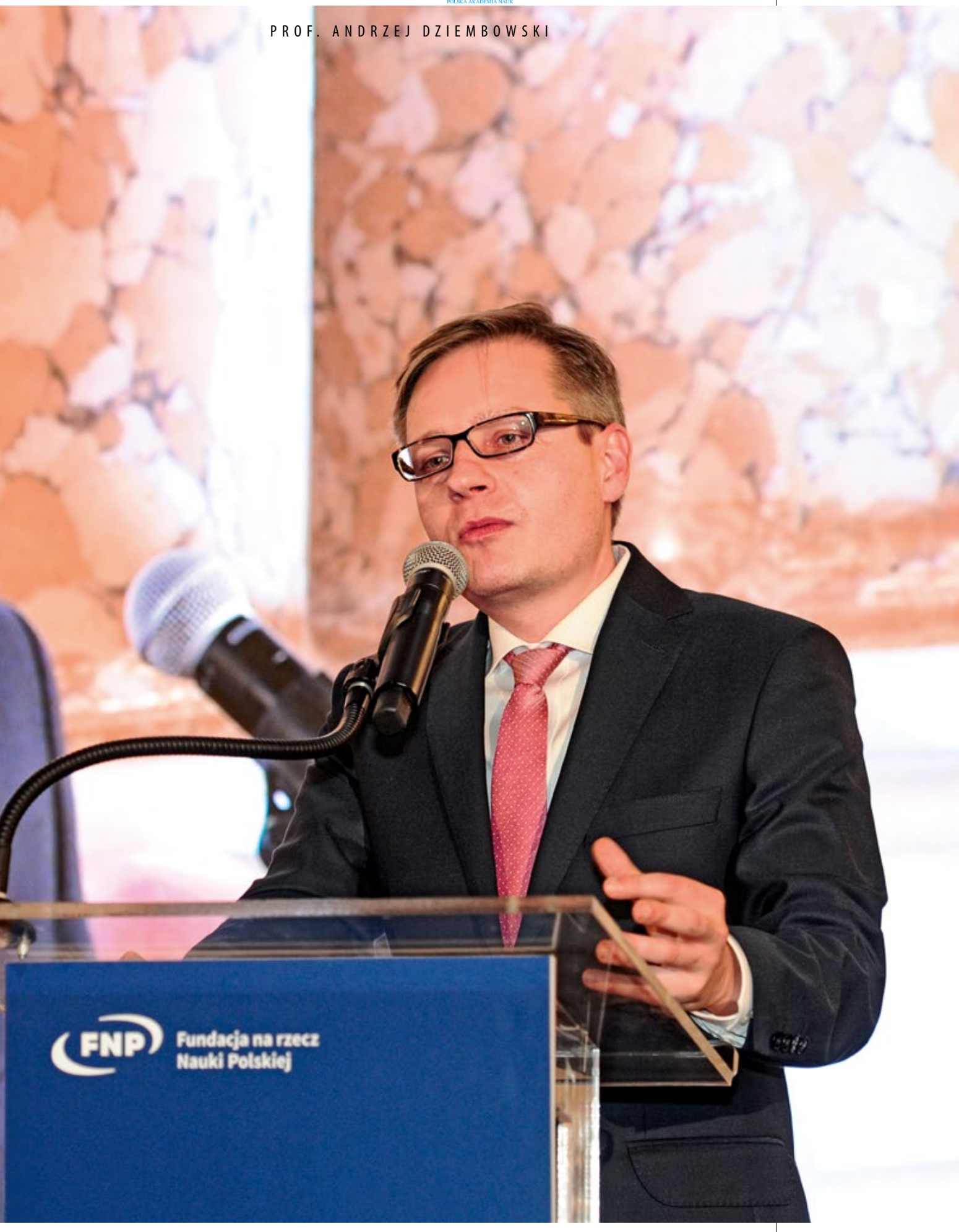


Curiosity Must Come First

Prof. Andrzej Dziembowski of the PAS Institute of Biochemistry and Biophysics, laureate of this year's Prize of the Foundation for Polish Science (FNP), talks about RNA-degrading enzymes, the role of yeast in studies that help humans, and two different types of scientists.

PROF. ANDRZEJ DZIEMBOWSKI



ACADEMIA: **The Foundation for Polish Science has honored you with its FNP Prize, known colloquially as the "Polish Nobel," for "explaining the function of key enzymes involved in RNA degradation, the dysfunction of which leads to pathological states." What are those enzymes and what is their mechanism of action?**

ANDRZEJ DZIEMBOWSKI: All information about the human body is encoded in the genes. A genome consists of parts that code for proteins and other, non-coding parts, which may fulfill regulatory functions. In order for a protein to be produced based on genes, information from the DNA must be copied into RNA in a process that is called transcription. The RNA molecule then undergoes quite complicated processing that involves adding certain elements at both of its ends and cutting out certain sequences in the middle. Such a mature molecule – called messenger RNA, or mRNA – is exported from the nucleus to the cytoplasm, where it serves as a template for the production of the protein.

> Using a mouse model, we can not only demonstrate that a specific RNA mutation indeed causes a disease, but also try to understand why this is so.

Since RNA processing is complicated, it may result in the production of defective molecules, which the cell must eliminate. Defect-free molecules, after they are transported to the cytoplasm, are used for the synthesis of proteins, but they have specific lifespans. For example, the RNAs that code for regulatory proteins controlling cell division are short-lived, whereas the RNAs coding for basic enzymes involved in cellular metabolism have longer lifespans. Many different proteins are involved in RNA degradation. The most important of these are enzymes called RNases. It is thanks to their activity that some mRNA molecules stay in the cell for a long time and others for shorter periods. They may work independently or create larger complexes composed of many subunits. In our research, we have devoted a lot of attention to one such complex, called exosome.

RNase mutations may result in different diseases. Damage to one of the RNases we studied is linked to Perlman syndrome, which is characterized by fetal gigantism and a predisposition for contracting cancer. This RNase is responsible for the degradation of the RNA molecules involved in the regulation of the cell cycle, so its dysfunction leads to pathologies, namely overgrowth. A different RNase, the DIS3 protein, which is part of the RNA degrading exosome in the cell nucleus, is often mutated in multiple myeloma patients. We're conducting intensive studies to figure out why this is the case.

Why is the exosome so important?

The human genome has around 20,000 genes, the same number as the mouse genome. Nematodes and fruit flies have fewer, and yeast still fewer, at around 6,000. These organisms are incomparably less complex, but humans have only four times as many genes. Consequently, the difference between people and, say, yeast boils down not to the number of genes, but to more complex mechanisms of gene expression regulation.

Most of the human genome consists of portions that are not genes, which means they don't encode information about proteins. They are also copied into RNA – it is now estimated that around 70% of the genome is transcribed. In this way, a lot of non-coding RNAs are produced in the cell. Some of those molecules fulfill regulatory functions, but many have no function. These needless RNAs must be eliminated, and the exosome is involved in this process. It also destroys the needless or defective mRNA molecules.

The exosome consists of a ring of nine proteins without enzymatic activity, to which different RNases are attached. Among these proteins, the most important function is fulfilled by the DIS3 protein, mutations of which are linked to myeloma.

What does this mutation involve?

We knew from previous studies that this mutation did not cause the complete inactivation of the enzyme, because its absence leads to cell death. In multiple myeloma, therefore, the exosome works, but not correctly. We have shown that this mutation weakens its exoribonucleolytic activity.

That's a difficult term.

RNA is a linear molecule – it has a middle part and two ends, called the 5′ end and the 3′ end. We can distinguish between two types of RNases, depending on the end from which they start degrading RNA. Endonucleases cut RNA in the middle, exonucleases from the ends. The exosome's main activity involves degrading RNA from the 3′ end. But this is a large protein, so there is additional endonucleolytic ac-

tivity, which means cutting the RNA molecule in the middle.

In multiple myeloma, the exonucleolytic activity of the DIS3 protein is weakened. If the endonucleolytic activity is additionally inhibited, such a defect will be lethal, it will cause the death of the cell. We have demonstrated this using a mouse model. Mice in which endonucleolytic activity was switched off were fine, but they died when exonucleolytic activity was switched off. Consequently, the blocking of endonucleolytic activity in cells with DIS3 mutations will lead to the death of cells, but only myeloma cells. And that's a concept for treating this type of cancer. Of course, this does not pertain to all cancers of this type, only to around 10% with the DIS3 mutation.

That was made possible by the technological progress achieved in recent years. One of the methodological revolutions involves developing DNA sequencing methods, which makes it possible to identify mutations even from single cells.

Do we know exactly how this mutation in the exosome is linked to cancer? What does it take to go from the ascertaining that a certain mutation is more frequent in patients suffering from a specific disease to the discovery of the mechanism governing it?

The exosome complex is somehow involved in the complicated process of antibody maturation and class switching in B lymphocytes. During its course, non-coding transcripts are produced, and the exosome is involved in their degradation. It appears that a mutation of the DIS3 subunit causes some changes in lymphocytes during this process. Probably, they act as a mutator, which means increasing the risk of further mutations, but we are just working on explaining this mechanism in more detail.

In order to determine this, we have used a specific research model. First, we worked with scientists from France to create mice with the same mutation as in multiple myeloma cells in humans. After that, we asked ourselves whether such a manipulation increased the risk of multiple myeloma in mice. When we established that it did, we could move on to the mechanism of action. By using a mouse model, we can not only demonstrate that a specific mutation indeed causes a disease but also try to understand why this happens.

The best known regulatory non-coding RNAs are very short molecules, right?

We call them micro RNAs – these are short, 20-nucleotide fragments of RNA whose role involves starting the process of mRNA degradation. The repertoire of microRNAs varies considerably between tissues, and in order to understand their mechanism of action, we must look at the structure of mRNA. We've said that the main activity of RNases involves degrading the molecule from the end, whereas the activity of endonucleases is very weak, almost negligible. Therefore, on the ends of a mature mRNA, there are parts that protect it from degradation. On the 5′ end, there is the 7-methylguanylate cap, whereas on the 5′ end, there is a sequence of adenosines called the poly(A) tail. In order for an RNase in the cytoplasm to be able to start the degradation of the RNA from that end, this tail needs to be removed first. This is possible thanks to microRNA molecules.

How does a microRNA molecule "know" what mRNA it should attach itself to? Is this process selective or rather random?

A microRNA does not exhibit perfect complementarity with the mRNA molecule that should undergo degradation. In eukaryotic cells, in which many very different processes are ongoing at the same time, there is nonetheless not too much room for randomness. A cell is not a bowl of soup in which various components just float chaotically. It uses a lot of energy to control the processes that take place within it, to avoid accidental interactions between proteins, and to eliminate defective proteins.

> Thanks to studies on test, we learned about the foundation of the action of selected RNA-degrading enzymes. So asking about analogous mechanisms in humans was the natural next step.

Of course, some processes in a living organism are to a certain degree random. For example, the results of cell differentiation may vary, this is often not predetermined. The production of transcripts from DNA is also random to a certain extent – I've said that most of the genome is copied into RNA, and shortly after that this RNA is degraded. We're just starting to study this randomness thanks to such technologies as single-cell RNA sequencing. If we apply this method to different cells within a tissue, we may detect these random differences between the individual cells that form it.

You've said that humans differ from yeast specifically in terms of the complexity of these control mechanisms.

Many of the molecular processes that have been discovered in yeasts are also found in humans. Some mechanisms, such as those related to microRNAs, are not found in yeast of the species *Saccharomyces cerevisiae*, but they are found for example in *Schizosaccharomyces pombe*. In my research work, I deal with processes taking place at the molecular level, and they are quite similar. However, there are further levels of complexity, such as the way a cell is organized, which is not fully influenced by the processes of expression, and they are quite unlikely to explain that. The same holds true for neurobiology: we know very well how a single neuron works, but this does not necessarily translate into understanding how the brain operates as a whole.

You began your career studying yeast. How did you move on to studying diseases that affect humans?

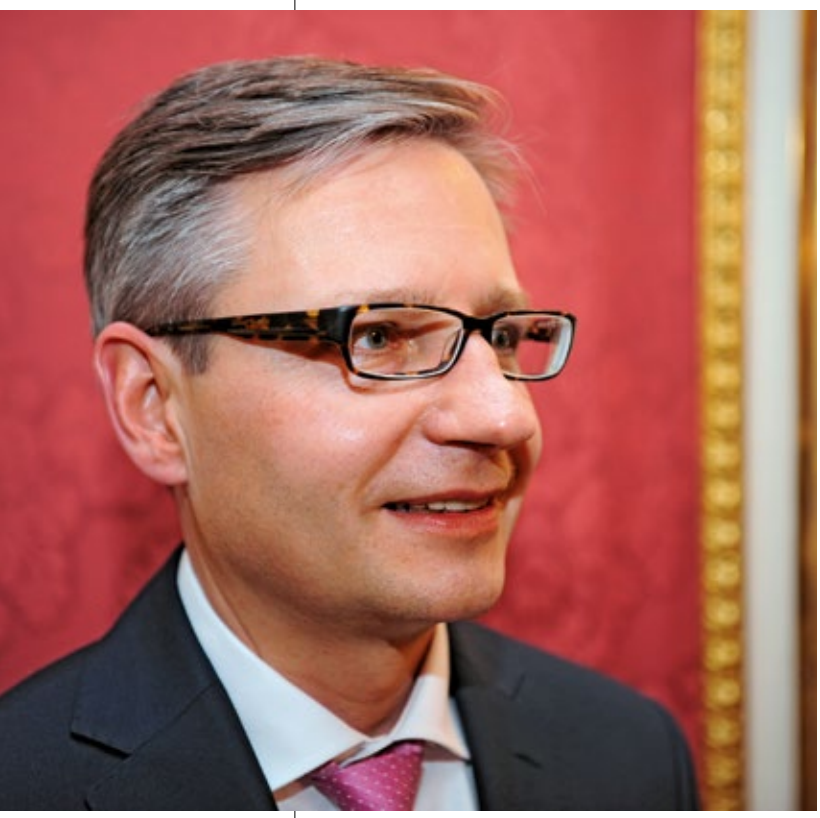
Twenty years ago, studies of yeast offered unique possibilities, because their genome was already known – it was the first organism whose genome was sequenced in whole. Aside from that, yeast have specific features that make it easy to introduce specific mutations, for example the disruption of any gene. It's also possible in mouse or human cells, but that's a lot more difficult. Thanks to studies on yeast, we learned about the foundations of the action of selected RNA-degrading enzymes. So asking questions about analogous mechanisms in human cells was a natural next step. Needless to say, these processes proved a lot more complicated there. For example, yeast have one crucial subunit responsible for RNA degradation by the exosome, while humans have two.

Enormous progress in research work was made thanks to the CRISPR-Cas9, which makes it possible to easily introduce mutations into any organism, such as mice. For instance, these may be the mutations that were identified in cancer patients or in genetic diseases in humans. In this way, we can provide insight into the mechanism in which such mutations have pathological effects at the level of both the entire organism and individual cells.

We introduced the CRISPR-Cas9 method a few years ago to create mutations in mice in collaboration with Prof. Ewa Borsuk. The method proved to be so effective that we organized a core facility to provide new animal models for other researchers working in Poland.

Is it a commercial project?

Not really, it's more about providing services to research centers. We will use the revenue to introduce and develop new methods – we have received a TEAM-TECH Core Facility grant for that purpose



Prof. Andrzej Dziembowski, PhD, DSc

is a molecular biologist, biochemist, and geneticist. He studied and received his doctorate at the Faculty of Biology, University of Warsaw, where he still teaches courses to students. He received his DSc degree (*habilitacja*) in 2009 and was awarded the title of a professor in 2014. He is in charge of an independent laboratory at the PAS Institute of Biochemistry and Biophysics in Warsaw. He worked for several years in the CNRS Center for Molecular Genetics in Gif-sur-Yvette. In addition to national grants and fellowships, he received the prestigious Starting Grant funded by the European Research Council (ERC) and grants under the European Union's 6th and 7th Framework Programmes. His work has earned him two Prime Minister's Prizes, the Award of the National Science Center, and the Commander's Cross of the Order of Polonia Restituta.

He has published research papers in prestigious journals in the field of molecular biology such as *Nature*, *Cell*, *Nature Structural & Molecular Biology*, *Nature Communications*, *Genes & Development*, *Molecular Cell*, *The EMBO Journal*, and *EMBO Reports*. He has coauthored numerous review papers in peer-reviewed international journals and specialist books. He is also a scientific reviewer.

from the Foundation for Polish Science. Potentially, our mice could also be used by pharmaceutical companies.

Probably all biologists dream of finding practical, therapeutic applications of their work.
Absolutely, but I must stress that in my team we focus on basic research. We try to explain mechanisms of action, not to develop a potential therapy, but we are of course very pleased if medical applications are possible.

In biological sciences, all members of a team typically contribute to its success. When you speak, you also use the plural – you say "we've studied," not "I've studied."
When I started my independent career, my lab was initially very small, then gradually grew larger, and it's now quite big. I think that this is one of the greatest things about experimental work – to work in a group, to look for a solution to a specific problem with others. Every member of the team may come up with the idea that will be followed by the entire group. That can't be planned, decided in advance. That's why the papers we publish typically have several authors, each of whom made his or her intellectual contribution.

Surely, I can't say that the publications I have coauthored are only my achievements – they are the achievements of all us. I've been a little lucky in my career. My doctorate, which I conducted under Prof. Stępień's supervision, was quite successful. During my post-doctoral training, I studied the exosome complex, I devoted my subsequent work to this topic, and the follow-up studies were also very successful. I was lucky not to suffer any spectacular failures, not to spend several years working on a project that yielded no results.

Leading a team is always difficult. The bigger the team, the higher the risk of conflicts. People who work in science have very different personalities, some have bigger egos, others smaller ones. So I try to give my associates a lot of independence. Also, the research team is now undergoing changes, the staffing composition is changing, we're currently drifting towards new research questions.

Is there strong competition between your team and other teams?
I can feel strong pressure, because there is indeed intense competition in our field. So there is always this fear that we've overlooked something or that someone will describe a certain mechanism earlier. Unfortunately, all the credit goes to the first person to describe a specific phenomenon. That's a race which it's easy to lose.

Luckily for us, we outrun others more often than they outrun us, but these fears grow over time. We once did a five-year project that ultimately brought a prestigious publication in the journal *Cell*. But the closer we were to the end of our work, the more we feared that someone would be faster than us. Fortunately, we were first, but this shows that there is a certain random factor in experimental research, it's impossible to plan success in advance.

With such strong pressure, can we still talk about any community in the world of science?
For sure, there is a community of people involved in science, because we understand our goals and motives. Each of us conducts research, plans experiments, writes applications for grants, so we have similar experiences. But there is competition within this community, and this competition is not always ethical. I'd rather say that a community may be formed within a specific discipline. There's a group of people I see at conferences – although we only meet in professional situations, we've seen each other so many times that we form a certain community.

> Some scientists are motivated by the sense of competition, by a desire to be first. Others just enjoy discovering how the world works.

However, it can't be said that there is a community of ideals in the world of science. For sure, scientists are not more ethical or nobler than society in general. Ambitions come into play. Why do people become scientists? Curiosity about the world, a willingness to understand how it works are crucial. But there is simultaneously competition and pressure, and we want to be first. Success brings a certain position in the scientific milieu, which drives the ambition to consolidate this position. So it's not true that all scientists want to make a single discovery in concord – we're all taking part in this race. This aspect is a key driver to some people, but there are also people who don't have such ambitions, and they just enjoy discovering how the world works. I'd rather side with the latter approach. Curiosity overpowers the need to outrun others.

Interview by **Olek Michalski**
photography by **Jakub Ostałowski**