GUARDIANS OF THE GENOME

TEXT: NORA LESSING

It can take hours and hours to solve a puzzle with a thousand pieces. It took Martin Beck from the Max Planck Institute of Biophysics in Frankfurt 20 years to complete a very special puzzle: the researcher and his team uncovered the arrangement of the thousand protein molecules that make up each nuclear pore – protein complexes that form a tunnel-like passage through the membrane of the cell nucleus. The proteins serve as both gates and gatekeepers: they connect the nucleus with the surrounding cell and actively control what is allowed in and out. Viruses, for example, have to remain outside.

Power plants for energy production, factories for everyday items, incineration plants for toxic waste: every cell in the body operates like a large city. Countless specialists and specialized facilities ensure that everything runs smoothly around the clock: protein factories – called ribosomes – produce new proteins, power plants supply energy, quality control experts like chaperone proteins help misfolded proteins adopt their correct shape. And logistics specialists – proteins that each recognize a specific type of cargo – deliver building materials exactly where they are needed.

The foundations for this activity are hidden in the nucleus, which is surrounded by a protective membrane. It's here that blueprints for most of the proteins and the RNA molecules of the cell are stored. However, only selected molecules are allowed to study the files: to enter the nucleus, they must first pass through one of the many nuclear pores – and these are strictly guarded. The only molecules that get through are the ones the pores explicitly admit.

Martin Beck from the Max Planck Institute of Biophysics in Frankfurt is studying just how these tiny tunnels are structured and what properties they have. This is a mammoth task in the truest sense, as the pores are not only made up of about a thousand individual proteins, but they also interact with many other proteins in the cell. And on top of that, the microtunnels can vary between different cell types, and even the same cell can have different kinds of nuclear pores.

Arms race with pathogens

What's behind this diversity? "It's possibly the result of a constant arms race between nuclear pores and pathogens," explains Martin Beck. A key role of the pores is to protect the nucleus from invaders like viruses and bacteria. They want to take the →

ILLUSTRATION: LOUISE DÜVER, SHAU CHUNG SHIN, MPI OF BIOPHYSICS

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The rich inner life of a cell: Surrounded by the cell envelope (light green), compartments perform various functions for the cell. The nucleus, enclosed by a membrane (cutaway sphere), acts as the command center. It contains, among other things, the DNA molecule – the blueprint for most cell proteins. Pores in its membrane regulate the import and export of molecules. Proteins are produced, modified, and sent off in vesicles within stack-like compartments (green, pink), while other compartments (orange) generate energy.

DNA stored inside and hijack it for their own purposes. Time and again, however, pathogens manage to trick the cell's defense mechanisms, using a fake entry pass to get through the pores and enter the nucleus. They smuggle in their own genetic material, causing the cell to primarily produce proteins needed by the invaders for their reproduction. This continues until the body's immune system detects the mistake and destroys the cell, or the cell dies from exhaustion. "But if nuclear pores keep changing due to evolution, bacteria and viruses must constantly find new ways to access the nucleus," says Martin Beck. To put it simply: with a bit of luck, pathogens that were deadly for one cell will be defeated by the nuclear membrane gatekeeper of related cells.

A nuclear pore is structured like a tunnel into which a variety of tentacles extend. These are water-repellent and repel most of the molecules in the cell. And this is how the nucleus keeps unwanted guests at bay. Only a few highly specialized proteins are not intimidated by the tentacles: they serve as docking points for proteins that can shuttle specific molecules through the pore. These "importins" and "exportins" can then place their cargo into the pore channel.

Beck's colleague Dirk Görlich, Director at the Max Planck Institute for Multidisciplinary Sciences in Göttingen, has studied these kinds of importins. Importins take specific proteins as cargo and transport them into the nucleus. When molecules need to be transported out of the nucleus, on the other hand, exportins are there to handle the task. "It's highly likely that there are specialized nuclear pores that focus on a specific type of cargo and are each built accordingly in a slightly different way," explains Martin Beck.

A nuclear pore is made up of 35 different proteins. Altogether, this adds up to around a thousand molecules. Martin Beck and his team have spent many years investigating how the individual molecules need to be arranged to form a functional pore. Since the pores are so tiny that light-based microscopes cannot make them visible in sufficient detail, the researchers examine them with an electron microscope. First, however, they must essentially freeze the pores in place – only then will the ubiquitous water molecules not have time to form ice crystals that would destroy the structure of the pores. Additionally, the molecules remain still in their frozen state for imaging.

Blurred images

The researchers then take electron microscope images of the prepared samples from various angles. Next, they combine the images to create three-dimensional models of the pores. "Despite all our efforts, for a long time our images were not sharp enough for us to identify the individual proteins. That's why it was so difficult to figure out how the individual components come together to form the shape we see in the images."

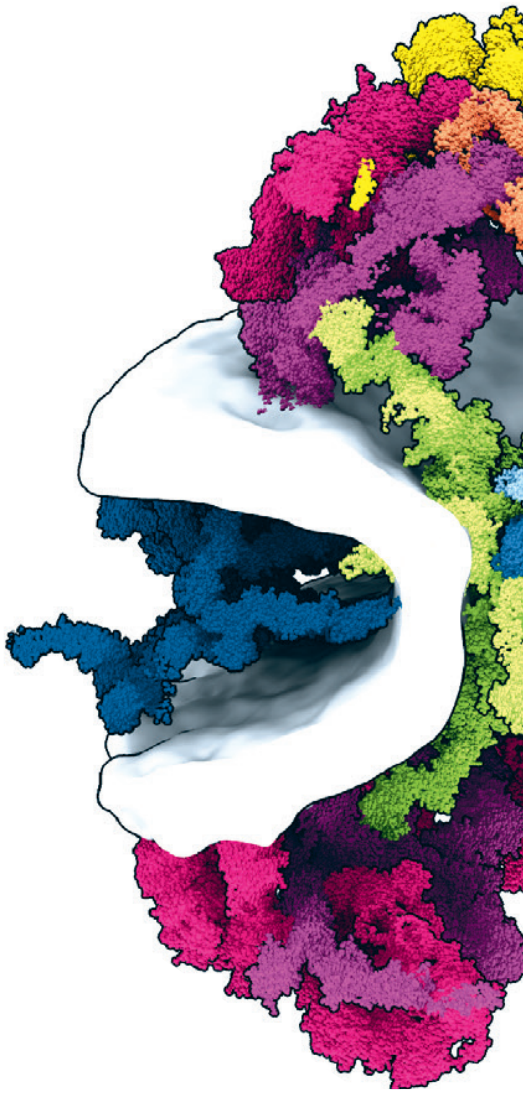
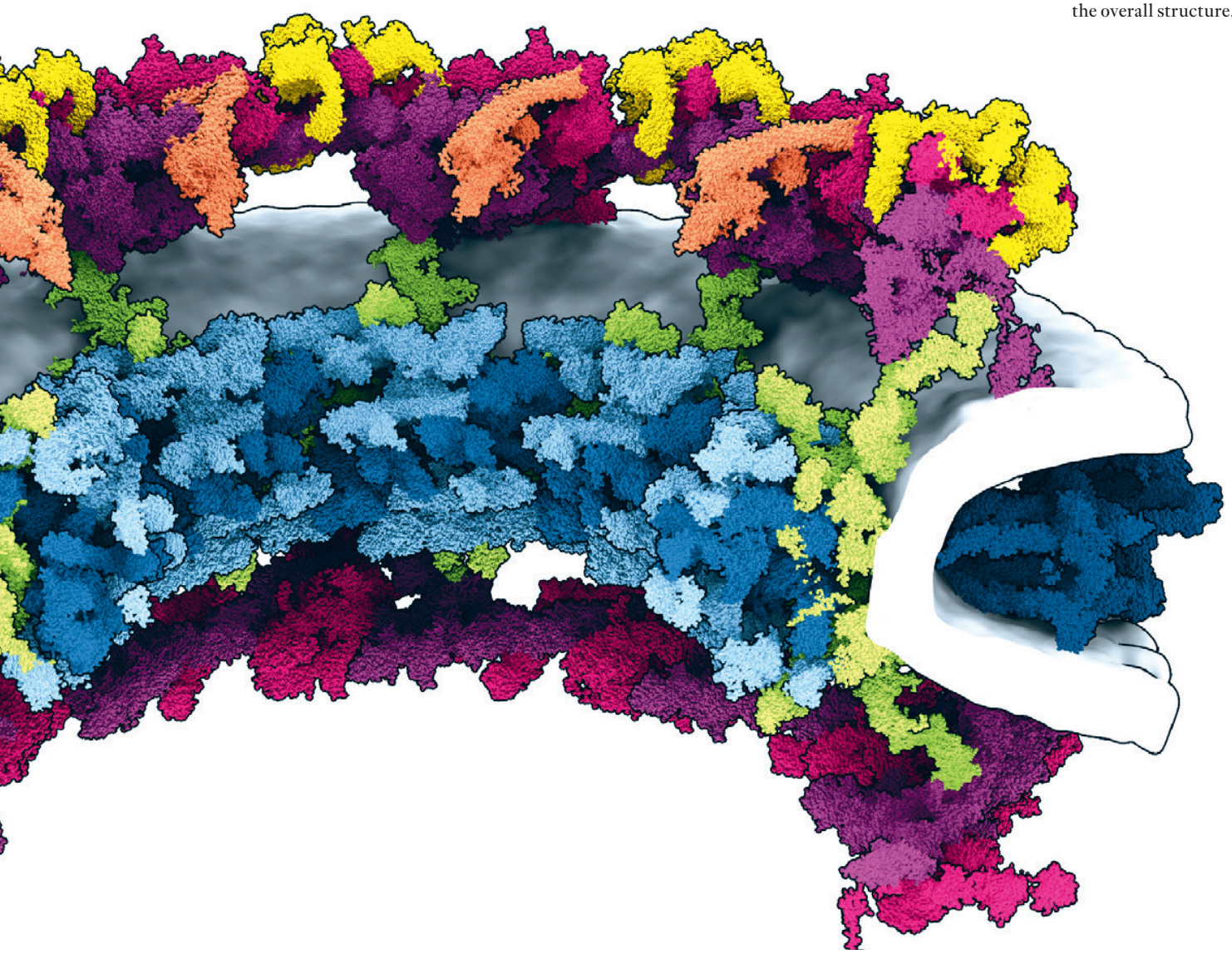


IMAGE: FROM SHYAMAL MOSALAGANTI ET AL., AI-BASED STRUCTURE PREDICTION EMPOWERS INTEGRATIVE STRUCTURAL ANALYSIS OF HUMAN NUCLEAR PORES. SCIENCE 376, EABM9506 (2022).

"No animal, no plant, no fungus can survive without nuclear pores. And as a defense mechanism against pathogens, they are extremely important."

MARTIN BECK

A nuclear pore is made up of about a thousand protein molecules. Complexes of several proteins (shown here in color) form different parts of the pore, such as the outer ring (top), the middle ring (center), and the inner ring (bottom). The analysis of the 32 Y-complexes – each made up of ten proteins (red, pink) – was key to deciphering the overall structure.



To appreciate the challenge of the task Martin Beck and his colleagues took on, it's important to know that the pore proteins have distinct and varied shapes. For instance, they can be small or large, have protrusions or indentations, and feature either smooth or wrinkled surfaces. Electrical charges and other chemical properties cause proteins to either attract or repel one another. Thus, a protein 3D puzzle with a thousand pieces results in an almost overwhelming number of possible solutions – even when the final shape is known. "At one point in my life, I thought about nothing else – and it nearly drove my wife crazy," recalls Martin Beck, laughing. "Sketches were scattered all over our house that I used to help me imagine how the pieces might fit together."

The breakthrough for the researcher and his team came from a specific structure within the human nuclear pore: the Y-complex. "We knew there was a Y-shaped structure in the nu- ⟶

clear pores of yeast cells. After a series of very elaborate experiments, we were eventually able to detect the same shape in human nuclear pores," recalls Martin Beck. For the researchers, this was an absolute stroke of luck, as the Y-complex is made up of ten proteins in humans, making it relatively large. In addition, its characteristic shape makes it easy to identify in electron microscope images. Moreover, there are 32 such Y-complexes in each human nuclear pore. With the Y-complexes, the researchers were able to immediately pinpoint the location of 320 proteins, thus revealing the structure of one-third of the pore. "Using an algorithm, we tested all possible orientations, angles, and positions for the Y-complexes and calculated how well each matched our images," reports Martin Beck. In the end, it turned out the 32 Y-complexes form two rings together – the basic structure of the tunnel that passes through the nuclear membrane. "Once we determined the position of the Y-complexes, everything became easier. Today, we know the exact positions of most of the protein components in the nuclear pore."

Simple structure

Looking more closely at the puzzle's solution, one thing stands out: the structure of the human nuclear pore is surprisingly simple! It's a startling conclusion after all the painstaking effort that went into solving the puzzle. "If I were to rebuild it, I'd say: let's design three proteins. One holds the membrane open (the tunnel entrance), one forms the ring (the tunnel wall), and one makes the tentacles. Done." Beck shrugs. "Why it takes a thousand proteins to create such a straightforward structure – that's a question I've been wrestling with for years and still can't answer." At least it's now confirmed that the tentacles inside the nuclear pore aren't identical – they have different characteristics. Some speculate this could play a role in transporting different types of cargo. "It's also possible that there are many more variations and that nuclear pores vary much more between cell types than we've realized so far. To achieve such diversity, all those different components might just be necessary."

SUMMARY

Nuclear pores are not rigid tunnels in the nuclear membrane; they are able to contract and expand. Presumably, they balance tensile forces on the nuclear membrane and pressure differences between the nucleus and the cell when external forces deform the cell.

There are many variations of the nuclear pore complex. They may be the outcome of an evolutionary race in which the pores repeatedly had to change to remain impenetrable to viruses and bacteria.

It's still a mystery why a simple membrane channel like the nuclear pore complex is made up of around a thousand protein molecules. One explanation could be that this gives the nuclear pore greater flexibility, allowing it to adapt more quickly.

Basic structure or not – recent research shows that nuclear pores are far more than just passive channels. Martin Beck's working group recently demonstrated that nuclear pores can contract and expand. "This is likely a response to mechanical stress transmitted through the nuclear membrane to the pore proteins. Imagine, for instance, a macrophage – a relatively large immune cell – that has to squeeze into a blood capillary. The pressure on the cell membrane compresses the nucleus. To prevent damage to the nucleus or rupturing of the nuclear membrane, the pressure must be balanced, and nuclear pores might act as pressure valves." It's also conceivable that these tiny tunnels behave like springs: when the tension subsides, the "springs" contract again. With financial support from the European Research Council (ERC), researchers in Frankfurt are currently investigating whether this hypothesis holds true. They also aim to precisely determine how nuclear pores manage to change their diameter and whether expanded pores transport different types of cargo from narrower ones.

Viruses in the nucleus

Together with Martin Beck's colleagues Gerhard Hummer and his team, alongside Hans-Georg Kräusslich's group at Heidelberg University, researchers have discovered how HIV inserts its genetic material into the nuclei of immune cells. The virus consists of an RNA molecule encased in a conical protein shell, which enters the cell. Once inside, it makes its way to the nucleus. "For a long time, it wasn't clear how the capsid docks onto the pore and passes through it. This was partly because researchers needed to observe the right place at just the right moment: the virus only infects a subset of cells. Moreover, each nucleus contains thousands of pores the virus could use to enter." After countless attempts, they finally got a snapshot at the critical moment. "The capsid is actually a little too large for the channel. But in our images, we could see that it still manages to squeeze through the tunnel intact." But why can't the pore's tentacles fend off this deadly invader? Dirk Görlich's team discovered that the capsid's surface properties are to blame. They mimic the properties of importins and are therefore not repelled by the pores. Additionally, the capsid enters with its narrow end first. It wedges into the pore like a spike, destroying the rings as it goes.

"No animal, no plant, no fungus can survive without nuclear pores. And as a defense mechanism against pathogens, they are extremely important," emphasizes Martin Beck. "There are plenty of reasons why we ought to have a better understanding of the pores of the cell nucleus." Changes to nuclear pores could, for example, be a cause of increased susceptibility to illness in old age: the pores become more permeable over time and allow more unauthorized substances access to the "Holy of Holies" of the cell. Recent research findings also suggest that the structure and behavior of these channels seem to be altered in cancer. "Insights from basic research could therefore help in developing new cancer therapies."

Martin Beck is confident that the coming years will bring numerous new insights into nuclear pores – not least thanks to increasingly powerful computers, artificial intelligence, and improved sample preparation techniques. "Today, we can observe how HIV enters a nuclear pore – something that would have been completely impossible 15 years ago. At the same time, what we know today is just the tip of the iceberg. The universe still to be discovered within cells remains largely unexplored." ⟵

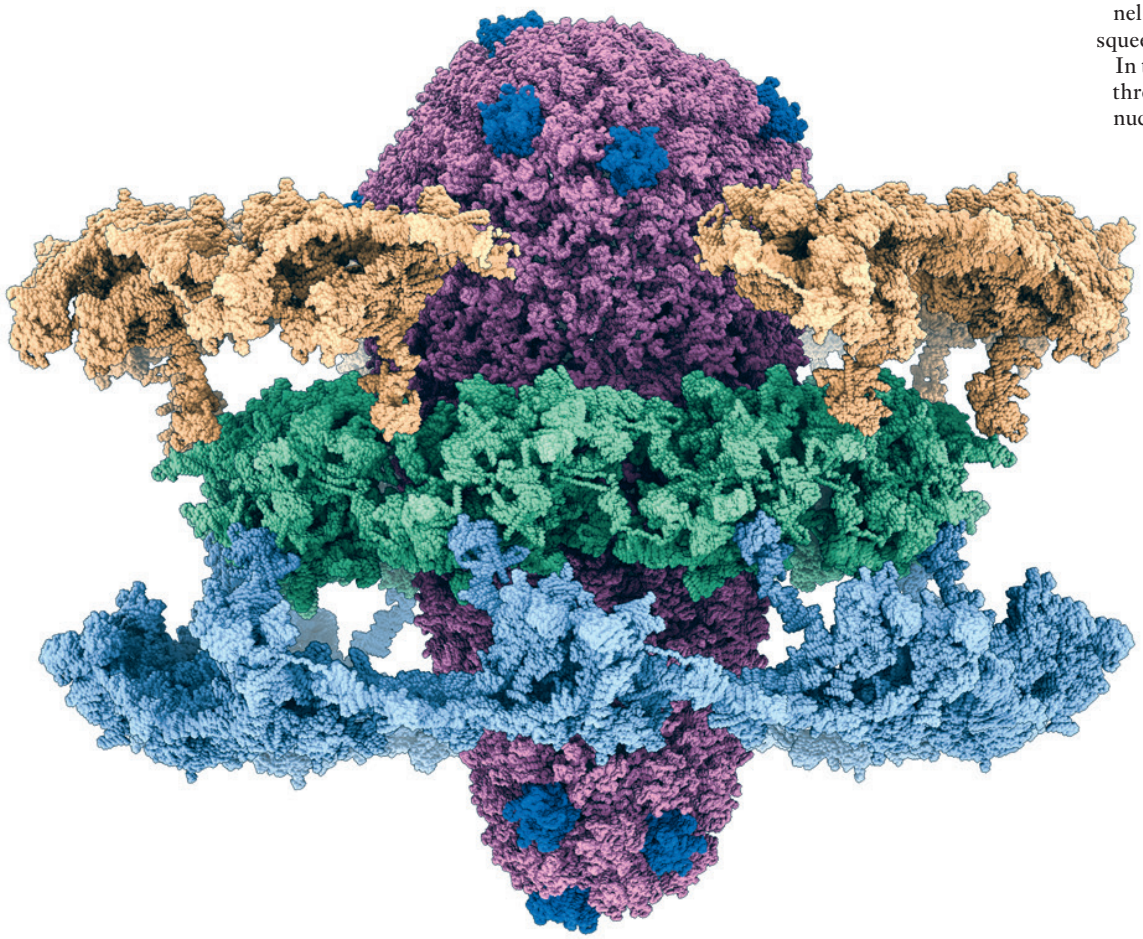


IMAGE: JAN PHILIPP KREYSING / MPI OF BIOPHYSICS

For the first time, researchers have succeeded in capturing an image of an HIV entering the nucleus of a cell. Since the virus capsule is slightly larger than the pore channel, the pathogen has to squeeze through the pore. In the process, it bursts through the rings of the nuclear pore one by one.