OUR INNER TAILS

PHILIP BEALES

NewScientist
INSTANT EXPERT
36

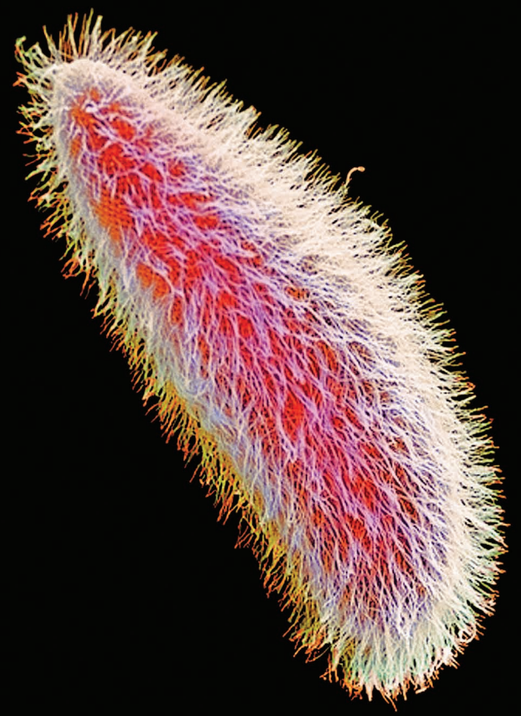
Cells' tails are not just for swimming, they also have some very surprising roles

A ROTATING TAIL

Many bacteria have tails too. These bacterial tails (pictured left) look like smaller versions of the flagella of complex cells (bottom) and so were called flagella too, but that is where the similarities end. Bacterial tails are made of different proteins put together in a different way. They also vary a lot from species to species: of the 40 different kinds of protein found in *E. coli's* tail, for example, only half are common to all the other bacterial flagella studied so far.

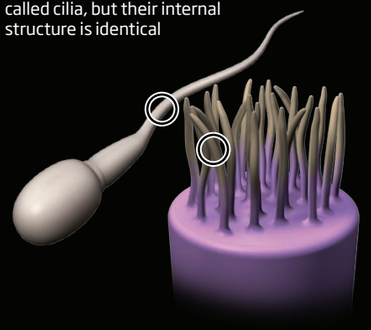
Whatever proteins they are made of, all bacterial flagella work in a fundamentally different way to eukaryotic tails: they rotate like a propeller, rather than beating back and forth. These flagella have a helical shape and revolve between 200 and 1000 times a minute, propelling bacteria as fast as 60 cell-lengths per second. A sprinting cheetah can achieve only about 25 body lengths in a second.

The third branch of life, archaea, also often possess rotating tails, but the similarity of archeal tails to those of bacteria may be a case of convergent evolution. In other words, cellular tails appear to have evolved independently on at least three occasions, in archaea, bacteria and eukaryotes.

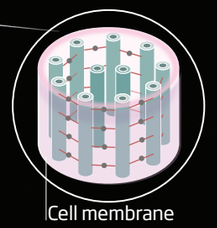


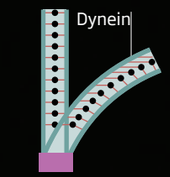
"Their body is furnished with diverse incredibly thin little feet, which were moved nimbly"

BEATING TAILS
Long tails like those of sperm cells are called flagella, while shorter ones like those on the cells lining our airways are called cilia, but their internal structure is identical

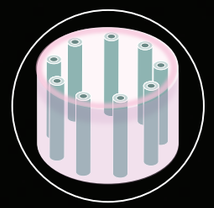


NON-BEATING TAILS
Almost all cells in the body have a single tail that only moves passively. The structure of primary cilia is slightly different to that of beating tails, with no central pair of microtubules. Their purpose long remained elusive





The beating motion is generated by the dynein arms linking the microtubules



PHOTOS: SCIENCE PHOTO LIBRARY

A SURPRISING TAIL

Think of a sperm cell. It consists largely of a tail, which wiggles steadily to propel the sperm in its quest for an egg. You might know that a few other kinds of human cells have tails, too. What will almost certainly surprise you is that of the hundreds of known types of human cells, more than 99 per cent have tails. For over a century, the purpose of most of these tails remained a mystery. Some dismissed them as mere relics, but it turns out tails play a major role in everything from development to learning and vision

THE TALE OF OUR TAIL

In 1648, at the age of 16, Antonie van Leeuwenhoek took up an apprenticeship with a textile merchant where he learned to count thread densities in cloth using some of the earliest magnifying glasses. Van Leeuwenhoek took to grinding his own high-quality lenses, achieving the highest level of magnification of his day – enlarging objects up to 270 times actual size. He had created the first microscopes.

One day, in his hometown of Delft in the Netherlands, van Leeuwenhoek placed a drop of water from a pond under one of his prototype microscopes. He was astounded to find it full of minute creatures darting here and there. He called them animalcules. He spent much time observing these animalcules over the following years, and saw that many swam by means of tiny beating tails or, as he called them in a letter to the Royal Society in 1676, "feet": "Their body is… furnifhed with divers incredibly thin little feet, which were moved very nimbly, and which I was not able to difcern till after feveral obfervations."

As microscopes improved, biologists discovered that all kinds of cells have beating tails. They are found not only on free-living "animalcules" and sperm, but also on the cells inside multicellular organisms. Some cells have short tails, between 2 and 15 micrometres long, which were called cilia after the Latin for eyelashes. Cilia do sometimes occur in rows like eyelashes, and can also occur more densely on part or all of the surface of a cell. In your body, up to 200 beating cilia are present on the exposed surface of each cell lining a liquid interface, such as in the windpipe.

The movement of these beating cilia is surprisingly complex. Somewhat like an arm of someone swimming breaststroke, a cilium usually goes through a sophisticated three-dimensional cycle that includes a power stroke and recovery stroke.

Some cells, such as sperm cells, have much longer tails – between 20 and 100 micrometres in length – which usually occur singly. These are called flagella, after the Latin for whip, and their motion is simpler: they move back and forth generating a two-dimensional wave-like motion.

RIGHT: BRIDGEMANART.COM FAR RIGHT: DR GOPAL MURTI/SPL

Despite their different appearance and motion, the beating cilia and flagella of all complex cells are structurally identical. Stretching the length of the tail is a hollow cylinder made of nine long fibres, or microtubules, joined along their length. In the centre, there is usually another pair of microtubules. The arms that link the microtubules together can "walk" up or down the adjoining microtubule, and this is what generates movement. The whole structure is enclosed by the cell membrane.

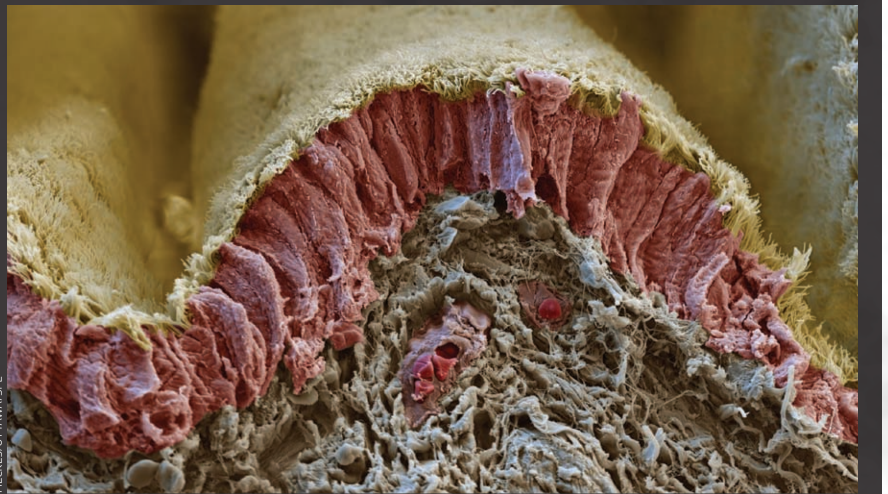
Tails with this characteristic structure are present in just about every kind of organism with complex cells, from single-celled protozoa in ponds to algae, fungi and animals. The only exceptions are most seed-producing plants, but as they still possess many tail-related genes their ancestors must have had them. So it seems clear that the last common ancestor of complex cells, or eukaryotes, must have possessed a beating tail.

While the purpose of beating tails is evident, in 1867 Alexander Kovalevsky reported that some cells in animals have a single small tail protruding from their surface that does not actively move. So what were they for? Swiss anatomist K. W. Zimmerman reported similar findings in mammalian cells in 1898 and suggested a sensory role. But these tails were so tiny they could barely be seen with optical microscopes, so they were hard to study. Most biologists paid no attention to them until the 1960s, when electron microscopes that could peer into their inner structures became available. Then the work of biologists Denys Wheatley, Barbara Barnes and others confirmed the presence of non-moving tails in various cell types including, intriguingly, nerve cells.

Sergei Sorokin at Harvard University, meanwhile, noticed that these non-beating tails were slightly different to moving tails – they lacked the central pair of microtubules found in beating cilia. He dubbed these tails "primary cilia", because they were the first kind of tails to appear on cells as rat lungs formed, ahead of beating tails.

Over the next few decades, much was learned about the development and structure of primary cilia. They were found in just about every cell type in the human body, apart from the hepatocytes of the liver. Despite their prevalence, some biologists dismissed them as evolutionary relics. Others thought it highly unlikely that these structures would be found in so many cell types if they did not do something useful. But what? In the 1990s, the burning question still remained: "What are primary cilia for?"

WAG THIS WAY



MECKES/OTTAWA/SPL

While non-beating tails have turned out to be crucial cellular sensors, beating tails also have unexpected roles. In our bodies, moving cilia (the brown "fur" pictured above) are found on the lining of many tubes and cavities. They help keep our windpipes and lungs clear by beating in unison 7 to 22 times a second to sweep mucus and particles along. They perform the same role in the nasal cavities and the Eustachian tube, which drains the middle ear. Beating cilia also circulate the cerebrospinal fluid around our brains and spine, and help move eggs along the fallopian tubes to the womb, where they can be fertilised by sperm swimming using their tails.

Impaired cilia cannot clear mucous properly, resulting in repeated infections of the sinus, throat, lungs and ears, and ultimately permanent lung damage. Smoking can damage cilia, for instance, while some individuals inherit genetic mutations that prevent cilia beating normally.

Very confusingly, inherited diseases caused by tails failing to beat properly are called primary ciliary dyskinesias, though they have nothing to do with primary cilia. As a rough estimate, around 1 in 32,000 people suffer from these disorders, but the true figure could be higher. In the most severe cases, the only treatment option is lung transplantation. With early diagnosis, regular physiotherapy can drastically reduce infections and slow the accumulation of permanent damage. Sadly, the disorders are often not diagnosed until a late stage.

Some people with faulty cilia not only suffer from recurrent infections and lung damage, but also have situs inversus, in which the position of the internal organs is reversed. This condition is known as Kartagener syndrome. Organs can develop in the opposite configuration – with the heart on the right and so on – without any health consequences. In other cases, not all the organs flip sides together, leading to serious problems such as heart defects or gut malrotation. For many decades, this remained a mystery. Why should people with faulty cilia also have reversed left-right symmetry?

We now know that the asymmetry of our bodies is established very early on. A few days after fertilisation of the egg, the embryo appears to be a symmetrical cylinder with a top and bottom but no left, right, front or back. Then, however, over the course of a few hours an asymmetry is established as genes are activated on one side but not on the other, and the shape of the embryo changes rapidly.

What triggers this? At one end of the embryo there is a pit, called the "node", which is lined with cells that each have a single beating tail on their surfaces. These tails all start to sweep in a rough circle in a clockwise fashion, and this motion generates a net leftward flow. This flow establishes which side is the left and which the right, and therefore the customary position of organs, called situs solitus. The clinching evidence came in 1998, thanks to some mice in which a gene related to the growth of cilia formation had been disabled. In these mice there were no cilia on the node cells and the establishment of left-right symmetry was random, with half having reversed symmetry.

Much still remains to be discovered, however. In particular, it is unclear how this leftward flow leads to asymmetric gene activation. One idea is that the flow creates an imbalance in the level of a signalling molecule, which may be detected by non-beating cilia on cells on the periphery of the node. Another idea is that the primary cilia on the periphery sense the direction of flow rather than a concentration gradient. Either way, the establishment of asymmetry would require both kinds of tails.

LINES OF COMMUNICATION

Two decades ago, some biologists were still arguing that the non-beating tails found on most cell types were evolutionary leftovers. No one would make that claim today. It is now clear that these primary cilia play a key role in the development and functioning of pretty much every organ in the body. Beating tails, too, have turned out to play a bigger role than previously appreciated. Much of the evidence has come from studying hereditary disorders caused by mutations in tail genes

"Our non-beating tails act not just as sensory antenna but also as communication hubs"

TAILS AS ANTENNAE

Why does nearly almost every kind of human cell in the body have a single, non-beating tail protruding from its surface? For decades many biologists dismissed these primary cilia as unimportant, but a few thought they must be useful. In 1985, for instance, Anthony Poole suggested that they were nothing less than "cellular cybernetic probes". And around the turn of the century, the evidence to support this view finally began to emerge.

Inside our kidneys, fluid flows through tiny tubules. The cells lining these tubes have non-beating tails protruding from their surface, that were spotted bending in response to the speed of flow. Further work showed that this bending produces a response in the cell, in the form of an influx of calcium ions.

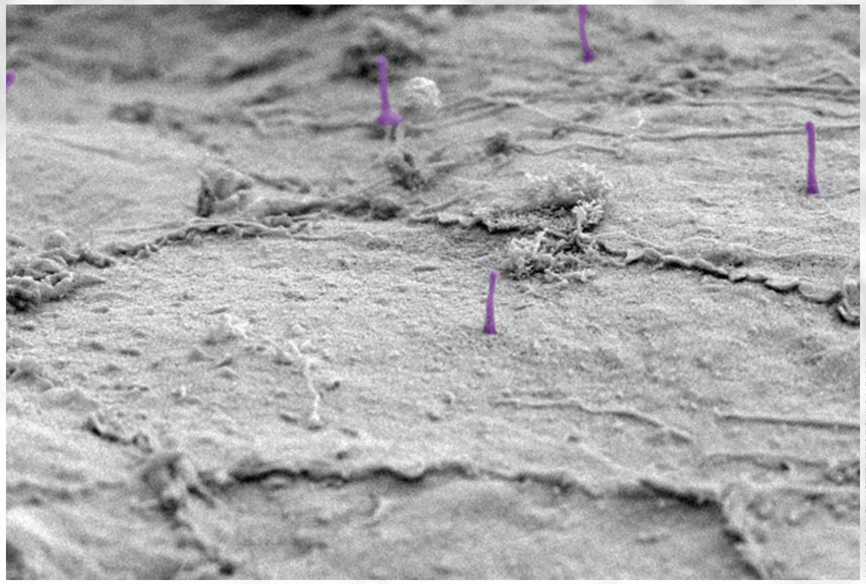
Meanwhile, other biologists had been studying a common genetic disorder known as polycystic kidney disease, in which large fluid-filled cysts form in the kidneys (pictured right), eventually destroying them. Its cause was traced to mutations in two proteins that form calcium channels, called polycystin-1 and 2. In 2002, it was shown that these proteins are usually found in the membrane of primary cilia, but are missing or misplaced in people with polycystic kidneys. These different strands of research pointed to a clear picture: kidney cells use their tails to sense fluid flow, and if these sensors are not working properly things go awry.

The findings made other biologists sit up and take notice. In the past decade, a series of landmark studies have shown that primary cilia can have all kinds of surprising sensory abilities. Besides fluid movement, some sense chemicals, osmotic concentration, temperature and even gravity. For instance, we detect odours through the many olfactory receptors on the primary cilia of olfactory neurons.

These sensory capabilities also turn out to be far more important than anyone imagined just a few years ago. The evidence comes from a growing list of diseases being traced to mutations in the genes coding for these sensors. The symptoms range from blindness to obesity to learning problems to kidney failure to short limbs to narrow ribs (which cause respiratory failure in infants).

The symptoms vary so much because primary cilia play a role in so many different processes. During embryonic development, for instance, cells are constantly on the move, migrating to form new tissues and organs. It is through cilia that these cells are able to sense their environment and so change their behaviour accordingly. Even in adults, the maintenance of organ function requires continuous feedback. In bone and cartilage, for example, the primary cilia detect pressure, switching on the genes needed to maintain or strengthen these tissues.

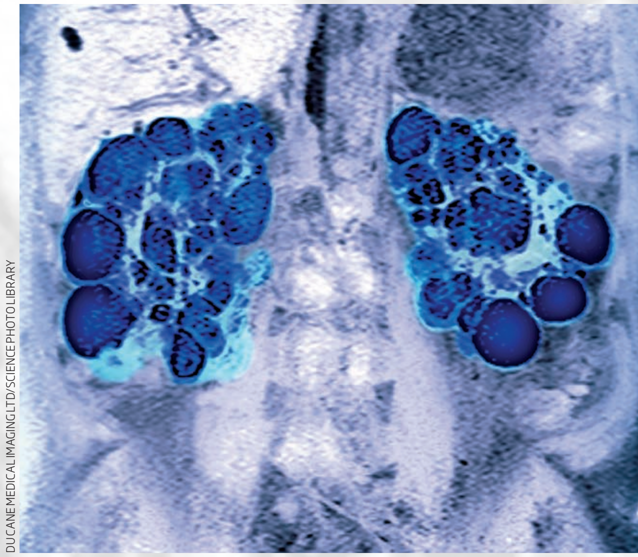
What's more, primary cilia are more than mere sensors. One way cells talk to each other involves the so-called "hedgehog signalling pathways" – a chemical communication system that plays a vital role during development. It has recently become clear that in vertebrates, the hedgehog signalling system depends on primary cilia, with many parts of this system being found on these tails. Our inner tails, then, are not just sensory antennae but also communication hubs.



These tiny tails, called primary cilia, are highly sophisticated sensors

PATRICIA S. CONNELLY/NATIONAL HEART, LUNG, AND BLOOD INSTITUTE; NATIONAL INSTITUTES OF HEALTH; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

ANA ROLO, UCL INSTITUTE OF CHILD HEALTH



Kidney trouble: cysts filled with fluid form when cells have faulty tails

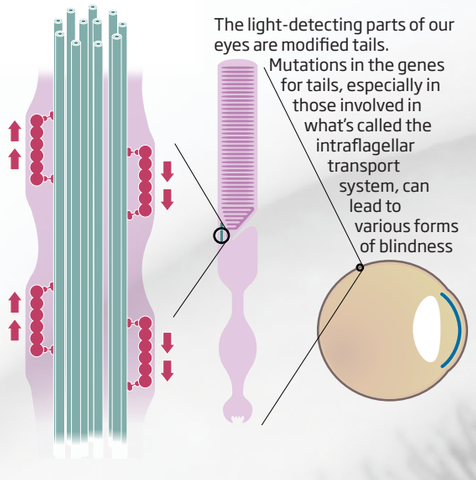
DUCANE MEDICAL IMAGING LTD/SCIENCE PHOTO LIBRARY

THE TALE HAS ONLY JUST BEGUN

Some of our inner tails still do what you expect a tail to do: wag. But the solitary tail found on most cells cannot actively move. Instead, these tails have taken on a very different role as cellular sensors. They play a role in the development and maintenance of all kinds of tissues, and even in learning and memory. As a result, mutations that impair the function of tails or prevent them from developing altogether can have serious consequences, from cognitive defects to blindness. While genetic diseases by their very nature are difficult to treat, developments in other fields and the rapid advances in our understanding of these diseases are opening up new possibilities

A MOST EXTRAORDINARY TAIL

Our inner tails have a sophisticated transport system. Self-assembling vehicles haul their cargoes up and down the microtubules like a train on a track



FRANK CHMURA/PLAINPICTURE

Growing a tail poses a challenge for cells. How do you get the building blocks of tails from the body of the cell, where they are made, to the tip of the tail where they are needed? Large molecules cannot move freely along cilia and flagella, so some kind of active transport is essential. The answer evolution came up with was a kind of train that travels up and down, using one of the microtubules that make up the tail as a rail.

These trains assemble themselves at the base of the tail and pull themselves along it, carrying cargoes such as microtubule components and receptors destined for the cell membrane. At the tip, they drop off their cargo and rearrange themselves, swapping "up tail" motors for "down tail" motors. They then move down the tail carrying cargoes such as signalling proteins destined for the cell interior.

This highly sophisticated "intraflagellar" transport system is essential for making and maintaining tails, and, through its role in carrying receptor and signalling proteins, for sensing the environment. Some recent studies suggest it may even play a direct role in some kinds of cell movement: the trains may attach themselves to proteins in the cell membrane that are in turn bound to something outside the cell. That means each train is anchored in place, so when the engine is active the trains remain stationary and the microtubule rail moves instead – and with it the cell.

Because tails play such a wide range of roles in the body, mutations that affect the intraflagellar transport system can produce a wide range of defects, from kidney diseases to developmental abnormalities and even various forms of blindness. But why should a faulty system for transporting things along tails lead to blindness? Well, it turns out that the light-detector in our eyes is essentially a highly modified tail.

The tip of this tail – the outer segment of photoreceptor cells – is greatly enlarged and contains all the light-detecting machinery. But the rest of it, the part that connects the outer segment to the main body of the cell, still consists of a narrow tube, known as the connecting cilium. The upshot is that all the proteins needed for detecting light have to be carried along this cilium to the outer segment.

Light-detecting proteins are frequently damaged, so there is a very high turnover of proteins in the outer segment. As a result, our vision depends on the intraflagellar transport system working well. A number of degenerative diseases that lead to blindness in childhood or adulthood, including those known as retinitis pigmentosa, have now been linked to mutations that disrupt the transport system, possibly leading to a buildup of toxic waste products.

While the precise mechanisms still aren't fully understood, treatments are already being developed for a number of these disorders. For instance, a team of doctors at London's Moorfields Eye Hospital and University College London is conducting the first human gene therapy trials to treat Leber's congenital amaurosis, a type of inherited childhood blindness caused by a single faulty gene that affects intraflagellar transport. The results so far have been promising.

"It turns out that the light detector in our eyes is essentially a highly modified tail"

HOW TAILS AFFECT OUR MEMORY AND LEARNING

It seems our inner tails play a key role in all kinds of processes, from development to vision. We can now add learning and memory to the list. Both primary and moving cilia are abundant throughout the brain, where they are found both on neurons and on the various kinds of support cells.

Somatostatin receptors located on brain-cell cilia are required for mice to learn to recognise new objects or to recall familiar ones. Mice without working receptors lose the ability to recognise objects they have seen before. Cilia in the part of the brain central to memory, called the hippocampus, are also required for the formation of adult neural stem cells. Without a fresh supply of stem cells learning is impaired. Mice with cilia dysfunction cannot find their way around.

Primary cilia are also important for the migration of brain cells and so are vital for the developing brain. Several inherited disorders caused by mutations in cilial genes are associated with cognitive defects. In Joubert syndrome, for instance, there are major posterior brain abnormalities resulting in muscle weakness, poor coordination and an abnormal breathing pattern.

VIKTOR PRAVDICA/ALAMY

Child's play: not so easy to figure out if brain cells lack the cilia they need for learning

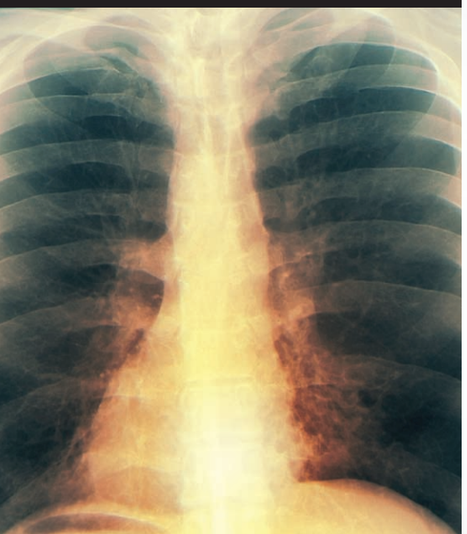
FIXING FAULTY TAILS

The list of disorders known to be caused by faulty tails, called ciliopathies, is growing rapidly. Because these disorders are caused by genetic mutations in the genes coding for cilia, there seemed little prospect of developing effective treatments. But the prospects have brightened with recent advances in biology.

In particular, it now seems likely that it will be possible to use gene therapy to prevent the degeneration of retinal cells leading to blindness, as caused by some cilial mutations. The cells in the retina are easily accessed for both treatment and monitoring, and several human trials are already in progress (see "A most extraordinary tail", right).

Using gene therapy to restore cilial function in solid organs such as the kidneys is a much greater challenge. However, in at least some cases it may be possible to find drugs to alleviate symptoms or even slow the progress of a disease. Some trials are now under way involving drugs already approved for other purposes. The use of such drugs is very attractive because their mode of action is usually known and they have already passed safety tests. Because cilial mutations can have very different effects in different tissues, some people might need to take a combination of drugs to treat their symptoms.

Last but not least, it might be possible to use small-molecule drugs – which can be swallowed in pill form – to at least partially compensate for the underlying genetic defects. An antibiotic called gentamicin can force cells to ignore mutations that halt protein production prematurely, before a protein is complete. This antibiotic has some nasty side effects, but newer, safer compounds have been developed and will be used to try to treat diseases such as Duchenne muscular dystrophy. Where ciliopathies are due to these kinds of mutations, this approach might work too.



ZEPHYR/SCIENCE PHOTO LIBRARY

Change of heart: faulty tail genes reverse organs and damage lungs in Kartagener syndome

Philip Beales

Philip Beales is professor of medical genetics at University College London's Institute of Child Health, and a consultant in clinical genetics. He is head of the Cilia Disorders Laboratory and Director of the Centre for Translational Genomics at UCL. He is co-editor of *Ciliopathies: A reference for clinicians* (Oxford University Press, 2013)

A CAUSE OF COMMON DISEASES?

In less than two decades, our view of our inner tails has been totally transformed. They have gone from being seen as minor players in a few tissues to playing a central role in the development and maintenance of just about every part of the body.

To date, about 30 disorders have been traced to mutations that affect the function of our cilia and flagella. With the exception of polycystic kidney disease, these ciliopathies are rare, affecting fewer than 1 in 2000 people. However, it is quite likely that the causes, at least in part, of some common diseases will also turn out to involve cilia. It may be that subtle variations in the genes that code for the building blocks of cilia contribute to these more common and familiar conditions.

For instance, cilia located on specialised neurons are important for the regulation of appetite, and severe obesity is one of the features of ciliopathies such as Bardet-Biedl syndrome and Alstrom syndrome. Although the precise cause of the obesity associated with these syndromes is still being investigated, there is nothing to suggest that this type of obesity is any different from more common forms that have reached epidemic proportions in Western society. Genetic studies also suggest that some cilial gene variants may be associated with obesity. To give another example, cilia located on cartilage in joints could be important for maintaining joint integrity and preventing arthritis.

Cilia may also play a role in some kinds of cancer and conditions that can lead to cancer, such as von Hippel-Lindau and Birt-Hogg-Dubé syndromes. As we have mentioned, the "hedgehog signalling system", so important during development, involves cilia. And it is these pathways that are disturbed in certain types of cancer, including medulloblastoma, pancreatic cancer and basal cell carcinoma. Although further work is required, cilia biology may yet provide important therapeutic opportunities for these types of tumour.

RECOMMENDED READING

Ciliopathies: A reference for clinicians (Oxford University Press 2013)

A systems-biology approach to understanding the ciliopathy disorders (***Genome Medicine***, vol 26, p 59)

Ciliopathies (***New England Journal of Medicine***, vol 364, p 1533)

WEBSITES

Ciliopathy Alliance, www.ciliopathyalliance.org

Cover image

G. Moscoso/Science Photo Library