the CRICK

HELLO FROM THE DARK GENOME

The genetic ghosts that haunt and help us

Africa:
A new lens on health

Justice by genomics:
The typos turning loss into hope

Drugging the undruggable

Protein origami and the cell's folding masters

Issue 01 Autumn 25

04 AN ‘ACCIDENTAL GENOMICIST’

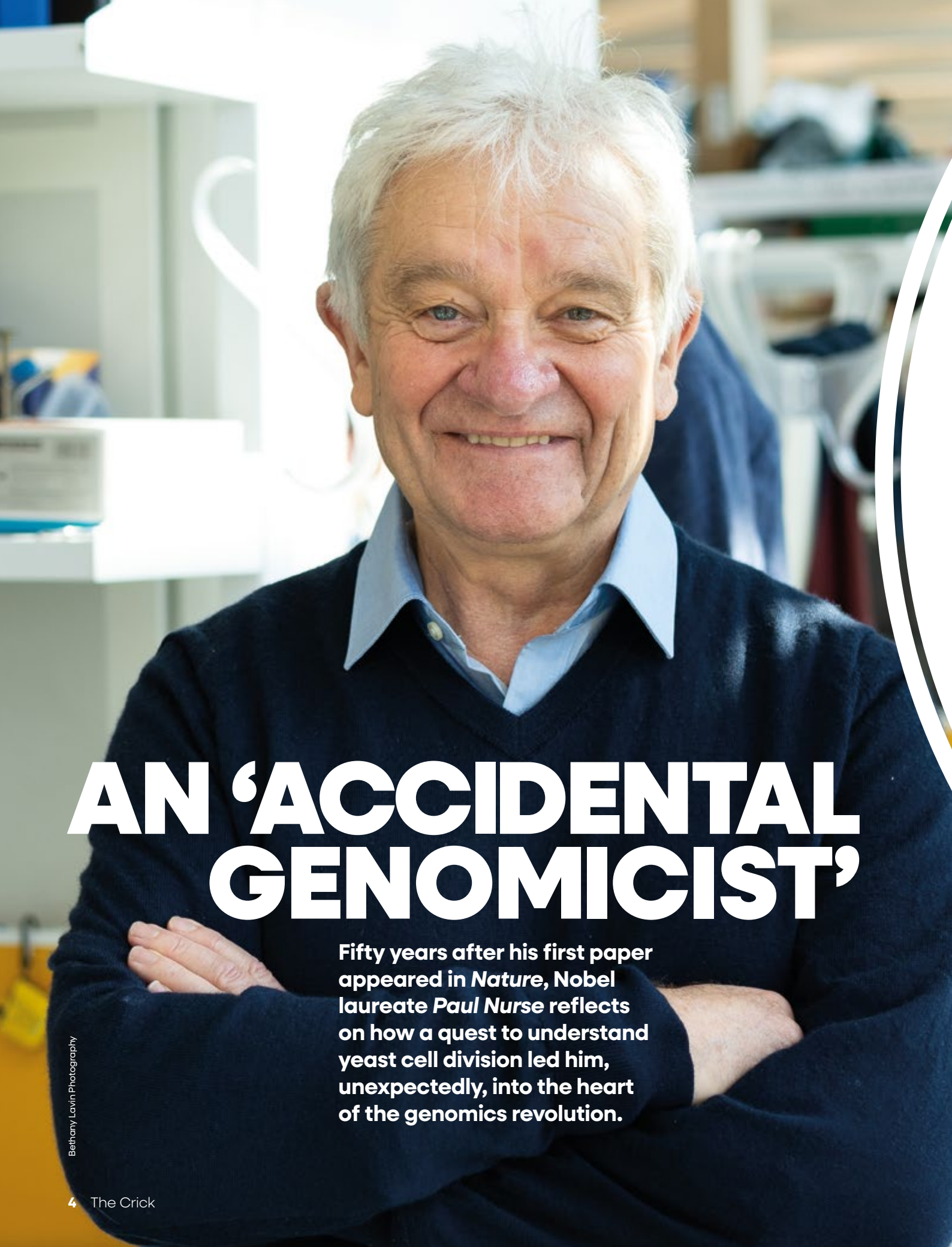
Paul Nurse reflects on the impact of genomics on his Nobel Prize-winning work on the mechanisms of the cell cycle.

22 EDITH HEARD

The Crick’s new director shares 10 lessons from a life in science, including her work on epigenetics and the X chromosome.

Since the human genome was first sequenced in the early 2000s, our understanding of its secrets has deepened at remarkable speed.

Yet even as genomic medicine enters the mainstream, and our tools grow ever more sophisticated, the genome continues to guard many of its mysteries.



AN ‘ACCIDENTAL GENOMICIST’

Fifty years after his first paper appeared in *Nature*, Nobel laureate *Paul Nurse* reflects on how a quest to understand yeast cell division led him, unexpectedly, into the heart of the genomics revolution.

Bethany Lavin Photography

Over the years I have realised that I am a sort of 'accidental genomicist'. Accidental because I never set out to do genomics research. I was just a geneticist trying to understand how yeast cells control their reproduction through a process called the cell cycle, but that turned out to require work which also contributed to genomics.

Genomics approaches emerged from molecular genetics and the emphasis that work put on DNA. The molecular genetic revolution, embracing techniques such as the isolation and amplification of specific genes (cloning) and the manipulation of the DNA molecule in cells (gene editing), occurred a few years after I started research. So my scientific career was quickly involved in developing molecular genetics for fission yeast, and subsequently that led to genomics. The reason for this was that early work focussed on single genes important for cell cycle control, but these were only part of a wider network of genes and that required genomic approaches to identify them and find out how they worked.

Mapping life's machinery

It all began for my lab around 1980. For the previous seven years I had used classical genetics – which studies individual genes and the effects they have on living organisms – to identify around 30 *cdc* (cell division cycle) genes which were required for the cell cycle of the single celled micro-organism fission yeast. I had chosen yeast to work with because it is ideal for classical genetics, great for isolating mutants, and a eukaryote, so basically not very different from mammalian cells. This work allowed us to describe the underlying logic of how all these genes interacted to control the cell cycle, but we had no way of working out what they actually did within the cell. That all changed with the new methodologies of gene cloning, combined with yeast transformation (the ability to introduce exogenous genetic material), and precise gene manipulation (gene editing). I developed these methods for fission yeast in partnership with my colleague David Beach at the University of Sussex, which allowed us to clone the genes and sequence their DNA. This led us to discover the *cdc2* gene (which encodes a protein now known as CDK; cyclin dependent kinase), as well as other cell cycle controlling genes. In 1984 I moved to the Imperial Cancer Research Fund labs in London's Lincoln's Inn Fields – one of the Crick's precursor institutes – where we used our ability to transform and manipulate genes to transfer them between organisms, allowing them to be studied in different genomes. This allowed us to show that functionally equivalent genes to *cdc2* were present in a second form of yeast – budding yeast – and also, crucially, in human cells (work done by one of my postdocs, Melanie Lee). These approaches demonstrated that CDK was a part of a universal cell cycle control mechanism operative in all eukaryotes, and they also set the stage for us to start work on fission yeast's genomics.

> **"In the 1990s, sequencing was a major task, quite different to today."**

Sequencing the whole fission yeast genome seemed to me the best way to identify all the *cdc*-like genes and get a molecular understanding of how the cell cycle worked. In the 1990s, sequencing was a major task, quite different to today. It required a grant specific for that purpose, but when I applied for these resources from UK funders, they said fission yeast was not part of their overall genomic sequencing strategy. To get round this block I visited my friends Bart Barrell and Fred Sanger at the Sanger Institute near Cambridge. Bart was a very experienced DNA sequencer who had worked with Fred, a double Nobelist and pioneer of early sequencing methods. Bart had a Wellcome Trust grant to sequence budding yeast which was not being fully utilised, so, with Fred's encouragement, we used part of that grant money to start sequencing the fission yeast genome. Progress was quite fast, as the fission yeast genome is small, and within months about half of the task had been completed. I applied to the Wellcome Trust for support to finish the sequence with Bart, but they got cross with us for sequencing fission yeast without their permission and would not allow a grant application. The ever-resourceful Bart suggested we apply to the EU and finish the sequence by co-ordinating about 10 labs in continental Europe. This 'cottage industry' approach took much longer, but in the end we finished the fission yeast genome.

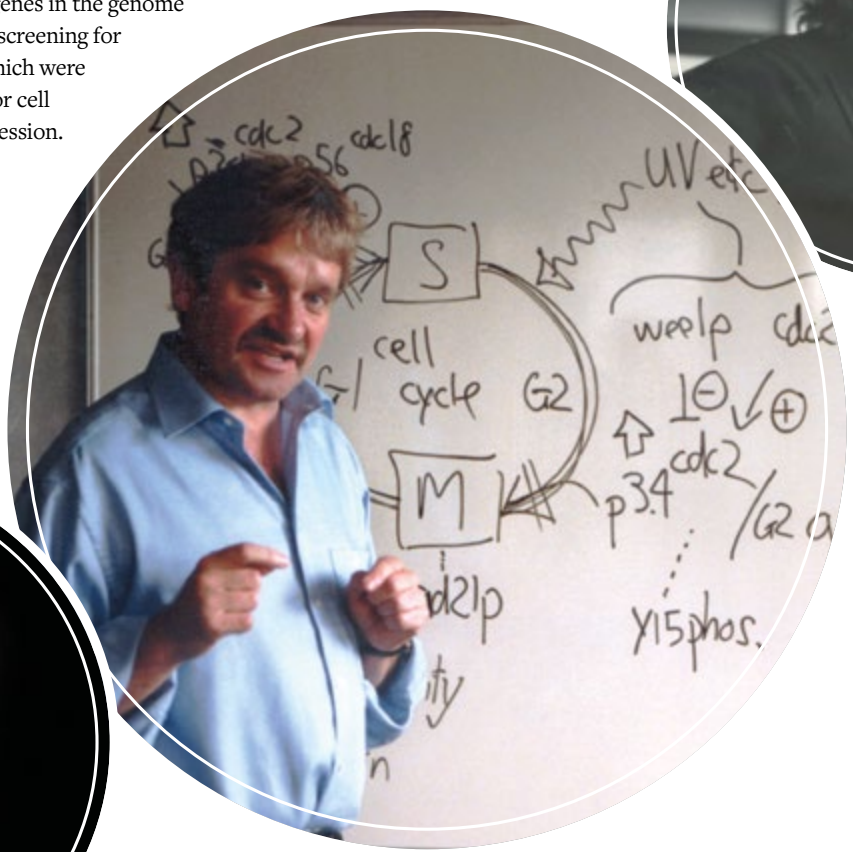
It was only the third eukaryotic genome to be fully sequenced at high accuracy and consisted of about 5,000 genes. The sequence revealed a number of interesting things. My favourite one was a comparison of the three eukaryotic genomes that had been sequenced, budding yeast, a nematode worm and fission yeast, with those from simpler (prokaryotic) bacterial genomes, that had already been sequenced. These comparisons revealed which genes were obviously specific to eukaryotes. Satisfyingly, this short gene list included *cdc2*!

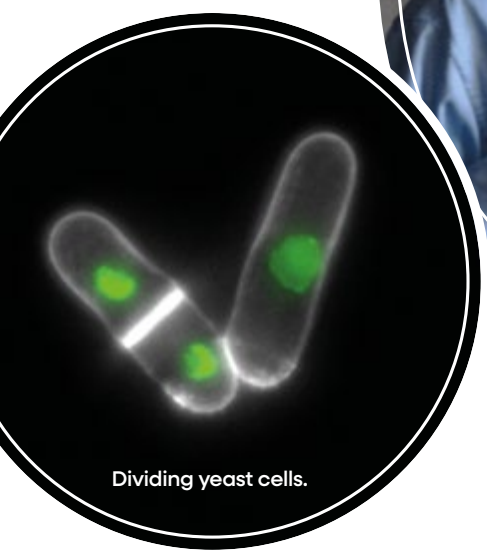
Cracking the cycle

What I really wanted the genome sequence for was to delete all the genes one by one, and so identify all the cell cycle genes required to reproduce a eukaryotic cell. Such a complete gene deletion collection would also be a valuable resource for investigating many other cellular activities. However, once again this was blocked by funders, this time because fission yeast was not part of the overall genomic gene deletion strategy. No UK or US research funder would support the work, so this time my lab set up a consortium with labs in South Korea funded by a Korean Biotech company. Genes were deleted in the South Korean labs and these strains were then shipped to my lab where Jacky Hayles (my first graduate student, who has only recently retired from the Crick) identified all the *cdc* genes in the genome by visually screening for mutants which were defective for cell cycle progression.

This was a major task, but was successful in identifying around 300 essential cell cycle genes and another 200 which influenced the cell cycle but were not essential. This collection also formed a valuable resource for many other cell and molecular biology investigations, and is still the most comprehensive genetic resource for studying the eukaryotic cell cycle and its control. Mission accomplished, but it had taken around 20 years, much of it in the ICRF and Cancer Research UK London Research Institute and subsequently at the Crick. It was very much a programme that embraced genomics although obviously was never a part of the strategic genomics endeavours. However, it has been very useful for understanding control of the eukaryotic cell cycle. The work on cell cycle control in the budding and fission yeasts laid the groundwork for understanding how cells in more complex organisms such as ourselves control their cell cycle. These insights continue to underpin biomedical advances demonstrating how simple modern organisms can help unlock universal biological principles. ■

Paul Nurse





Dividing yeast cells.

PROTEIN ORIGAMI AND THE CELL'S FOLDING MASTERS

Tomas Voisin

> **"There's so much we still need to learn about how chaperones help proteins fold."**

Chaperones are the molecular guides that help proteins fold correctly, and their choreography is central to the dance between health and disease.

Just as a misshapen key can't open a lock, the proteins in our cells need to have the correct 3D shape to function properly. And defects in a protein's structure can cause diseases.

Proteins are initially stitched together inside cells by large molecular machines called ribosomes, which 'grow' the protein by adding a sequence of small amino acid building blocks one after the other. This creates linear chains, which fold, origami-like, into the correct 3D structure.

However, while origami involves folding a fully made sheet of paper, a protein chain starts to fold while it's still being stitched together. This poses challenges, as regions of a protein that need to end up in close proximity in the final structure aren't always synthesised at the same time.

One way evolution has tried to solve this challenge is through chaperones – specific proteins that help other proteins fold correctly, including while they're being made. But despite chaperones' importance in determining a protein's structure and function, researchers still don't fully understand how they work.

"There's so much we still need to learn about how chaperones help proteins fold," says David Balchin, who leads the Crick's Protein Biogenesis lab. "A particular mystery my lab has been focusing on is the series of events that occurs as the nascent protein chain starts to emerge from the ribosome itself, and how chaperones might stop it getting tangled and misfolded".

Folding choreography

Recently, researchers in David's lab have used a range of techniques to map out in exquisite detail, how particular chaperones coordinate their action during protein synthesis in bacteria.

They discovered that, as a new protein chain emerges from a ribosome and reaches about 100 amino acids in length, it's grabbed by a chaperone called Trigger factor.

As the protein continues to emerge, Trigger factor performs some clever choreography, attaching itself to regions that have yet to finish folding, and detaching from those that are nearly fully folded. Through this dance of binding and unbinding, Trigger factor shields partially folded regions from the rest of the cellular environment, which may help prevent mistakes forming.

David's team has also started to reveal how this shielding effect helps coordinate other chaperones. When Trigger factor first binds to an emerging chain, it prevents other chaperones from binding. But as the chain grows, some regions escape from Trigger factor and new chaperones can then engage in the folding choreography.

"We're carefully starting to dissect the series of events that allow chaperones to coordinate protein folding as a chain emerges from a ribosome, revealing how features of proteins that are being created in real time dictate the complex and dynamic coordination of chaperones," says David.

Understanding how living organisms have evolved mechanisms to enable protein folding during synthesis is important to gain insight into how the molecular machinery of life works correctly. And it could, one day, identify ways to manipulate proteins to develop treatments for different diseases. ■

BRIGHT SPARKS IN CANCER RESEARCH

Beth Askham

An unconventional idea is revealing a previously hidden layer of tumour complexity. Leanne Li's lab is merging cancer biology with neuroscience, with electrifying results.

"I always wanted to do something outside the box, but people didn't believe that my ideas would work," says Leanne Li, leader of the Crick's Cancer-Neuroscience Laboratory.

That was five years ago, before her interview to join the Crick. At that time, Leanne had spent months preparing for the interview. She had carefully planned her answers and tailored her research proposal to match what was considered the safest route to success – working on fashionable topics in cancer research.

On the morning of the interview, however, during a brief meeting with the Crick's director, Paul Nurse, which was meant to discuss her career at the Crick, she unexpectedly found herself confiding to Paul her long-lasting desire to work on science that truly fascinated her – rather than jumping on the 'hot topics' bandwagon.

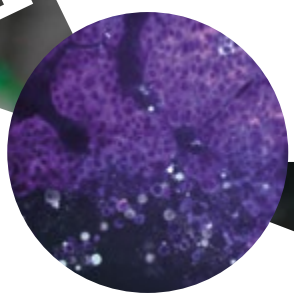
Later that day, just five minutes before the interview, Paul appeared with a big smile, and said, "There's a change of plan – I want you to talk about only one thing that really fascinates you, research where you truly believe in the science."

"I was completely shocked, because usually we pitch three different research projects," remembered Leanne, "but none of those projects I'd prepared actually fit with Paul's criteria."

"My mind went blank, and I thought, what should I do now? Within those 30 seconds I had to decide, should I go for the well-prepared, 'safe' projects, or should I go for my crazy idea."

Leanne's 'crazy idea' was about a relatively uncharted area of science: the interaction between cancer and the nervous system, and whether that could one day be harnessed to treat the disease.

"It was an unprepared project. But it was the scientific dream that has inspired me for the past decade."



Slice of lung cancer tissue (purple) under the microscope showing that some cancer cells have ongoing electrical activity (seen in white).

"And I thought, OK, where else but the Crick could I do what I really want to do in science?" says Leanne.

She went for it, got the job, and today, that 'crazy' idea has now become a hot topic in cancer research.

Where cancer meets the nervous system

The path that Leanne took to studying the cancer-nervous tissue interaction began in Switzerland around 15 years ago, when she did her PhD with renowned cancer biologist Douglas Hanahan and discovered, surprisingly, that the ability of pancreatic cancer cells to spread relied on a well-known signalling pathway involved in learning and memory, usually active in brain cells.

This led her to start longstanding collaborations with neuroscientists who introduced her to the field of electrophysiology.

So when Leanne started at the Crick, she took advantage of her past training, combining cancer biology and neuroscience to investigate how tumours communicate with the rest of our body.

Electrifying cancer research

"At the Crick, I'm supported and encouraged to do bold and creative research," says Leanne. "I spend quite a lot of time with neuroscientists, embedded in their labs, discussing topics and learning their techniques. I'm interested in gaining a different perspective of cancer through their research lens."

Recently, members of Leanne's lab, postdocs Paola Peinado, Marco Stazi and Claudio Ballabio, showed for the very first time the direct, causal evidence between electrical activity and cancer progression. They found that certain lung cancer cells can generate their own electrical activity and build their own electrical network within a tumour. This network appears to exist independently of the body's main electrical network, including the nerves surrounding the tumour.

This work has focused on one of the most aggressive types of lung cancer called 'small cell' lung cancer, a form of the disease in which tumours are often made up of two distinct subtypes of cancer cells, neuroendocrine cells – which have neuron-like features – and non-neuroendocrine cells that play a supporting role. It's mainly the activity of the neuroendocrine cells that drives the tumour's ability to grow and spread.

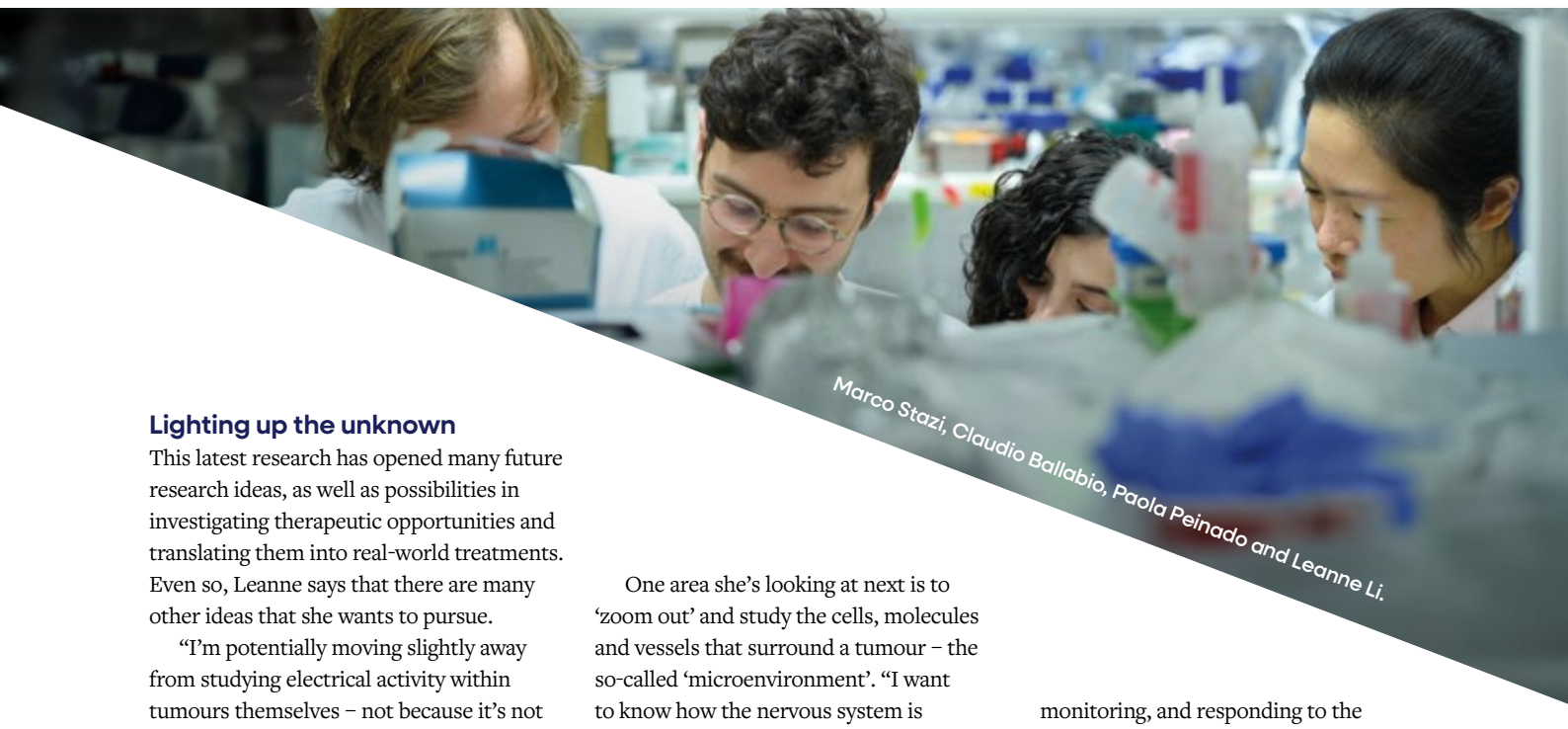
Leanne's lab noticed these two cell types seemed to have a similar relationship mirroring that of two types of cells in the brain – neurons and astroglia. Neurons are the main cell type generating electrical signals, while astroglia are 'housekeeping' cells that support them.

And just as in the brain, in small cell lung cancer they found that the non-neuroendocrine cells were fuelling their neuroendocrine neighbours with a metabolite called lactate, allowing them to generate electrical signals which, in turn, drive tumour growth.

"We knew that some cancer cells can mimic neural behaviour, but we didn't know how developing an independent electrical network might impact the disease outcome. By combining neuroscience and cancer research techniques, we've been able to look at this disease from a different perspective," says Leanne.

"There's still a long way to go to understand the biological impact of this electrical activity, and the specific disease mechanisms that make the tumour more aggressive and harder to treat. But we hope that in understanding the way these cancer cells are fuelled, we can also expose vulnerabilities that could be targeted with future treatments," she adds.

> **"... where else but the Crick could I do what I really want to do in science?"**



Marco Stazi, Claudio Ballabio, Paola Peinado and Leanne Li.

Lighting up the unknown

This latest research has opened many future research ideas, as well as possibilities in investigating therapeutic opportunities and translating them into real-world treatments. Even so, Leanne says that there are many other ideas that she wants to pursue.

"I'm potentially moving slightly away from studying electrical activity within tumours themselves – not because it's not interesting, but because what really excites me is the unknown. So now we've proved that electrical activity can directly drive cancer progression, what's the next big mystery in the field?" she says.

One area she's looking at next is to 'zoom out' and study the cells, molecules and vessels that surround a tumour – the so-called 'microenvironment'. "I want to know how the nervous system is impacting the whole construction of the microenvironment, how that is impacting therapeutic resistance," says Leanne. "And then ultimately, we want to look at the brain itself. How is the brain sensing, monitoring, and responding to the tumour formation? Could we one day treat cancers by treating the brain?

"There are just so many other things that are unknown and I want to go for those." ■

A MESSAGE FROM THE DARK GENOME

THE GENETIC GHOSTS THAT HAUNT AND HELP US

Roger Highfield

Long dismissed as genetic junk, the dark genome is stepping into the spotlight, revealing how ancient viral remnants and rogue DNA elements impact evolution and disease.

Within our genetic code lurk ghosts of ancient parasites, viral fragments and rogue elements that plagued our ancestors long ago. These DNA revenants stir in the shadows of a vast and mysterious realm known as the dark genome – a genetic underworld that is only now yielding its secrets.

The light genome, first illuminated a quarter of a century ago, comprises the 20,000 or so genes that build our cells – the known, the named, and the charted. The remaining 98% was initially dismissed as junk, yet within this shadowed script lie powerful fragments. Scientists investigating this dark domain are finding that, among the shattered genes and decayed DNA, there are viral fossils, inactive jumping genes and other shadowy elements that can shape our biology.

While the light side accounts for just 2% of our DNA, 5% of the dark genome is made up of endogenous retroviruses, which are the remnants of ancient infections. Remarkably, this percentage rises to almost half of our DNA if you include other virus-like elements. Some of these parasites still whisper instructions from the shadows, offering answers to many riddles of evolution, development, and disease.

As I discovered during a tour of the Crick, the story of the dark genome shadows many key developments in genetics. More than seven decades ago, its extraordinary influence on evolution was glimpsed by a pioneering scientist, though was initially ignored by the establishment. Over the years, various Nobel Prizes have recognised discoveries that either directly or indirectly relate to the dark genome, which has been implicated in autoimmune disorders, neurological diseases and more besides.

Though many of the ghosts in the dark genome have been tamed during our evolution, not all rest peacefully. The role of these crepuscular elements in cancer is being studied by teams led by George Kassiotis and Samra Turajlic at the Crick, supported by the labs of Charlie Swanton and Julian Downward. With new insights and a battery of novel techniques, they hope to harness the dark genome to change the future of cancer medicine.

Dave Guttridge

The genome's hidden allure

"Your genome has more viral hitchhikers than it does genes," says George Kassiotis. We are sitting in his office, which gives little away about his life or science, amplifying the sense of mystery. With its off-white walls and mottled dark grey carpet, the furnishings are stark, include ranks of empty box files (so the room is not so echoey) along with a computer. But George says he has everything he needs to explore the dark genome.

After he completed his PhD in immunology, George continued his research at the National Institute for Medical Research in Mill Hill, in northwest London, where he eventually set up his own lab to study the immune reaction of mice to a particular retroviral infection. Retroviruses use RNA as their genetic material and they insert a DNA copy of their RNA into the host cell's genome. An infection of this kind stimulates an immune response including from a kind of white blood cell, called a T cell.

Puzzlingly, the T cells of the infected mice George was studying behaved as if they had already encountered the retrovirus they had just been inoculated with. He subsequently discovered that they had mounted a response to proteins that were made by a remnant of another retrovirus that had integrated into the mouse genetic code: he had stumbled across an endogenous retrovirus, or ERV, part of the dark genome. "I was captivated – how do we recognise new retroviral infections, when we have so many endogenous ones, embedded in our DNA?"

George found that in immune-deficient mice unable to produce antibodies, the peaceful co-existence between the host and endogenous virus was disrupted – defective and dormant ERVs were 'resurrected', leading to retroviral infection and spread. "This suggested to us that immune responses to ERVs are not only possible to induce but must also be kept under control," George tells me.

Most ERVs in mice – and humans too – have accumulated mutations and deletions that mean the endogenous viruses have lost the ability to infect. They are also repressed by what are called epigenetic mechanisms, chemical modifications that turn genes on and off in cells. However, the hundreds of thousands of defective copies of ERVs that lurk in our genome have between them the necessary components to build a virus particle, with defective parts in one virus complemented by non-defective elements from another. "Particles made by ERVs are not infectious or even complete, but they may still be perceived as a threat by the immune system," explains George. The immune system is tricked into believing that there is a genuine retroviral infection, so it responds by doing what it has evolved to do: get rid of the 'infected' cells.

Immune responses against ERV particles or components are part of the body's natural defences against cancer. As cells mutate, the escape of ERVs from the restraint of epigenetic controls can act as 'red flags' for the immune system, which, in its attempt to eliminate the newly sensed virus, also destroys the cancer cells.

The dark genome runs up more flags in a cancer than just the ERV proteins. "The dysregulation of the dark matter in the transformed cells in a tumour produces a swathe of aberrant products – the result of mixed-up codes from virus and human – which can also be recognised and targeted as foreign by the immune response," George says.

There are other ways that viral genetic ghosts influence cancer: they can interfere with genes related to immunity or the response of cancer cells. Some tumours hijack these viral sequences, using them to promote the uncontrolled cell growth that is the hallmark of all cancer. "For example, there is a cancer-causing form of the much-studied ALK kinase (a protein that controls cell growth) that is driven by one of these viral sequences in melanoma" says George. "There are many cases like that. In fact, ERVs were first discovered because they were causing cancer in mice and in chickens," he adds.

> **"Your genome has more viral hitchhikers than it does genes."**

To study how they sway the development of tumours, George works with cancer researchers around the world to study the trove of DNA data from cancer cells and how it is turned into the instructions to make proteins and other working molecules (the transcriptome). His collaborations include large-scale projects such as TRACERx (TRAcking Cancer Evolution through therapy/Rx), which tracks detailed changes in a cancer's genetic makeup and behaviour. Using the Crick's computer cluster, which harnesses "sizeable computational power", George's team can interrogate the activity of dark elements in a few days, a job which would take decades on an office PC. From this, he can learn how the dark genome helps a tumour outwit the body's defences, establish a blood supply and spread. "Tumours use this adaptability for their own mini-evolution inside one human body," says George.

Samra Turajlic and George Kassiotis.

Dave Guttridge

The viral legacy in our DNA

Scientists recently put together the most complete sequence of human DNA ever, revealing nearly half of it (about 46%) is made up of virus-like elements: LINEs (Long Interspersed Nuclear Elements) are long repeating messages that make up about 20.7%: shorter SINEs (Short Interspersed Nuclear Elements) 12.7%, LTRs (Long Terminal Repeats) which include endogenous retroviruses (almost 5%) constitute 8.8%; and DNA transposons, jumping genes, are 3.6%. There is also a complex mix of virus-like elements known as SVAs (SINE-VNTR-Alus), only about 0.15% of your DNA.

The rogue elements within

Aside from ERVs, another oft-cited example of how the dark genome can shape evolution was discovered almost a decade before the structure of DNA was even known. That glimpse came in the form of evidence that genomes contained mobile DNA elements, transposons or jumping genes, so named because these parasites can hop from one region of DNA to another, disrupting other genes in the process.

While working in Cold Spring Harbor Laboratory in New York, Barbara McClintock studied maize, where transposons make up 85% of the genome, and first observed them in 1944. She published her findings in 1950, and called them 'controlling elements', acknowledging their important role. "The idea was that they were not only jumping, but they were also affecting the function of the gene near where they planted," says George. "that's actually how McClintock discovered them, because they changed the colour of a maize kernel."

But this first hint of their regulatory role was dismissed by her peers. "Scientists of that time could not easily understand or accept the concept of a 'controlling element'," George says. "She faced criticism, to the point that she had to drop the term 'controlling elements', and rename these mobile genetic elements transposons." McClintock would eventually win the 1983 Nobel prize for physiology or medicine for her discovery.

These movable pieces of genetic material now make up just 3.6% of the human genome but they've left a lasting impact. One example of how DNA transposons have shaped us can be traced back hundreds of millions of years, when a transposon was domesticated in a common forebear of humans and fish to create the ancestor of the modern-day RAG1 and RAG2 genes, crucial components of the recombinase enzyme found in the B and T cells of the immune system. RAG recombinase is essential for immune diversity, generating a varied repertoire of B and T cells that can detect and respond to the multitudes of different microbial invaders we encounter during our lives.

Hidden throughout our DNA are many other traces of ancient genetic parasites that have changed in influence over millions of years. These are remnants of our genetic history.

Rise of the genetic underworld

Long before humans walked the Earth, a war raged in the genomes of ancient life. In plants like maize, mobile genetic elements had mastered the trick of transposition, moving themselves across the DNA within a single cell.

Over generations, these early genetic parasites were reinforced by more insidious invaders. DNA transposons were joined by retrotransposons, stealthy agents that copy themselves into RNA, then, using an enzyme called reverse transcriptase, paste themselves back into a new location in the genetic code, occasionally causing havoc.

Some transposons evolved to be even more ambitious. They hijacked proteins from their hosts, constructing viral envelopes that allowed them to slip between cells, like ghosts gliding from room to room. As they evolved, they developed keys to pick cellular locks – molecular receptors – that let them invade with eerie precision.

> **"They are a force of evolution, a very powerful force."**

Dave Guttridge

Flow cytometry detector array and filter set that measures the emission of fluorescent signals from labelled proteins on or in cells as they pass through the flow cytometer and are interrogated by lasers.

> **“The coolest aspect of the dark genome is that it enabled humans to have a placenta.”**

Among them were the retroviruses, so named because they, like their sister retrotransposons, convert their RNA code into DNA, before inserting into the genome of a host cell to duplicate. When retroviruses infect cells that develop into egg or sperm cells, viral DNA can become a permanent fixture down the generations. It is estimated that around one in 100 human births acquires novel retroelement DNA and, over the eons, an uneasy cooperation has emerged between living things and their genetic phantoms: while cells provide retroelements with the means to multiply, retroelements arguably repay by churning the genetic code to help their hosts evolve.

Today, thanks to the efforts of George and many others, we know more about the function of at least some of the around four million or so remnants of retroelements that are scattered across our DNA. Some researchers argue that the ability of ERVs and retrotransposons to reshuffle genetic material has sped up the evolutionary process, allowing our ancestors to adapt more rapidly to environmental challenges. “They are a force of evolution, a very powerful force,” he says.

Humans are darkly complex

Towards the end of the 20th century, the regulatory role of the dark genome took on greater significance as molecular biologists found that humans seemed bewilderingly simple: water fleas, tomatoes and wheat, among other ‘simpler’ kinds of life, possess many more genes than we do.

It turns out that human complexity relies on how our 20,000 genes are used, for instance through alternative splicing, when parts of the same gene can be spliced together in different ways to create more proteins. In this way a person’s 20,000 genes can, when used in specific patterns, generate up to 400,000 proteins, by some estimates, to build an immune system, organ or whatever. Retrotransposons can become entangled in this process.

“The coolest aspect of the dark genome is that it enabled humans to have a placenta,” says Rachael Thompson, a postdoctoral research assistant in George’s lab.

To create a placenta, mammals in effect stole genes that retroviruses use to bind with and enter a cell, fusing their membranes in the process. In this way, a once-harmful viral invader was captured and turned into a genetic architect. Quite different placental creatures have stolen equivalent genes but from different viruses, “which is pretty insane”, Rachael says. Successive retroviral invasions of the genome of our placental ancestors gave them the opportunity to use a better retroviral envelope than the previous one to make their placentas.

This dark knowledge has shed light on pregnancy complications: a team at the Pasteur Institute found that, during serious infections, proteins induced by signalling molecules called interferons not only block infection but can interfere with the legacy viral machinery that today makes a placenta.

The dark genome also shapes how a baby develops. One ancient piece of viral DNA, called HERV-H, helps with looping, where DNA folds to bring distant parts of the genome together, like a long string bending to connect important sections. This looping helps turn genes on and off at just the right time, making sure the baby’s tissues grow and form correctly.

"An ERV integration near one of the amylase genes, which make enzymes that break down starch, drives amylase secretion in saliva, so bread tastes sweet," says George "They've taken away our tail, due to a mutation of the responsible gene by a transposable element. They've changed our skin colour, too, by affecting the production of the pigment melanin. And they've provided us with antiviral factors that helped fight off viral epidemics."

Other facets of the dark genome seem to play a role in neurodevelopment, potentially influencing conditions such as schizophrenia, addiction and autism. One theory suggests that viral sequences helped drive the evolution of complex brain functions, providing genetic material that enabled rapid advancements in cognition, memory and social behaviour; in this way, the dark genome may have helped to make us human.

Shedding light on kidney cancer

While George Kassiotis explores viral ghosts and immune sentinels within the dark genome, I visit his Crick colleague Samra Turajlic, who is studying renal cell carcinoma, the main kind of kidney cancer. "My interest in the dark genome came about because of kidney cancer, which is a fascinating disease for many reasons," says Samra, who is also a consultant medical oncologist. "Intriguingly, one aspect that has really come into focus over the past decade is the paradox of why immunotherapy works in kidney cancer."

The immunotherapy in question inhibits immune checkpoint proteins – molecules that evolved to prevent excessive immune responses to pathogens but are frequently co-opted by cancer cells, which may appear 'foreign' to the immune system, to protect themselves from immune attack. Checkpoint inhibitor drugs take the brakes off the immune system and have been successfully used to treat melanoma, a form of skin cancer caused when the ultraviolet in sunlight leaves a trail of mutant proteins ('neoantigens') that the immune system can spot like foreign flags. But kidney cancer harbours far fewer such mutations, so should not be easily targeted by immunotherapy. That mystery led Samra to suspect the answer lay in the genome's shadowy vaults.

She began to suspect that immunotherapy worked because it wasn't targeting conventional mutations at all but proteins linked to the dark genome. To test this idea, she approached George for help.

Samra focused on the cellular machinery that monitors whether cells have enough oxygen to thrive, which is disrupted by cancer. Normally, when there's low oxygen, cells turn on emergency signals.

> **"They've taken away our tail ... and they've provided us with antiviral factors that helped fight off viral epidemics."**

Thanks to work by the 2019 Nobelists Bill Kaelin, Peter Ratcliffe (another Crick colleague), and Gregg Semenza, and others, it's clear that a mix-up happens where cancer cells think they have no oxygen – even when they do. This 'false low-oxygen alarm' (called pseudo-hypoxia) flips on the dark genome, activates blood vessel growth and more, and helps cancer cells to multiply and spread.

Using data from the TRACERx study, Samra's team found that an important milestone foreshadowing the evolution of kidney cancer is damage during adolescence to a key part of a cell's oxygen sensor. Called the VHL gene, it is the recipe for a protein that helps turn off the hypoxia response.

What does it mean to be human?

Anyone who thinks humans are unique might be troubled that nearly half of our DNA comes from ancient retroviruses and retrotransposons. But our bodies depend on other 'invaders'. The mitochondria 'batteries' that power our cells are thought to have originated when a distant ancestral cell engulfed a bacterium. Moreover, while the body contains roughly 30 trillion human cells, we also contain a similar number of bacterial cells, along with other microbial residents, notably fungi and, of course, viruses.

Dave Guttridge

Work in the Retroviral Immunology Laboratory and Flo Cytometry Facility.

Fortunately, all normal cells have two copies of the VHL gene, but the second copy can be damaged, usually many decades after the first VHL gene copy is lost – and this induces pseudo-hypoxia. Samra, along with Bill Kaelin, discovered that pseudo-hypoxia triggers the manufacture of certain endogenous retroviral elements, which are pervasive in kidney cancer. Another reason that upregulation of part of the dark genome occurs is that cancer affects how the DNA in cells is packaged in a form called chromatin, which organises the DNA 'recipe book'. But in cancer the chromatin is loose, so it is easier to flip to a page, allowing more of the genome, both light and dark, to be read.

"The biggest change in the transcription of endogenous retroelements happens very early on in cancer evolution and then remains quite steady," says Samra. To pick out the dark genome proteins presented to the immune system, which she likens to hunting a needle in a haystack, Samra uses immunopeptidomics, in which mass spectrometry, which 'weighs' molecules to identify them, is used to study immune-peptide complexes, the molecules "shown" by a tumour cell to mark it out for clearance by the immune system. "We are going to have to understand which ones are good for the immune system and which ones are good for the tumour," she says.

The implications of solving this 'big puzzle' go much wider than kidney cancer. "If we can unlock this kidney cancer conundrum, we can unlock it for other types of cancer as well," says Samra. That is why the Cancer Grand Challenge programme, run jointly by the US government's National Institutes of Health and Cancer Research UK, has announced that it intends to funnel £20m into 'dark proteome' research.

To complement her detailed work on kidney cancer, Samra is leading a four-year study of thousands of patients, called MANIFEST, to understand immunotherapy responses and its side effects more generally, funded by the Medical Research Council, Office for Life Sciences, and industry. "We are conducting a deep study into many elements of patients' coding genome, the dark genome, immune system, the blood, the gut microbiome and the tumour environment," says Samra. "It's a kitchen sink approach because these interactions hold the key – we are taking the dark genome and contextualising it."

Architects of discovery

In the southeast corner of the fourth floor of the Crick, I visit some of the 20-plus members of Samra's cancer dynamics team. Most are busy working at ranks of desks with computers, though a few are in neighbouring laboratory spaces, where their studies focus on both kidney cancer and melanoma.

One of them, Taja Barber, one of the Crick's laboratory research scientists, tells me how she has to use barcodes, computers and more to keep track of the many samples from cancer patients that flow through Samra's laboratory, from storage in liquid nitrogen to analysis. Scattered around her is the usual molecular biology lab paraphernalia: centrifuges, Rainin pipettes (ergonomic, to prevent repetitive strain industry, says Taja), PCR (to multiply DNA) and so on. These can be found in greyish boxes near biosafety cabinets to handle and grow cells, both animal and human, in phials suspended in reddish nutrient media.

Taja shows me one impressive box of tricks that is used to shed light on the dark genome: the PhenoCycler, a concoction of white and clear blue plastic that contains a powerful microscope to highlight cellular details inside tissue, or a tumour, not just the cancer itself but the immune cells that invade it. Running the PhenoCycler takes two technicians, Steph Hepworth and Anne-Laure Cattin. They add glowing tags (made of a fluorescent molecule linked to an antibody that can target a particular molecule) to different parts of the sample, take pictures, then erase the tags and repeat the process.

Two floors below, in the northwest corner of the Crick, George shows me millions of pounds worth of instruments, called flow analysers, which are also expertly tended by technicians. These powerful instruments can study thousands of cells per second, identifying the parts of the immune system that dark elements activate using antibodies honed to dock with specific molecules, each carrying a fluorescent tag that glows under lasers of different wavelengths.

> **"If we can unlock this kidney cancer conundrum, we can unlock it for other types of cancer as well."**

In one room, a confection of black tubes, silver drums and yellow laser warnings make up an analyser that can not only detect labelled cells but divert a single cell into a well for further study, or to be multiplied. This is one of many of the Crick's 'science technology platforms', each operated by a dedicated team – in this case, 12 technicians, led by Andy Riddell. "We are very proud of this facility," says George, "it is one of the largest in the country." Using these instruments, the Crick teams have gathered evidence that targeting the dark genome can provide new avenues for cancer treatment.

From the shadows, next generation medicine

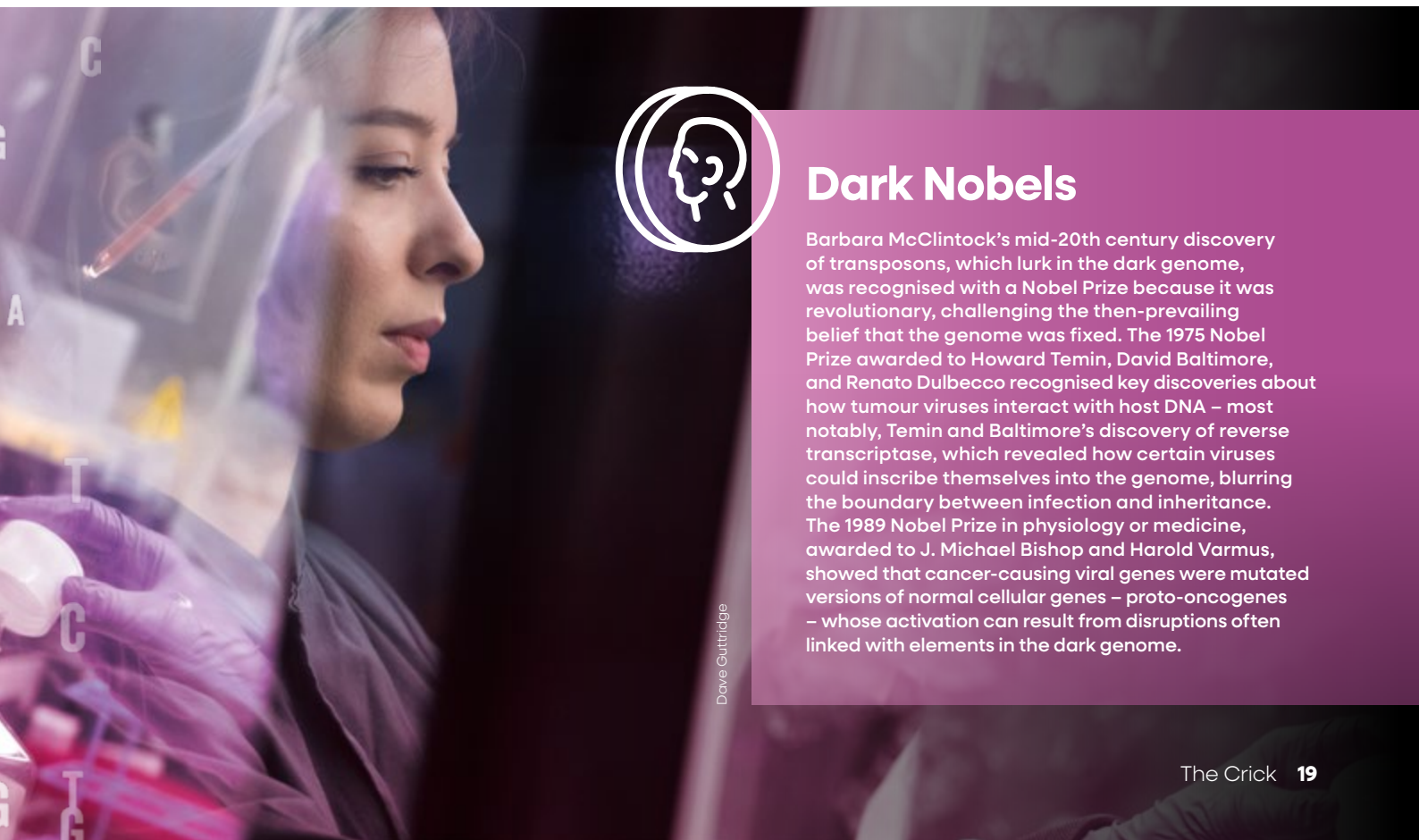
When it comes to cancer, the good news is that the immune system can recognise dark viral elements when they are reactivated, so that these endogenous elements can, in the words of George, "act as a kind of alarm system." Efforts are under way to detect them in blood, providing early warning of cancer, though he emphasises that proven, reliable, practical tests remain some way off.

Various companies have also been set up to make potential cancer vaccines that target the proteins made by dark retrotransposable elements. Among them is one that George has helped to set up, called Enara Bio, though again he is the first to caution that it can take decades to turn basic scientific insights into practical treatments.

As we uncover more details of the dark genome, expect more shockwaves across medicine. Scientists are already exploring ways to silence harmful ERVs, potentially offering new treatments for other conditions such as autoimmune diseases. Others are investigating whether beneficial viral sequences could be harnessed for gene therapy, using ancient viruses as tools to repair faulty genes.

"They have been linked to age-related inflammation," adds George, "even to the point that some have suggested we should be on antiretroviral therapy to slow down ageing and age-related inflammation."

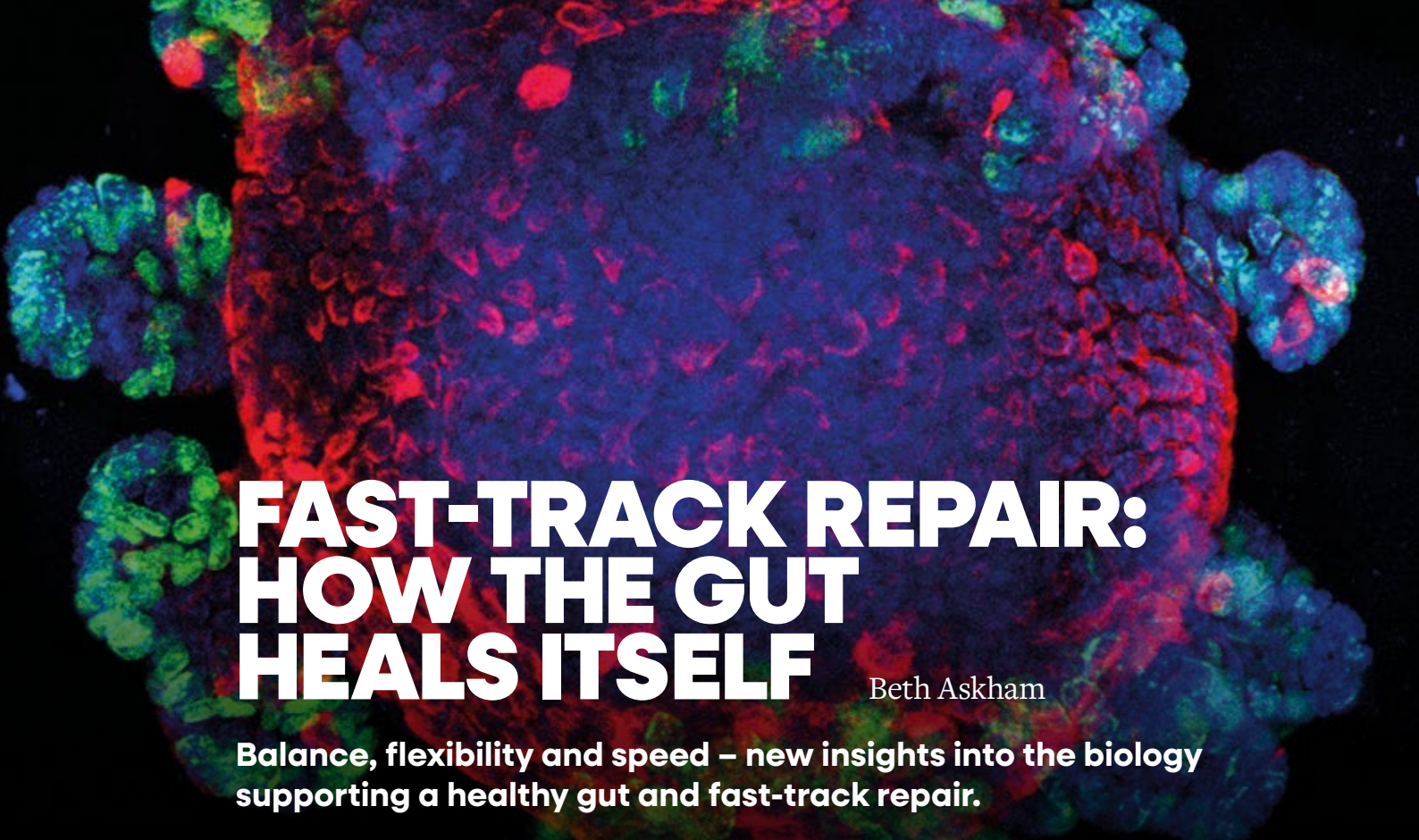
Recent technological advances, and studies by a global network of researchers, have transformed our understanding of these parasitic dark elements, says George. As they uncover the dark genome's secrets, scientists will reshape our understanding of evolution, development and health. Rather than fearing what elements lurk in the shadows of our genome, we should recognise them for what they are: not just the architects of our past and present but also of our future. ■



Dave Guttridge

Dark Nobels

Barbara McClintock's mid-20th century discovery of transposons, which lurk in the dark genome, was recognised with a Nobel Prize because it was revolutionary, challenging the then-prevailing belief that the genome was fixed. The 1975 Nobel Prize awarded to Howard Temin, David Baltimore, and Renato Dulbecco recognised key discoveries about how tumour viruses interact with host DNA – most notably, Temin and Baltimore's discovery of reverse transcriptase, which revealed how certain viruses could inscribe themselves into the genome, blurring the boundary between infection and inheritance. The 1989 Nobel Prize in physiology or medicine, awarded to J. Michael Bishop and Harold Varmus, showed that cancer-causing viral genes were mutated versions of normal cellular genes – proto-oncogenes – whose activation can result from disruptions often linked with elements in the dark genome.



FAST-TRACK REPAIR: HOW THE GUT HEALS ITSELF

Beth Askham

Balance, flexibility and speed – new insights into the biology supporting a healthy gut and fast-track repair.

Intestinal stem cell culture (organoids) labelled with markers of dividing stem cells (green), matured cells (red) and cell nuclei (blue).

Cancer Research UK

The cells that line our guts work hard – they absorb vital nutrients that sustain us and keep us healthy. But they're also continuously exposed to pathogens and toxins, so are easily damaged. They need a process for fast recovery.

Thankfully, the gut wall can repair itself at incredible speeds. And researchers are learning that this rapid repair process is thanks to a carefully maintained balance of different cell types in our gut wall. "This is a finely-tuned balance which is crucial to repair the gut and keep it functional," says the Crick's Vivian Li, whose team is using cutting-edge techniques to identify the cells involved in maintaining the health of our gut lining. Her work has chiefly focused on the gut's stem cells – specialised cells that generate new gut wall cells to replace those that get damaged.

As well as allowing us to understand the gut's vital repair process, Vivian's work is leading to crucial insights into diseases that affect the gut, such as inflammatory bowel disease (IBD) and bowel cancer.

The key to gut repair

Recently, Vivian's team found there is a carefully maintained balance between three types of cell – stem cells, gut wall cells, and a third type of cell that is essentially a transition between these two cell types, known as transit amplifying (TA) cells. Like stem cells, these seem to have the flexibility to turn into different cell types, a process called 'differentiation'. TA cells turn into gut wall cells during normal tissue maintenance, but when stem cells are challenged and lost, TA cells can reverse to de-differentiate back to stem cells.

A key question has been to understand what governs the balance between these three types of cell, and how a cell 'decides' whether and when to differentiate. Vivian's lab has recently highlighted a protein called ARID3A as a key player in this process.

> **"This is a finely-tuned balance which is crucial to repair the gut and keep it functional."**

Without ARID3A, the balance between the cells is lost, and the gut cannot repair itself as quickly.

"We've identified the control gene for the decision moment of whether to keep producing more cells," says Vivian. "Diseases affecting the gut in humans can involve a chronic state of injury, so next we plan to explore whether loss of ARID3A also plays a role in preventing the human gut from repairing itself in long-term conditions like IBD," she adds.

In the future, Vivian's lab plans to further expand on this work and map out all the genes and molecules that regulate this repair process and maintain the balance of a healthy gut. ■

DRUGGING THE UNDRUGGABLE

Dani Diaper

At the frontier of drug discovery, a new approach to targeting the toughest diseases using cyclic peptides.

Over the past decade, the number of new drug approvals has plateaued across the developed world. This is despite growing investment in drug discovery, and a deeper knowledge of which processes or proteins to target.

A major reason for the plateau is that many of the most promising targets are difficult to develop drugs against. "Mostly, drugs have been developed for the low-hanging fruit," says the Crick's Louise Walport. "Now we need to go after the harder targets – those that play a key role in disease but are extremely difficult to work with."

Much of the challenge lies in the molecular targets themselves. Many disease-related proteins – including those involved in cancer and neurodegenerative disorders – have long been considered 'undruggable' using conventional techniques. Some are inherently unstable, making them hard to work with. Others reside in parts of the cell that are hard to reach. Many lack the well-defined 3D pockets that traditional drugs bind to.

Targeting these proteins requires solving multiple challenges.

Why promising drug targets remain out of reach

One challenge is the early-stage drug development process, which typically involves purifying the target protein to test potential drug candidates. But purifying tricky targets is often impossible, due to their unstable nature.

A second lies with 'traditional' drugs. Most current drugs fall into two main categories: 'small molecule' drugs, and biologics.

Small molecule drugs – such as statins or aspirin – are relatively simple, inexpensive chemicals that usually bind to defined pockets. However, they require relatively high doses and often attach to other molecules, causing unwanted side effects.

Biologics, by contrast, are larger and more complex – typically whole proteins, such as antibody-based drugs or hormones. They are harder to deliver into cells but can bind more specifically to challenging targets, often with fewer off-target effects.

A new class of molecules takes aim

Louise's team is trying to solve these challenges by working with a new class of drugs called cyclic peptides – small fragments that combine the best qualities of traditional drugs. Importantly, they can bind to featureless surfaces, making them well-suited to tackling 'undruggable' targets.

To solve the 'purification problem', they're using a cyclic peptide discovery approach, called mRNA displays, in a novel way that bypasses purification entirely.

First, they genetically engineer cells to make high levels of a given target. Then, they produce a collection of trillions of different cyclic peptides, each linked to a unique RNA fragment that acts as a barcode.

Finally, they crack open their engineered cells and expose their contents to the vast collection of labelled cyclic peptides.

Redefining what's druggable

"If a cyclic peptide binds tightly to the target, we can identify it by reading its RNA barcode," Louise says. "The advantage of this method is that we don't need to purify the target at all. We're finding cyclic peptides that stick to it in its natural, biologically relevant form."

This is more than just a technical tweak. Side-stepping purification avoids a process that can distort a protein's natural shape. For complex targets, maintaining a realistic cellular environment is key. "Many promising cancer targets have disordered regions that make them nearly impossible to purify," Louise explains. "For years we've called these targets 'undruggable', but that's only within the limits of traditional drug discovery. It's no longer true."

Having demonstrated proof of principle, they can now focus on diseases with unmet clinical needs, such as hard-to-treat cancers. Future steps include refining the technique to broaden the technology's reach.

Her team's approach is one of many exciting new innovations that could redefine modern drug discovery – where 'undruggable' is no longer part of the vocabulary. ■

LIFE LESSONS

In conversation with Roger Highfield

Edith Heard was described by the *Financial Times* as "the epitome of a European scientist."

The Crick's new Director shares 10 lessons from a life in science, spanning curiosity, courage, coffee and the secrets of our genetic code.

Born and raised in London, Edith Heard has a British Greek background and dual British French nationality. During her Natural Sciences studies at the University of Cambridge, where she graduated in 1986, she shifted her attention from physics to genetics, later earning a PhD at the Imperial Cancer Research Fund. She spent nine years at the Institut Pasteur, followed by a year as a visiting scientist at Cold Spring Harbor. In 2001, she set up her own group at the Institut Curie to investigate the principles of gene regulation and epigenetic processes such as X-chromosome inactivation. She went on to become the director of the Genetics and Developmental Biology department in 2010, and then, from 2019, led EMBL as Director General.

I'm not fazed by not understanding things. As a scientist, that can be incredibly helpful because there's so much to know, and one can never know everything. I was born in London to a Greek mother and a British father, my first language was Greek, and for the first few years of my life I really couldn't understand much of what was going on outside of my home environment. I found I could overcome it by hard work, and being receptive. My life philosophy probably stems from that: everything is out there to be understood.

Our house was packed with the sick (there were a lot of Greek people coming to London to get treated), or people who were in exile because it was the time of the junta or Regime of the Colonels (a right-wing military dictatorship that controlled Greece from 1967 to 1974). That made me understand that freedom matters. When I became a scientist, I realised that that is one of the wonderful things about science – it can facilitate freedom. People are encouraged to think openly, encouraged to cross frontiers, to work in different countries. That is also why I am involved in the PAUSE programme, or "Programme d'accueil en urgence des scientifiques et artistes en exil," a French national programme that supports scientists and artists in exile, who come from countries where they are at risk of being imprisoned or bombed, or receiving death threats and facing many other hardships.

I started out very mathematical and I think that that really helps to think about biology.

In biology, most of the time we take things apart. We crush up cells to look at their DNA or we dissociate things and believe that if you can rebuild them, you can understand them. But if you can integrate the physics and the chemistry of molecules and biological processes, you can really start to better understand what drives life.

I was lucky that I went to a girls' school where I was encouraged to do what I wanted, and we were encouraged to do science. I realised that I like to understand how things work. I had an analytical mind, so it was natural for me to end up becoming more scientific.

I originally went to university to do astronomy. Instead, I fell in love with looking down a microscope at cells and the tiniest things, but I could have equally been as interested in looking at planets, galaxies and the largest things.

> **"I fell in love with looking down a microscope at cells and the tiniest things, but I could have equally been as interested in looking at planets, galaxies and the largest things."**

I don't like too much change, but I know that it's led to big leaps in my progression as a scientist and as a person. A step out of my comfort zone was moving from the UK to France, where I could not speak the language, where I fell in love with the science I was working on.

I was lucky enough to work in another temple of biology, Cold Spring Harbor, for one year and that was transformative for both my career and my personal life. I followed my partner, as he went there for a sabbatical, and I went as a visiting scientist, along with our two kids. I worked in the lab of David Spector. That year at CSH was amazing. It opened up my eyes and launched many new areas of research and collaboration for me. When we came back to France, I set up my own lab at the Curie.

Another big step was moving to EMBL where I went from running my lab and department in the comfortable environment of the Institut Curie, to running six institutes in five different countries, with 30 member states. There were challenges not just about language but a whole different way of doing things, working with multiple stakeholders.

Michael Bowles

I've become more confident in choosing the problems that I want to work on and not baulking at big challenges. Over the years, I've realised that one should always try to ask the big questions. I am not frightened of challenges, and I would say that's one of the reasons I took on the leadership role at EMBL, which is capable of answering some very big questions. The same goes for the Crick.

For me, it has been important to be a spokesperson for my field, epigenetics. When asked what epigenetics is about, the first thing I always say is what do you mean by epigenetics? The term is used in many different ways. Even when you go to an epigenetics meeting, you realise that different scientists are not actually talking about the same thing.

I work on X-chromosome inactivation, which is considered a classic epigenetic process as it involves stable silencing of the genes on one of the two X chromosomes during female development. The question is how do you stably shut down one X chromosome, even when it is identical at the DNA sequence level, to the second copy? When the genome got sequenced scientists realised that genes don't tell the whole story. Then everyone suddenly got excited about the idea that epigenetic changes – which can be easily reversed or modulated, unlike genetic mutations – might be key to explaining life.

> **"I have found that in some contexts, as a woman in a senior leadership position, one has to talk 10 times louder and 10 times longer."**

Scientists should always challenge dogmas, but you have to be brave and resilient. I wanted to understand an essential process, X inactivation, yet for the first five to 10 years of my career, first as a postdoc, and then as a group leader, many of the things that I was stumbling on people said: "No, no, that can't be true. It doesn't fit, doesn't fit with the dogma." As a result, I did not publish in high-profile journals. But these were some of my best papers and I was committed to publishing our findings and making people understand that what we were seeing was valid, even if it did not fit with existing models and dogmas.

Since I started my lab, we've come up with many new hypotheses, published on them, then in some cases we have gone on to kill a hypothesis because the data told us to. That's what science is about. Being rigorous and being brave and not being frightened of being challenged, even challenging one's own science.

I have a strong sense of justice. I find it frustrating when people are not fairly treated, or if someone comes up with a hypothesis that is different to what people are thinking about and it gets ignored. I can be brave enough to speak up when others don't necessarily. I look around and think: "Well, why didn't anyone else say it? It's so obvious." Having said that, I choose my battles carefully.

I don't feel I've often been challenged because I'm a woman, only on scientific issues. However, there are occasions, particularly in my most recent job, where I could tell that if I had been a guy wearing a suit and tie, some people would probably have talked to me in a very different way, or listened more attentively. I have found that in some contexts, as a woman in a senior leadership position, one has to talk 10 times louder and 10 times longer.

I love to sleep. I don't get enough of it. If I don't get my caffeine first thing in the morning, I'm completely dysfunctional. I used to carry caffeine tablets because if I go into the day or a meeting without having had my early caffeine shot, the whole day is gone. Recently, my lab even gave me a portable, mini-espresso machine as a Christmas present.

To relax in our current family base in Paris, I listen to music or go to the cinema. I could watch three films in a day. In fact, there's no limit to how much cinema I can watch or music I can listen to.

It's kitsch, but I do think it's amazing to have children. Having my children was a personal high point. It's almost like a drug – everything they do I find fascinating maybe because I'm a scientist and I am rather analytical about things. I watch their decisions in life and I think, "Oh my God, and now they did this!". I do have a bit of an addiction to my kids. I can never see enough of them. Having said that, I could also imagine my life as a scientist without children. In my particular case, and given the incredible partner I have, I think it was the right choice – and they provide balance.

During any crisis, there is a silver lining and opportunities. I've never had to deal with as many crises as in the past five years. When it comes to my toughest period professionally, it has to be these past few years dealing first with the pandemic, then with what's happened with the Ukraine, the Middle East, and the recent geopolitics. None of these were of my doing, but I ended up in a position of leadership where I had to confront them and navigate many challenges for EMBL, while ensuring the organisation continues to deliver its multiple missions of research, service provision, and training.

> **When it comes to my toughest period professionally, it has to be these past few years dealing first with the pandemic, then with what's happened with the Ukraine, the Middle East, and the recent geopolitics."**

The pandemic was something scientists knew was coming, and could come, but when it hit us, it was still a shock. How naive could the world be? The first lesson I learned was we really should have been better prepared. As Director General of an intergovernmental organisation, I saw five different countries dealing with it in different ways, for better or for worse. So I learnt a lot about what it must take to govern a country through a medical crisis. I also learnt a lot about values, what is valued in a country, what is valued in an institution.

EMBL was in the process of negotiating our budget during the pandemic along with our new programme for the next five years. It was actually much easier for me to explain the relevance of EMBL's work and our new programme to ministries who normally would give us only five minutes. For example, our Hamburg site was dealing with the BioNTech vaccine tests. I found that the member states listened to us.

The best advice I ever received came from my PhD supervisor Mike Fried: you need mentors, especially as a woman. I can still picture us having coffee together and him telling me this in his Bronx accent. He did not really mean the kind of mentor we talk about today – someone you meet with regularly on a fixed basis. He meant finding people who will look out for you, especially at difficult moments. I have always tried to keep in touch with people who I trust and respect. The further you go in your career, especially in leadership positions, the lonelier it can become. All it takes is one or two conversations with people you trust to make you realise certain things, or to make you think again.

You can define success by how much you've allowed a new generation of science to happen. Aside from my kids, a high point is being able to follow the course of the people who've come through my lab. I'm more proud of them than even the science that I did or I'm doing. The scientists I'm most enamoured of, or respectful of, or impressed by, are the ones who also have produced great scientists, who encouraged and supported their colleagues. ■

Michael Bowles

VARIANTS: THE TYPOS TURNING LOSS INTO HOPE

Henry Scowcroft

Across the 3 billion 'letters' of our DNA, we each carry around 6 million variations. Researchers are unravelling their effects on our lives.

"The day before, we'd been scooting round Whipsnade Zoo. She was seemingly happy and healthy," remembers Nikki Speed, of the life-changing December day just before Christmas in 2013.

That morning, Nikki went to wake her two-year-old daughter Rosie.

But Rosie was never to open her eyes again.

There isn't a 'big enough word' to describe the impact on the family, says Nikki, who became one of many grieving parents who looked for the answers among the single-letter variations we all carry in our DNA.

Solving the mystery of how these variations affect our health – how we live and die – is a vital scientific quest. As well as providing answers for parents like Nikki, it could reveal the causes of inherited cancers, identify patients who could benefit from precision drugs, and help settle appalling miscarriages of justice.

And thanks to new gene-editing technologies, scientists are working out how to predict the effects of these subtle DNA changes before they even occur.

THE QUEST FOR ANSWERS

"I sat with my mum in her kitchen, and we went through the weeks leading up to Rosie's death – every mouthful of pesto pasta she ate – trying to find what I'd missed. We were just told we needed to wait for the post-mortem. So we just waited. We were numb."

But the post-mortem turned up nothing, nor did further investigations to rule out cardiac causes. "I also had a little boy who was nearly five. I was paralysed with fear for him, too," Nikki says.

Each year in the UK, around 40 children aged between one and 18 die in unexplained circumstances, often while asleep – that's more than one every fortnight. The phenomenon has a bland acronym: SUDC, Sudden Unexplained Death in Childhood.

In many cases the culprits are thought to be hiding in our DNA. And for that reason, a key technique in molecular biology – DNA sequencing – is being brought to bear on SUDC.

Since the first human genome was mapped in the early 2000s, researchers have sequenced the genomes of millions of people, discovering more than a billion individual variations in our genetic code. Some are common, most are rare. Some are slap-bang in the middle of vital genes, others lurk in the vast, 'dark' spaces between them. A landmark study in 2022, using data from the UK Biobank, estimated that we each carry around 6 million variants across our genome's 3 billion DNA letters.

But these variants can have very different effects on our health. At one extreme, so-called pathogenic variants can cause inherited forms of diseases such as cancer, and rare but lethal diseases that affect a handful of people worldwide. At the other end of the spectrum are benign variants – harmless genomic background noise.

Each year in the UK, around 40 children aged between one and 18 die in unexplained circumstances.

After Rosie's death, Nikki Speed turned to DNA sequencing for answers. And since it wasn't yet routinely available in the UK, she found hope in New York, where a project called the SUDC Registry and Research Collaborative was offering bereaved families genetic testing, based on current knowledge of genes linked to sudden death.

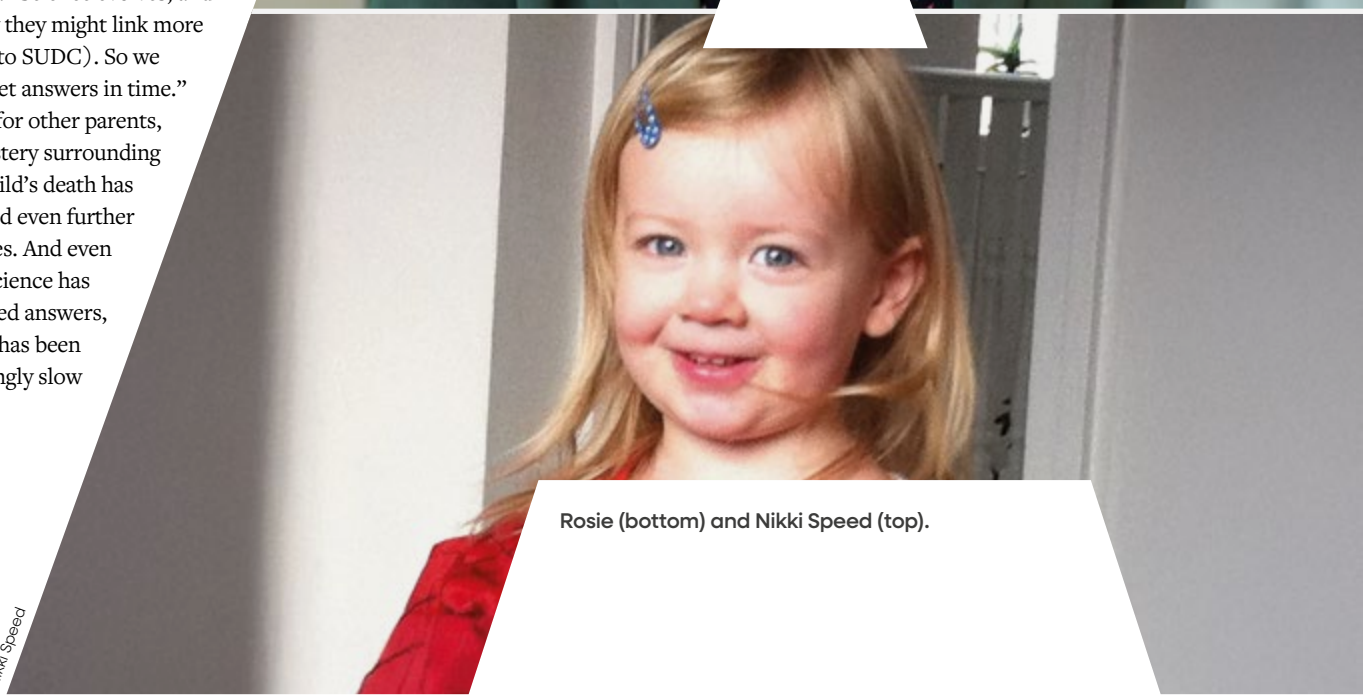
Being accepted onto the project was a "huge moment", Nikki says. "I felt like I was doing something".

The results, which arrived a year later during a family holiday in Florida, were frustratingly inconclusive. They'd done all they could.

Nevertheless, Nikki remains hopeful: "Science evolves, and one day they might link more genes (to SUDC). So we might get answers in time."

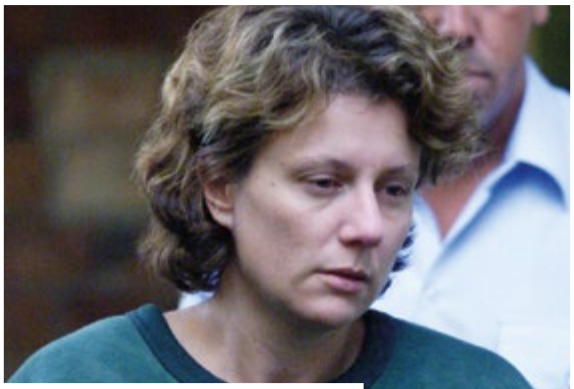
But for other parents, the mystery surrounding their child's death has triggered even further tragedies. And even when science has suggested answers, society has been agonisingly slow to act.

Michael Bowles



Nikki Speed

Rosie (bottom) and Nikki Speed (top).



Fairfax Media/Getty Images

Kathleen Folbigg leaving court after being refused bail in 2004.

Michael Bowles

Kathleen Folbigg at the Francis Crick Institute in 2025.

A STUNNING INJUSTICE

More than a decade before the human genome was deciphered, in February 1989, Kathleen and Craig Folbigg celebrated the arrival of their first child, Caleb. Less than three weeks later, in the middle of the night in their suburban flat in New South Wales, Australia, Caleb stopped breathing. He was just 19 days old. The cause of death was recorded as Sudden Infant Death Syndrome, SIDS. (SIDS is a term reserved for children under one; above that age, deaths are recorded as 'SUDC').

Over the next decade, the couple would lose three more children, all in unexplained circumstances. Patrick, born a year later, developed epilepsy and blindness, and died aged eight months old. Two years later, the couple's third child, Sarah, failed to wake from her sleep at 10 months old.

And in 1998, the couple welcomed their fourth child, Laura. A battery of post-natal tests came back normal, and a year later the family celebrated her first birthday. But several months later, Laura, too, failed to wake from an afternoon nap.

Four unexpected deaths in a single family might flag at least a suspicion of an inherited condition. But a pathologist recorded Laura's death as "undetermined" and raised the idea of homicide. After a police investigation, in 2001 Kathleen – then 34 – was arrested and charged with the murder of her four children.

A lengthy trial followed, and despite no direct evidence of smothering any of the children, in 2003 a jury convicted her of three counts of murder, one count of manslaughter and one count of grievous bodily harm.

Vilified in the media as "Australia's worst mother", the judge sentenced her to forty years in jail.

Carola Vinuesa.

SETTING THE RECORD STRAIGHT

When immunologist Carola Vinuesa, a principal group leader at the Crick, talks about Kathleen, it's with an intensity born of a deep commitment to justice.

"Right now, there are hundreds of women around the world facing criminal sanctions for hurting or killing their children," she says, but for many the courts have either failed to consider genetics, or, if they have, have only carried out limited testing. It's not hard to understand her

> **"Right now, there are hundreds of women around the world facing criminal sanctions for hurting or killing their children."**

frustration, given her experience: Carola led the team of researchers and legal experts who challenged Kathleen's conviction using evidence buried within the family's genome.

Carola's involvement began in 2018, while working at the Australian National University in Canberra. A former student in her department, now a lawyer, rang her to ask whether Kathleen's case might be helped by genetic testing.

Carola asked to look over their medical records, and was struck by several details. Caleb had laryngomalacia ('floppy larynx' – known to cause obstruction of the airways). Laura's post-mortem had revealed inflamed heart muscle, myocarditis. Her sister was being treated for a respiratory infection at the time she died. Together with Patrick's epilepsy, she felt there were alternative explanations. She took on the case.

Three months later, she and her colleague Todor Arsov had sequenced Kathleen's genome, and begun looking for variants in around 350 genes they had identified as either linked, or potentially linked, to sudden deaths in children.

They spotted something almost immediately: tucked away on the short arm of chromosome 2, a single-letter change in a gene called CALM2.

It would take years more to build proof sufficient to convince a court, but Carola is clear that, even back then, they strongly suspected this variation was harmful, thanks to several unique features of CALM2 and the protein it makes, calmodulin.

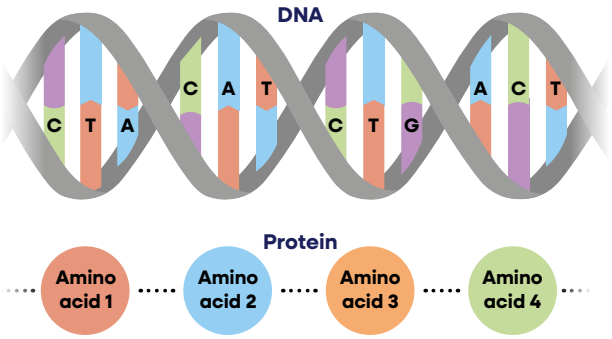
ISLANDS OF CALM

As researchers have sequenced DNA from organisms across the major branches of life – including higher organisms like plants, fungi and animals – they've discovered that certain genes are far less variable than others. And that's because these so-called conserved genes are fundamental to life.

Find out more about how scientists uncovered new genomic evidence in Kathleen's case

WHAT IS A VARIANT?

Our genetic code tells our cells how to make proteins out of building blocks called amino acids.

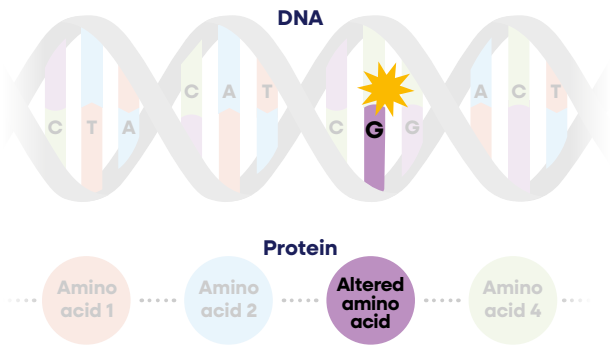


The order of DNA bases determines the order of amino acids in a protein

The order of amino acids determines **how the protein works.**

Variants are DNA changes that alter the sequence of letters. They can be **insertions, deletions, or substitutions.**

Variants **can occur naturally,** through mistakes as cells replicate their DNA, or can be caused by **cigarette smoke, UV radiation** or other exposures that damage DNA.



A single DNA change (variant) can change the amino acid in a protein

Sometimes, variants can **change order of amino acids** in a protein, causing it to **malfunction.**

They can also be experimentally induced by gene-editing techniques such as **CRISPR.**

Dave Guttridge

CALM2 is among these genes, and it's "extraordinary" for two reasons, says Carola.

First, humans have not one, but three separate CALM genes – prosaically called CALM1, CALM2 and CALM3. Usually, genes that exist in multiple copies have evolved subtly different roles, with subtly varying sequences. But, uniquely, each of our three CALM genes seems to make proteins that are 100% identical to each other – molecular triplets, unlike any other family in the human genome. In other words, says Carola, "calmodulin is absolutely vital."

That's backed up by medical science: rare CALM gene variants cause serious conditions, such as 'long QT' syndrome, which causes fast, chaotic heartbeats linked to fainting, seizures, and – occasionally – sudden death. In fact, whenever variants in CALM2 have been spotted, they're never benign – it doesn't seem to tolerate any changes to its sequence.

And second, across the entire tree of life, the equivalents of the CALM gene are strikingly similar. What's truly remarkable, says Carola, is how perfectly preserved the

calmodulin protein itself is – right down to its molecular building blocks. One amino acid in particular, glycine-114, stands out. "It's not just unchanged in all animals – it's conserved in plants and fungi too," she explains. "Glycine-114 is an essential part of an essential protein."

And back in 2018, the CALM2 variant she and Todor had spotted in Kathleen Folbigg's gene altered that essential, conserved glycine-114. That meant it was highly likely to be harmful.

A few months later a legal inquiry was launched, with Carola involved, and samples from the Folbigg children's DNA were analysed: both Sarah and Laura Folbigg carried the variant too.

But there was disagreement among geneticists at the inquiry. This precise CALM2 variant had never been seen in a human before. Some considered it a 'variant of unknown significance', whereas Carola and her team considered it was likely to be pathogenic.

Frustratingly, the inquiry judge concluded there was no possibility that the variant had caused Sarah and Laura's deaths.

However, through painstaking laboratory work led by a Danish team, and analysis of other children with glycine-114 variants in CALM3, in 2021 Carola and her colleagues published overwhelming, peer-reviewed evidence that the Folbigg family's CALM2 variant was pathogenic.

Speaking at an event at the Crick this year, Kathleen recalled the day she heard the news. "It was bittersweet," she said. "I cried for a long time, and there was a lot of soul searching. Because I'd been telling everyone 'I didn't do anything, I didn't do anything' ... but, genetically, I did."

The discovery led to the launch of another inquiry, and this time the judge was convinced. In December 2023, 20 years after her conviction, Kathleen's case was referred to the court of appeal, and her conviction was quashed.

She was, finally, free.

SLOW, STEADY PROGRESS

The thread that weaves Nikki and Kathleen's stories together – beyond the obvious tragedy – is the quest to unpick the human consequences of genetic variation.

Nikki is still waiting for answers that only research can provide. And for Kathleen, the uncertainty around a single, never-before-seen genetic variant held up justice for five years.

But what if we could systematically test large numbers of variants in the human genome before tragedies occur? What if we could sketch out the map in advance?

And that is what one of Carola's colleagues, Greg Findlay, is trying to do.

It was a sunny day in Boston, Massachusetts in 2012, when "something just clicked," says Greg. "I was sitting in a Harvard Medical School courtyard reading a paper and I just thought, wow, that's cool, that's where I want to take my career".

Then a lab technician, Greg had just read how researchers had used DNA sequencing in a novel way. Sequencing costs had plummeted from millions of dollars to thousands, allowing them to go beyond interrogating *whether* individual DNA sequences were present in a sample – i.e. a qualitative measurement – and instead quantitatively measuring *how much* of each different sequence was present, by sequencing the same sample many times and counting how many times each sequence was detected.

In Greg's mind, this opened the door to studying genetic variation on an unprecedented scale.

"Historically, it's been too laborious to test individual variants one by one," he says, so variants get tested after they turn up in someone's genome.

Using the new technique he read about that day, Greg went on to develop a way to catalogue human variants by making every possible single-letter DNA change along a given gene, then measuring all their effects at once – something he called 'saturation genome editing', or SGE. "With SGE, we can test every potential variant up front, then see whether they are spotted clinically afterwards. It's prospective, instead of retrospective, testing."

SGE relies on a technique called CRISPR-Cas9 to precisely edit individual letters in a stretch of a cell's DNA. Whereas previous techniques relied on artificial and limited systems to study variants, says Greg, "CRISPR changed everything. It allowed us to precisely place variants into an authentic context – a living cell's genome – and to study a much wider range of variants."

After multiple variants have been edited into cells' DNA, the next step in SGE is to use quantitative DNA sequencing to measure their effects, and so reveal potentially harmful variants on an unprecedented scale.

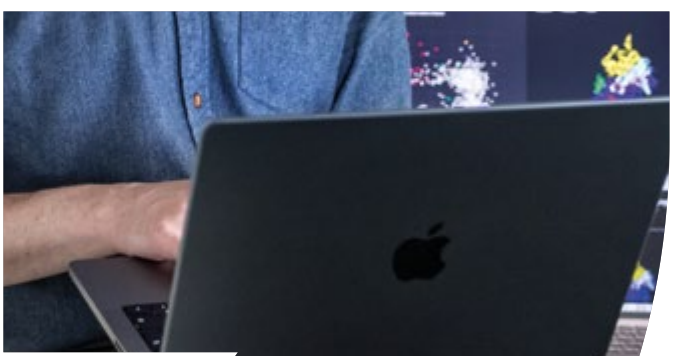
CRISPR changed everything. It allowed us to precisely place variants into an authentic context – a living cell's genome – and to study a much wider range of variants."

Greg first published a proof-of-concept of this method in 2014 and, at first, "no one really seemed to notice". That changed in 2018, when Greg and his colleagues carried out a landmark analysis of the infamous BRCA1 cancer gene.

MAPPING CANCER VARIANTS

First linked to the disease in 1990, people who inherit certain BRCA1 mutations have a greatly increased risk of a range of cancers – notably breast and ovarian cancer. Since then, tens of millions of people have had their BRCA1 genes sequenced, turning up more than 4,000 pathogenic variants and thousands more of unknown effect. Greg's 2018 study correctly, and independently, identified many of the pathogenic variants – but it went further, flagging hundreds of 'unknown' variants as potentially harmful.

Greg Findlay.



Dave Guttridge

"That's when people really took note, and started to use it in their own labs," he says. In 2020, Greg relocated from the US to the Crick to establish his own lab and refine the technology, using it to map another gene, VHL, linked to cancers including kidney cancer and a rare adrenal gland cancer called pheochromocytoma. As with BRCA1, they found "perfect accuracy" in identifying variants known to cause kidney cancer but also shed new light on how different VHL variants were linked to different cancers.

These discoveries have been hugely influential, says Clare Turnbull, a clinical geneticist at London's Royal Marsden Hospital. "The idea that you could pre-annotate every possible variant using functional data means that, if you encounter a variant clinically, you'd be more confident in classifying it as pathogenic or benign." And that would ultimately mean better information for people potentially at risk of inherited disease.

What's particularly hopeful, says Clare, is the prospect of blending Greg's methods with software that predicts variant effects 'in silico'. "This would help us interpret rare variants of unknown significance found in people tested in our clinics," she says.

It also starts to unpick a particularly thorny issue. "A lot of our information on genomics comes from large-scale population sequencing of predominantly White people," Clare says. That means we lack information on harmful variants that are more common in people of other backgrounds. Proactively generating data on all possible variants could level the playing field for people of all ethnicities.

As well as continuing to focus on cancer, Greg's team is further refining SGE. Recently, they adopted a newer gene-editing technique called prime editing, allowing them to overcome a major limitation of traditional CRISPR editing. CRISPR can only introduce variants across a 200-DNA-letter-long stretch of a gene, requiring multiple experiments to 'walk' along a gene's length to induce all possible variants. Prime editing allows SGE experiments that target much larger regions of the genome. "It's massively sped things up," he says.

And, rather than testing every single variant in each of the 20,000 genes in the human genome – something that would take "around 50 years or more" – he sees real promise in using SGE data to train computer models

> **“I’d like to think that the legal system could listen to scientists a bit more often.”**

to make much more accurate predictions. “But that’s going to take a lot of experimental data,” he says.

The implications of SGE go beyond identifying inherited conditions. It could help more patients get new targeted cancer drugs, which rely on spotting particular defects that arise in their cancer. “For example, if we can say that a previously uncategorised BRCA1 variant likely causes loss of function, that suggests the patient’s tumour might be sensitive to drugs called PARP inhibitors,” says Greg, opening the way for more people to benefit from these drugs.

FROM LOSS TO ACTION

Carola thinks technologies like SGE could hold the key to liberty for individual women around the world. And she hopes that the justice system can take a pragmatic view of the evidence they can provide. “We’re not talking about the greater than 90% certainty you need for a clinical decision like whether to have a mastectomy,” she says. “We’re talking about the fact there’s a woman in jail for having killed her children, and she’s got a mutation in a gene that is likely to cause early infant death.”

Since her release, Kathleen has taken a pragmatic approach to rebuilding her life. “It’s a good thing to be able to stand up and say, science did this, science gave me peace of mind. And science helped me sit here today to be able to talk to everyone,” she told the audience at the Crick. “I’d like to think that the legal system could listen to scientists a bit more often.”

Meanwhile, Nikki has co-founded a charity, SUDC UK, to raise awareness, fund research and support affected families. Thanks in part to their campaigning, in 2023, the NHS made genomic testing routinely available to these families, something that felt “really validating, like people finally understood.”

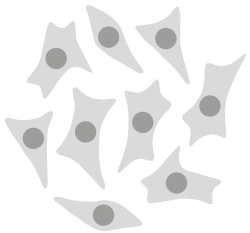
And like Kathleen, she has huge hopes for what research can offer, both personally – in that it may one day reveal the cause of Rosie’s death – but also for others affected.

“The ideal outcome would be that we identify biomarkers that can help predict and prevent SUDC,” she says, pointing out that since 1990, research on SIDS has been accompanied by an 80% reduction in deaths in children under one year old.

“We know what we need to do, we just need to do it. If we do the research, we’ll be able to save children’s lives.” ■

HOW DOES SATURATION GENOME EDITING WORK?

“Saturation genome editing, SGE, allows you to test thousands of different variants in a single experiment,” says Greg Findlay. “Consider a sample of cells, where you’ve used CRISPR to edit a different variant into each individual cell. You let them all grow for a while under a particular set of conditions, then you sequence everything at the end.”

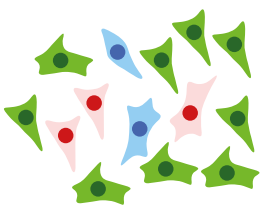


Edit cells with CRISPR to contain different variants

Cells carrying harmless variants thrive, while those with harmful mutations struggle to survive. By reading the final mix of DNA, researchers can see which variants helped cells cope – and which ones didn’t. This gives vital clues about which mutations are likely to cause disease in humans.

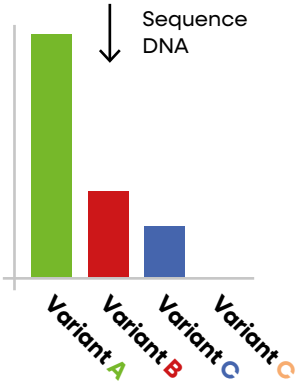
Allow to multiply

The cells that are unaffected are able to grow unhindered.



Sequence DNA

Because the sequencing can measure the relative levels of different variants, it reveals the variants that harmed a cell’s ability to grow, vital evidence as to whether they’re likely to be harmful or not in a human.



GENOME REVOLUTION:

FIVE DISCOVERIES REWRITING MODERN BIOLOGY

Rob Goldstone

As head of the Genomics Science Technology Platform at the Crick, I have had a front-row seat to the decade's most powerful genetic discoveries.

Dave Guttridge

One of the Crick's Illumina NovaSeq X DNA sequencers is prepared for use. It can sequence a genome for £200 – a fraction of the cost a decade ago.

THE £100 GENOME

The Human Genome Project cost more than £2bn, but since then costs have dropped dramatically. Just 10 years ago, our machines could sequence a genome for around £1,000. Today we can read a genome for around £200, and this might soon plummet even further – the latest machines can run for around **£100 per genome**.

LONG READS, BIG LEAPS

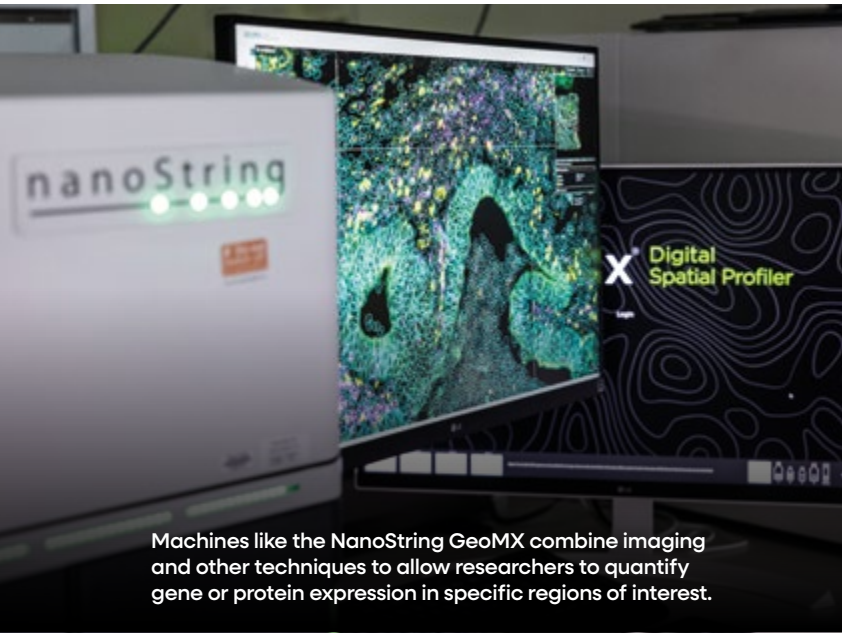
Genomes contain lots of repetitive sequences, which have traditionally been hard to sequence – the genomic equivalent of black holes. That all changed with the advent of techniques to read long stretches of DNA in one go – **long-read sequencing.** A decade ago, the technology was in its infancy and data strewn with errors. Today, advances in molecular biology and computational models have increased the accuracy to over 99.5%, enabling end-to-end sequencing of the whole human genome.

A nanopore flow cell is loaded for long-read sequencing. This machine can produce reads over 1 million bases with 99.5% accuracy.

CELL BY CELL

Until relatively recently, when we analysed DNA or RNA in a tissue sample, we had to analyse everything at once and take an average – so-called 'bulk' sequencing. But we can now sequence genetic material from individual cells, at scale – and it's been revolutionary. Early **single-cell techniques** were cumbersome, costly, complex and limited. But a decade ago, new technology allowed us to capture and individually analyse thousands of cells at once. And even newer techniques now allow us to look simultaneously at DNA, RNA and immune activity, even from archived tissue samples, offering an unprecedented cellular portrait of life, health and disease.

Complex single-cell techniques can look simultaneously at multiple types of molecule – DNA, RNA and proteins thanks to sophisticated automation platforms.



Machines like the NanoString GeoMX combine imaging and other techniques to allow researchers to quantify gene or protein expression in specific regions of interest.

THE TISSUE ATLAS

Traditional methods only told us what was in a sample, not where it came from. **Spatial genomics** changed that, combining genomics with imaging data to reveal exactly where gene activity is taking place in a tissue. A decade ago, capturing this picture was labour intensive and complex, and only allowed us to look at a limited number of genes at once. But since 2019, new devices have allowed us to create spatially detailed maps of the activity of thousands of genes, with incredible detail – even to allow true single-cell resolution. It's like going from a rough sketch to a high-res biological map.

BEYOND THE CODE

Our DNA contains information that goes beyond the sequence of As, Ts, Gs and Cs. The study of how cells chemically modify their DNA to regulate gene activity – **epigenetics** – has seen its own revolution over the past decade. A decade ago, studying these changes was slow and required large samples – early techniques to measure this, such as bisulfite sequencing and ChIP-seq were widely used but limited. Today, powerful new devices allow us to paint an incredibly detailed picture, running epigenetic studies at single-cell resolution in parallel with techniques that measure gene expression at the same time. This gives us a far more detailed picture of how genes are controlled and how that control can go wrong in disease.

Sophisticated equipment like this automated liquid handler simplifies manually intensive tasks, enabling the epigenome to be analysed in unprecedented detail.

AFRICA: A NEW LENS ON HEALTH

Dani Diaper

Scientists in South Africa are working with the Crick to pioneer imaging technologies that will redefine how they detect and study diseases affecting their local communities and accelerate the hunt for better treatments.

Samantha Reinders

Xolelwa Mabokela (right) and her friend Nobuhle Manqina (left) live in Khayshelita on the edge of Cape Town and both had TB while pregnant.

In 2018, Xolelwa started having debilitating back pain. She was eventually diagnosed with multidrug-resistant tuberculosis.

Months later, she discovered she was pregnant. Battling severe illness, including the removal of her left lung, Xolelwa gave birth to a healthy baby girl, Lilitha, and began the long road to recovery. "It was so hard being way from my baby," she says.

Xolelwa's friend, Nobuhle, also had TB while pregnant. "TB is still a stigma here and ultimately it is low on the list of people's priorities. No money, no jobs. People don't wash their blankets. We have to change our habits." says Nobuhle.

Xolelwa (left) and her friends.

Samantha Reinders

Xolelwa and Nobuhle's stories highlight the human cost of TB in South Africa, where the disease claimed more than 54,000 lives in 2022, one of the highest death rates in the world.

To fight back, researchers need a deeper and more sophisticated understanding of the bacterium responsible, *Mycobacterium tuberculosis*, and how it interacts with human cells.

Sharper views of a silent killer

Around 25km away, at Stellenbosch University, Ben Loos and his team are helping to pilot an ambitious microscopy technique that was funded by the Chan Zuckerberg Initiative and developed at the Crick. It could transform the way diseases such as TB are studied.

Advanced microscopy is often prohibitively expensive and inaccessible to most labs in Africa. But through collaboration with the Crick, Ben's team are pioneering a low-cost, high-resolution technology called the Visual Proteomics Correlative Light and Electron Microscopy Kit, or VP-CLEM-Kit. As well as testing how to install, run and maintain it in a typical lab, they're also using the kit to further their research.

For Ben and his team, this isn't just about scientific progress; it's about impact. By making high-end imaging more accessible, they're unlocking new opportunities for African researchers to tackle diseases such as TB.

The VP-CLEM-Kit technology combines two types of microscopy – super-resolution single-molecule localisation light microscopy, which allows scientists to visualise individual molecules inside cells, and volume electron microscopy, which captures the 3D structure of the cell in intricate detail. By superimposing images taken by both techniques from the same cell, it reveals life in unprecedented detail.

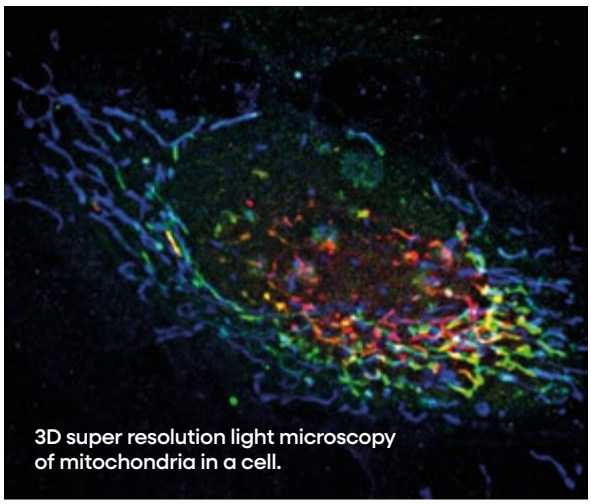
"The VP-CLEM-Kit will allow us to make strides in the way we understand many cellular processes," says Ben. "We'll get a more detailed view of the structures inside a cell. And that will reveal new mechanisms and treatment avenues for a range of conditions."

Among the structures studied by Ben's team are mitochondria, which release energy to power vital processes, including autophagy – essentially the cell's waste-clearance system.

"It turns out autophagy plays an important role in TB infection," explains Ben. "For us, that was really exciting as we can use the VP-CLEM-Kit to study local strains of TB directly".

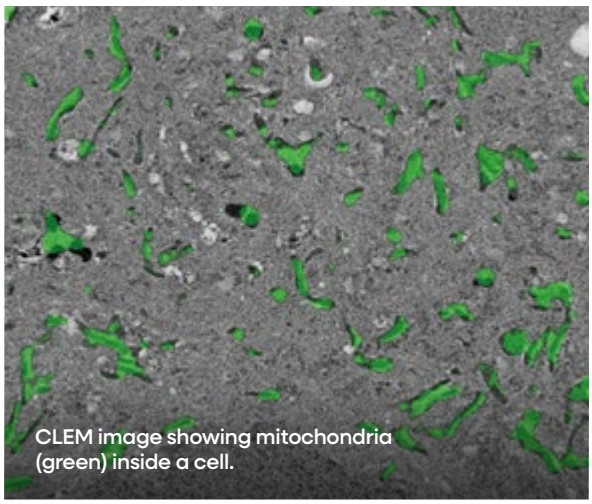
The team is now infecting living cells with a locally circulating strain of TB to examine the impact on mitochondria, and on the cellular autophagy compartments that are involved in clearing bacteria.

"The VP-CLEM-Kit will be able to look at this local strain of TB in incredible detail, and potentially reveal new treatment strategies that help fight the infection more effectively."



3D super resolution light microscopy of mitochondria in a cell.

Francis Crick Institute



CLEM image showing mitochondria (green) inside a cell.

Francis Crick Institute

Ben Loos and his team.

Samantha Reinders

Imaging innovation

The VP-CLEM-Kit project began more than a decade ago, when Ben and the Crick's Head of Electron Microscopy, Lucy Collinson, were discussing how high-end imaging technology like super resolution light microscopy and CLEM was often only affordable by well-funded, purpose-built facilities.

Currently, only one research group in Africa has the capability to perform CLEM.

Ben and Lucy joined forces with physicist Paul French and computational scientists Ricardo Henriques and Amy Strange to create a version of VP-CLEM-Kit that is affordable and easy to use outside of well-resourced facilities.

"The challenge we faced was how to replace an advanced imaging workflow, that requires complex sample preparation and expensive equipment, with something that can be operated more easily and housed in a standard lab or light microscopy facility," explains Lucy.

Prototype to practice

The new VP-CLEM-Kit arrived in Stellenbosch in September last year. Now, the team is taking on a new challenge: turning sample prep, high-powered microscopy and data crunching into one seamless workflow that other African labs could follow in the future.

As a member of the Crick Africa Network – an equitable programme that aims to increase research capacity to tackle health problems across Africa – Ben is keen to bring innovative technology to the continent.

"Running standard CLEM experiments requires equipment that is very expensive to buy and run. That is not an option for us, nor for the majority of the labs throughout Africa," says Ben's colleague, Nicola Vahrmeijer.

"So, this new approach looks at what is available in a standard lab – and how can we achieve unprecedented high-quality 3D imaging using what we already have access to."

A key issue is sample preparation – "an essential part of the new workflow," says Nicola.

One notable change to the VP-CLEM-Kit sample preparation process is that it replaces costly pressurised liquid nitrogen equipment with a much cheaper UV lamp in a standard freezer, making this step far easier and more accessible.

Beyond TB – studying environmental pollution and dementia

Researchers in Ben's lab are also studying the toxic effects of heavy metals such as manganese, released into the environment during industrial mining.

Manganese pollution alters mitochondrial function in ways not yet fully understood. "Our VP-CLEM-Kit will allow us to study changes in mitochondrial shape at super resolution, in 3D. Examining our samples in this way will bring an incredible level of precision, giving greater insight into what molecules are where and how this affects their function." enthuses lab member Asandile Mangali.

Other team members will use VP-CLEM-Kit to study neurodegenerative diseases such as dementia, by mapping how faulty autophagy and mitochondria contribute to cell ageing and cell stress, leading to diseases such as Alzheimer's.

Dementia is of growing concern in South Africa, where misunderstandings about the condition fuel stigma – and in some cases, have even led to people being wrongly accused of witchcraft.

Read more about how the Crick Africa Network (CAN) is increasing research capacity to tackle health problems on the African continent

Relocating from Ben's lab, Dumisile Lumkwana (left) joined Lucy (right) to lead the hands-on development of the project.

Michael Bowles

Nicola Vahrmeijer with part of the new VP-CLEM-Kit.

Naomi Okugbeni (centre) and Bethaba Shazi (right) are part of the team working on TB research at Stellenbosch University.

The bigger picture

Once Ben's lab has tested and validated the new VP-CLEM-Kit, it will be rolled out to other research institutes across Africa.

Meanwhile, Lucy and her collaborators at the Crick continue to refine and automate the technology. "Working with Ben's lab has been invaluable for making this technology truly practical," says Lucy. "We'd love to see the VP-CLEM-Kit in every research institute that needs it, anywhere in the world. Soon, the global south will be the experts, teaching the global north – a refreshing change."

"It's an immense privilege and opportunity for us to be part of this work," says Ben. "With the potential to roll this technology out across the continent, the impact for science in South Africa and beyond is an honour we take rather seriously.

"We see this as a transformative moment, and the beginning of something much bigger, empowering scientists across the continent to push past what they thought was possible."

> **"... the global south will be the experts, teaching the global north ..."**

Back in Khayelitsha, Xolelwa reflects on her TB treatment, "I was really lucky" she said, "there was medication I could take because I was pregnant, and even medication I could change to when I needed to breastfeed my child."

But TB still claims tens of thousands of lives, and with a crucial need for new diagnostic and treatment strategies, the South African Government has set a goal to eliminate TB as a public health threat by 2030.

By making high-end microscopy more accessible, VP-CLEM-Kit is unlocking new opportunities for African researchers to help hit that target. ■

Samantha Reinders

WHY DOES THE Y MATTER?

Clare Green

Researchers in the Sex Chromosome Laboratory.

Dave Guttridge

The Y chromosome is emerging as a major player in male health, far beyond its reproductive role. Could the Y hold the key to ageing, disease, and even longevity?

For Crick geneticist James Turner, when it comes to the Y chromosome, sex is only the beginning of the story.

Both sex chromosomes – the Y and its partner X – originally evolved from a pair of autosomes – the name for the other 22 pairs of chromosomes. Then, over the course of evolution, the Y lost almost all of its genes, carrying just 2% of DNA in a male cell.

To date, most Y chromosome research has focused on understanding a single gene it carries, which is responsible for testis development, rather than the other genes scattered along its length.

So James, along with then-PhD student Jeremie Subrini, set about investigating the role of the other Y chromosome genes, initially focusing on their impact on fertility in male mice. They used genetic editing to 'knock out' Y genes one at a time, creating several genetically different groups of mice.

The researchers then looked at how reproduction was affected when a given Y gene was knocked out, including effects on sperm count, sperm appearance and function, and number of pups produced.

Some genes were indeed critical for reproduction, as knocking them out caused infertility or reduced fertility. But many of these genes simply had no impact on mice's ability to reproduce. "We could finally demonstrate that if you knock out some genes on the Y, testes aren't affected," says James.

Every cell in the body, from the heart to the brain to the lungs, contains sex chromosomes, too. And as men age, researchers have spotted an unexpected phenomenon: these cells can start to lose their Y chromosomes.

The Y chromosome's second act

"We've known about Y loss with age for a while but we didn't really think it mattered. After all, it's needed for male reproduction; why would the Y be important for other areas?" muses James. "But over the past five years or so, a flurry of research has linked Y-loss with dementia, cancer and heart disease. Sadly, it looks like men losing their Ys are more likely to die younger."

It's both a problem and an opportunity. If scientists can work out what Y genes are doing in other cells in the body, can they see a pattern? Could these lost genes ever be replaced by genetic editing?

"The ultimate goal would be to identify a gene which, if lost, increases the risk of dementia, for example," James says. "Then maybe we could work out how the loss of this gene causes disease and potentially prevent that happening."

He's now kick-started the process by re-investigating the same genetically engineered strains of mice. This time the team is giving them a full-body MOT, investigating what happens in their heart, lungs, immune system and brain, when each Y gene is knocked out.

> **"Sadly, it looks like men losing their Ys are more likely to die younger."**

James's journey is a classic example of scientific serendipity – research can open doors into new and unexpected areas. He was investigating the Y's role in fertility long before groups looking at cancer and other diseases became interested in the punch this tiny chromosome packs.

"We've successfully outlined the role of genes on the Y in mouse fertility, critical information given one in six couples struggle to conceive," says James. "But it has also sparked interest in a new horizon of sex chromosome research: the role these genes play throughout the body." ■

HOW TO BUILD A HUMAN

Kathryn Ingham

All building work requires a site manager to coordinate construction, and a developing embryo is no exception.

During the earliest stages of human development, just three weeks after fertilisation, a crucial rod-like structure appears along an embryo's length: the notochord. This biological scaffold releases signals essential for instructing cells how to develop, as the embryo's spine and nervous system take shape.

Despite its critical role, exactly how the notochord forms has been a mystery that researchers, including the Crick's James Briscoe, have been trying to crack.

"Until now, it's been difficult to grow notochord tissue in the lab," says James. "This makes it hard to understand the complex processes that shape our bodies and understand medical conditions that arise from developmental disorders."

A puzzle of timing and sequence

James and his team have been working out how to grow the notochord from human stem cells. First, they studied development in a range of vertebrate species, including chickens, establishing the sequence of molecular signals that constitute the biological 'instruction manual' for the notochord.

Next, they introduced these signals to human stem cells, in the right order, and watched as they developed into a tiny scaffold, just over a millimetre in length. Importantly, this structure contained developing neural tissues and bone stem cells in the correct locations for embryonic growth.

Blueprint for the body

Researchers are now equipped with a model to observe early human development unfold in the lab. It's a profound shift for scientists studying developmental conditions, including scoliosis or spina bifida, and also degeneration of disks in the spine.

"The notochord defines human architecture, orchestrating the delicate and precise cell changes required to create a body," says James. "We hope our discovery is a vital piece of the puzzle in modern developmental biology." ■

Electron scanning microscopy image showing a very detailed look at pieces of trunk organoids.

HIJACKED HEALING

Kathryn Ingham

Scars and scabs are visible examples of the body's fascinating ability to regenerate. After injury, chemical messages provoke an influx of new cells to repair the damage, provoking the development of replacement cells and new blood vessels.

These phenomena – chemical signalling, migrating and dividing cells, and new blood vessel growth – are also hallmarks of cancer. So, one way to view cancer is the result of a healing process gone wrong, and this may explain how cancer cells can hijack the powerful biology of wound healing to create environments in which they can grow.

Of the around 30 trillion cells in the human body, the vast majority stay put, held in place by strict molecular instructions. Ilaria Malanchi and her team at the Crick are trying to decipher those instructions, to understand how cancer cells interact with and corrupt healthy cells, enabling their spread.

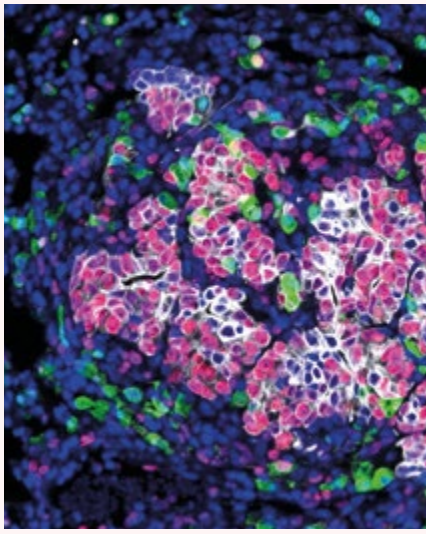
"Cancer affects more than just the part of the body where it first developed, it's a systemic disease that sparks changes to many aspects of our biology, even at the very earliest stages," Ilaria says.

Rewiring our defences

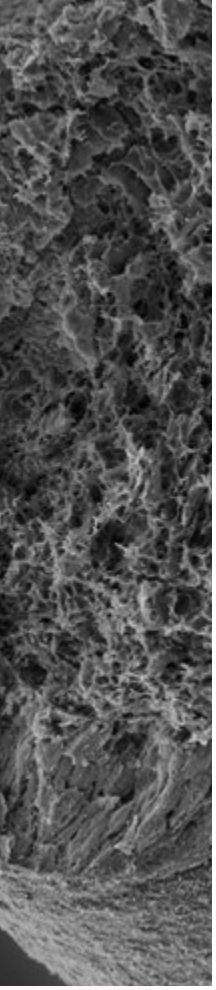
Her team recently examined what happens as breast cancer cells spread and reach the lungs in mice. They found that cancer cells prompt alveolar cells – normally the site of oxygen exchange – to de-specialise and enter an 'injury repair' state. This releases chemical messages that allow the cancer cells to successfully grow and divide in the lungs, effectively 'hijacking' the wound-healing processes.

This work will help researchers understand why some cancer types are more likely to spread, why some cancer types commonly spread to specific organs, and pin down the precise biological signals associated with these changes.

"If we think of cancer as hijacked wound healing, we can bring a sense of order to the apparent unpredictability of cancer spread, and maybe even prevent it," says Ilaria. ■



Cancer metastasis in the lung. Cancer cells can be seen in white, with proliferative cells in pink and type 2 lung alveolar cells in green.



Tiago Rito, Marie-Charlotte Domart

BIOLOGICAL ENIGMA

Clare Green

Borrowing from wartime cryptography, a new gene therapy uses cellular 'passwords' to unlock treatment only where it's needed.

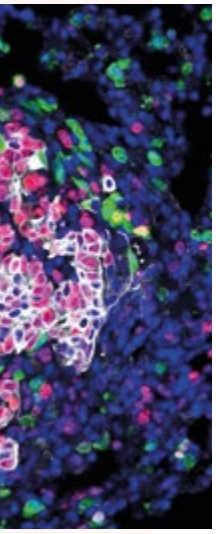
During the Second World War, German forces used the Enigma code to encrypt secret messages sent over radio waves. Famously broken by mathematicians including Alan Turing, the Enigma machine scrambled communications so only those with the right tools and settings decoded the message.

A similar challenge faces drug developers today – how to 'transmit' medicines around the body that are only 'understood' by cells that need treatment, leaving healthy cells unaffected.

This is the principle for an innovative technique by Crick and UCL researchers Pietro Fratta and Oscar Wilkins. They're developing a gene therapy (a method that introduces new DNA into cells to correct defects) which is only activated in certain cells. Essentially, it's encrypted.

The therapy is first being tested in cells affected by motor neurone disease (MND), a rare but devastating disease that causes a gradual decline in a person's ability to move and control their body, and eventually fatal paralysis. There's no cure, and current treatments only modestly slow down the disease.

"MND affects motor neurons, which are essential for us to move, speak, swallow and breathe, so they're probably the most critical cells in our body," says Oscar. "Despite this, less than 0.00001% of someone's cells are affected. The challenge is targeting treatments to this minuscule fraction of affected cells while avoiding the 99.99999% of healthy cells."



Tumour-Host Interaction Laboratory

Finding the right decryption tool

Pietro and Oscar centred their approach around a protein called TDP- 43, known to be involved in MND. In healthy cells, the protein resides near the DNA, helping to correctly interpret genetic instructions. But in cells affected by MND, TDP-43 clumps together in distant parts of the cell.

"Under normal circumstances, TDP-43 ensures that the DNA gets correctly 'photocopied' into RNA, the messenger carrying the instructions to make proteins," says Pietro. "But when TDP-43 clumps together, random sequences, called 'cryptic exons', get included in the RNA. This means that a wide range of proteins become faulty, with serious consequences."

"TDP-43 is often described as a 'Goldilocks' protein," says Oscar. "Its levels have to be just right. We can't allow the encrypted message to be read everywhere because there would be too much TDP-43 in otherwise healthy cells."

Pietro and Oscar have found an ingenious way around this. The gene therapy is activated by the presence of cryptic exons, which are only found in cells where TDP-43 has stopped working – in other words, cryptic exons act as a decryption tool.

Because the gene therapy restores levels of functioning TDP-43, it can then stop the decrypted message from being read further – a very smart way of self-regulation that may stop potentially toxic side effects.

"Focusing on MND is just a start," says Pietro. "We could tweak the method for other neurological conditions involving TDP-43."

Whether in neurodegenerative disease, cancer or heart disease, a key challenge is to find something unique about the diseased cells to exploit. And Pietro and Oscar's encryption system is, like the messages sent over radio waves using Enigma, a way to deliver a therapy that only cells with the correct decryption tools can use. ■

ANCIENT VIRUSES

Tom Calcraft

Retroviruses are viruses that have evolved the ability to write their genetic code into a cell's own DNA. The oldest known lineage of retroviruses, foamy viruses, emerged around 450 million years ago, before animal life moved onto land or even the evolution of trees.

Viral therapies

Today, scientists are harnessing this ancient ability to rewrite cells' DNA, in order to engineer retroviruses as cutting-edge gene therapies.

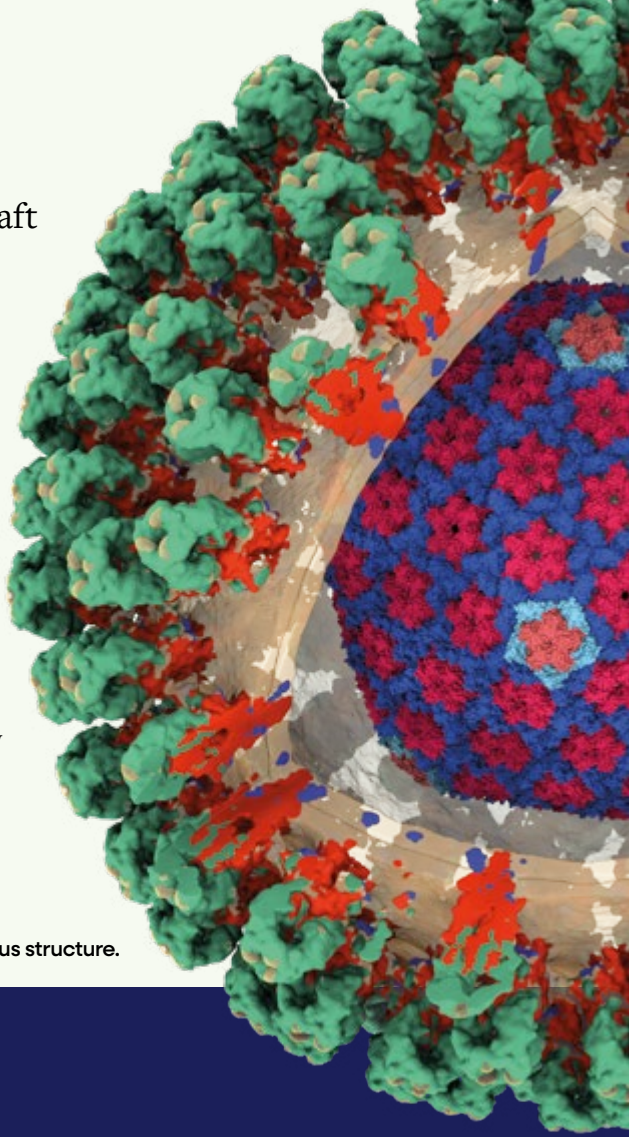
Foamy viruses can infect a wide range of cells and aren't known to cause disease so they're promising candidates for retrovirus-based therapies. But there is more we need to know about how they work, including how they infect cells, before we can realise that potential. That's where our team comes in.

Structural similarities

We have been studying foamy viruses using a powerful imaging technology called cryogenic electron microscopy (cryoEM). This allows us to better understand mechanisms of infection.

We recently revealed an unexpected connection: the surface proteins that foamy viruses use to enter cells appear to be structurally related to those of important pathogenic respiratory viruses, including modern viruses such as SARS-CoV-2.

This suggests that these viruses work in similar ways, revealing clues about how one might block modern viruses from infecting cells and advancing work to engineer foamy viruses to treat genetic diseases and cancer. ■



Tom Calcraft

Cryo electron microscopy image of the foamy virus structure.

GENETIC TIMELINES

Kathryn Ingham

In many aspects of our lives, we find meaning in the order in which events occur, making assumptions of the 'middle child' and talking of calm before storms. But when it comes to biology, there's an undeniable biological order driving diseases like cancer, where the sequence of molecular events can shape a patient's future, often before they've been diagnosed.

Each tumour is a unique patchwork of cells carrying different genetic mutations, evolving over time. It's why people diagnosed with the same type of cancer can experience wildly different outcomes.

Modern DNA sequencing technologies are uncovering a crucial additional layer of complexity; it's not just the presence of mutations in a tumour that affect its behaviour, it's the order in which they occur.

Take 'tumour suppressor' genes, the biological safeguards that prevent cells from growing without control. New research suggests that if loss of a tumour suppressor occurs at a particular time in a tumour's development, it can have an unexpected protective effect.

"It can be tempting to look at cancer mutations as good or bad, black or white," says Francesca Ciccarelli, who leads a research team at the Crick and Queen Mary University of London. "But that's not always the case."

Sequence shapes survival

Her team examined a tumour suppressor gene, *CDKN2A*, which is commonly mutated in a pre-cancerous condition of the oesophagus – Barrett's oesophagus.

They found losing this gene doesn't always trigger cancer, and can sometimes prevent Barrett's progressing. This is because cells that lose *CDKN2A* cannot tolerate subsequent loss of another key gene *TP53*, which is essential for Barrett's to progress.

But if the order is swapped and *CDKN2A* is lost *after* the cancer has already lost *TP53*, *CDKN2A* loss fuels growth of more aggressive tumours in the oesophagus.

This highlights how tracing the evolutionary history of cancers is key to identifying vulnerabilities in the course of disease, when treatments may be most effective or doctors could intervene to stop cancer developing in the first place. ■

Tell us what you think

Help us shape future issues of this magazine.

The Francis Crick Institute is a unique partnership between:

CANCER RESEARCH UK
IMPERIAL
KING'S College LONDON
UCL
UKRI Medical Research Council
wellcome

Thanks to Xolelwa Mabokela (pictured), Nobuhle Manqina, Ntombizanele Mabumbulu and Samantha Nongogo, and all at Eh!woza, a non-profit organisation based in Khayelitsha, Cape Town, working with communities with high rates of HIV and TB to raise awareness of the social impact of disease.

Samantha Reinders


THE
FRANCIS
CRICK
INSTITUTE