THE *SINS* OF THE FATHER

The roots of inheritance may extend beyond the genome, but the mechanisms remain a puzzle.

BY VIRGINIA HUGHES

When Brian Dias became a father last October, he was, like any new parent, mindful of the enormous responsibility that lay before him. From that moment on, every choice he made could affect his newborn son's physical and psychological development. But, unlike most new parents, Dias was also aware of the influence of his past experiences — not to mention those of his parents, his grandparents and beyond.

Where one's ancestors lived, or how much they valued education, can clearly have effects that pass down through the generations. But what about the legacy of their health: whether they smoked, endured famine or fought in a war?

As a postdoc in Kerry Ressler's laboratory at Emory University in Atlanta, Georgia, Dias had spent much of the two years before his son's birth studying these kinds of questions in mice. Specifically, he looked at how fear associated with a particular smell affects the animals and leaves an imprint on the brains of their descendants.

Dias had been exposing male mice to acetophenone — a chemical with a sweet, almond-like smell — and then giving them a mild foot shock. After being exposed to this treatment five times a day for three days, the mice became reliably fearful, freezing in the presence of acetophenone even when they received no shock.

Ten days later, Dias allowed the mice to mate with unexposed females. When their young grew up, many of the animals were more sensitive to acetophenone than to other odours, and more likely to be startled by an unexpected noise during exposure to the smell. Their offspring — the 'grandchildren' of the mice trained to fear the smell — were also jumpier in the presence of acetophenone. What's more, all three generations had larger-than-normal 'M71 glomeruli', structures where acetophenone-sensitive neurons in the nose connect with neurons in the olfactory bulb. In the January issue of *Nature Neuroscience*[1], Dias and Ressler suggested that this hereditary transmission of environmental information was the result of epigenetics — chemical changes to the genome that affect how DNA is packaged and expressed without altering its sequence.

Biologists first observed this 'transgenerational epigenetic inheritance' in plants. Tomatoes, for example, pass along chemical markings that control an important ripening

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gene[2]. But, over the past few years, evidence has been accumulating that the phenomenon occurs in rodents and humans as well. The subject remains controversial, in part because it harks back to the discredited theories of Jean-Baptiste Lamarck, a nineteenth-century French biologist who proposed that organisms pass down acquired traits to future generations. To many modern biologists, that's "scary-sounding", says Oliver Rando, a molecular biologist at the University of Massachusetts Medical School in Worcester, whose work suggests that such inheritance does indeed happen in animals[3]. If it is true, he says, "Why hasn't this been obvious to all the brilliant researchers in the past hundred years of genetics?".

One reason why many remain sceptical is that the mechanism by which such inheritance might work is mysterious. Explaining it will require a deep dive into reproductive biology to demonstrate how the relevant signals might be formed in the germ line, the cells that develop into sperm and eggs and carry on, at a minimum, a person's genetic legacy.

A mother might pass on effects of environmental exposures to a fetus during pregnancy. So, to study the phenomenon of transgenerational epigenetics cleanly, biologists are focusing on fathers, and have been looking at how sperm might gain and lose epigenetic marks. "In the past two to three years there's been a lot of new information," says Michelle Lane, a reproductive biologist at the University of Adelaide in Australia. But proposals for how it all works are themselves embryonic. "It's a huge black box," Lane says.

MONSTER PLANTS AND OBESE CHILDREN

The epigenetics revolution hit in the early 2000s, when scientists began reporting that environmental factors — everything from neglectful mothering and child abuse to a high-fat diet and air pollution — can influence the addition or removal of chemical tags on DNA that turn genes on and off. This idea of an environmentally responsive genome still stirs debate (see *Nature* **467,** 146–148; 2010). But the notion that epigenetic marks are transmitted across generations is even more provocative.

Swedish botanist Carl Linnaeus was among the first to spot changes resulting from this phenomenon. In the 1740s, he received a plant specimen that looked very similar to common toadflax (*Linaria vulgaris*), but with very different flowers. Linnaeus was shocked because this challenged his theory that plant species could be categorized by the structure of their flowers. "This is certainly no less remarkable," he wrote, "than if a cow were to give birth to a calf with a wolf's head." He named the plant *Peloria*, after the Greek word for 'monster'.

In the 1990s, plant biologist Enrico Coen at the John Innes Centre in Norwich, UK, found that in the monster plants, methyl groups litter a gene involved in flower structure called *Lcyc*, completely shutting it down. (DNA methylation usually turns genes off.) Coen's team also showed that these methyl marks pass through seeds to later generations[4].

The public first started to take notice in the mid-2000s, after large epidemiological investigations in Europe began to show transgenerational effects in humans. One study of Swedish historical records showed that men who had experienced famine before puberty were less likely to have grandsons with heart disease or diabetes than men who had plenty to eat[5]. Similar work with children in Britain reported in 2005 that fathers who had started smoking before the age of 11 had an increased risk of having boys of above average weight[6].

> "WHY HASN'T THIS BEEN OBVIOUS TO ALL THE BRILLIANT RESEARCHERS IN THE PAST HUNDRED YEARS OF GENETICS?"

But many scientists remained sceptical. Epidemiological studies are often messy, and it is impossible to rule out all confounding variables. In the past few years, however, several studies in rodents have supported these observations and begun to attribute the transmission of various traits to changes in sperm.

SPERM SIGNATURES

Male rats fed a high-fat diet, for example, beget daughters with abnormal DNA methylation in the pancreas[7]. Male mice fed a low-protein diet have offspring with altered liver expression of cholesterol genes[3]. And male mice with prediabetes have abnormal sperm methylation, and pass on an increased risk of diabetes to the next two generations[8].

"We and many other people have now shown these paternal effects," says Rando, who led the low-protein study. "And we're all having a hell of a time figuring out how they work."

The animal studies have triggered some strong debate. The most controversial results have come out of Michael Skinner's lab at Washington State University in Pullman. Skinner's team exposed pregnant rats to large doses of pesticides and fungicides, which led to organ damage in their adult offspring. The sperm of male offspring showed changes in DNA methylation that persisted for at least four generations[9].

But at least two groups failed to replicate the data, and in 2010, federal investigators found that one of Skinner's postdocs had fabricated data for a related paper, which the authors had retracted in 2009. Skinner says that some teams have replicated his results, and that those who have not were using inappropriate protocols. Last year, his own team reported successfully reproducing the results of the retracted paper[10].

METHYLATION MECHANISM

Explaining how transgenerational epigenetics works has been difficult in part because most studies track outcomes — such as changes in glucose, cholesterol and fertility — that can be affected by a range of factors, making it tricky to tease out cause and effect. By contrast, Dias and Ressler's work with acetophenone takes advantage of specific biology: the chemical binds to a particular receptor in the nose that is encoded by a single gene, dubbed *Olfr151*. "This is the massive pro of their study," Rando says.

Dias and Ressler do not claim to understand exactly what is going on, but they do have a working hypothesis. Somehow, the information about the frightening smell gets into a mouse's testes and results in lower methylation of the *Olfr151* gene in sperm DNA. The researchers even ran experiments using *in vitro* fertilization to make sure that the father was not in some way passing on a fear of acetophenone through interactions with the mother. The epigenetic tweak in the sperm is perpetuated in the offspring's DNA, leading to increased expression of the receptor in the animals' noses and, ultimately, enhanced sensitivity to the smell.

But the chain of causation is loose. "There are a lot of disconnects there," says William Kelly, a developmental geneticist at Emory. "It's not beyond the realm of possibility or plausibility. It's just right now we don't know enough about how information is transferred between generations."

The first question is how the effects of environmental exposure become embedded in an animal's germ cells — in this case, the mouse's sperm. Germ cells have been shown to express olfactory receptors[11]. So it is possible that *Olfr151* receptors in sperm respond to odorant molecules in the bloodstream and then change the methylation of the corresponding gene in sperm DNA.

Alternatively, after being exposed to the odour and the pain, a mouse might produce RNA molecules — perhaps in the brain — that make their way into the bloodstream and then selectively target the *Olfr151* gene in sperm. Many studies in plants have hinted at this sort of systemic RNA shuttling. RNA molecules expressed in a plant's leaf, for example, can travel through its vascular system to many of its other tissues and affect gene expression[12].

But creating an epigenetic mark in the sperm is only the first step. To pass down through multiple generations, the signal needs to survive multiple rounds of rigorous

epigenetic reprogramming. In mammals, the first of these happens just hours after conception, when most methylation is stripped from sperm DNA in the single-celled embryo. Then, as the embryo develops and divides, and cells begin to differentiate into various tissue types, methylation is gradually re-established. But even if some signal from the father were to survive this process, the embryo's own primordial germ cells, those that eventually become its sperm or eggs, undergo a second round of epigenetic scrubbing (See 'Without a trace').

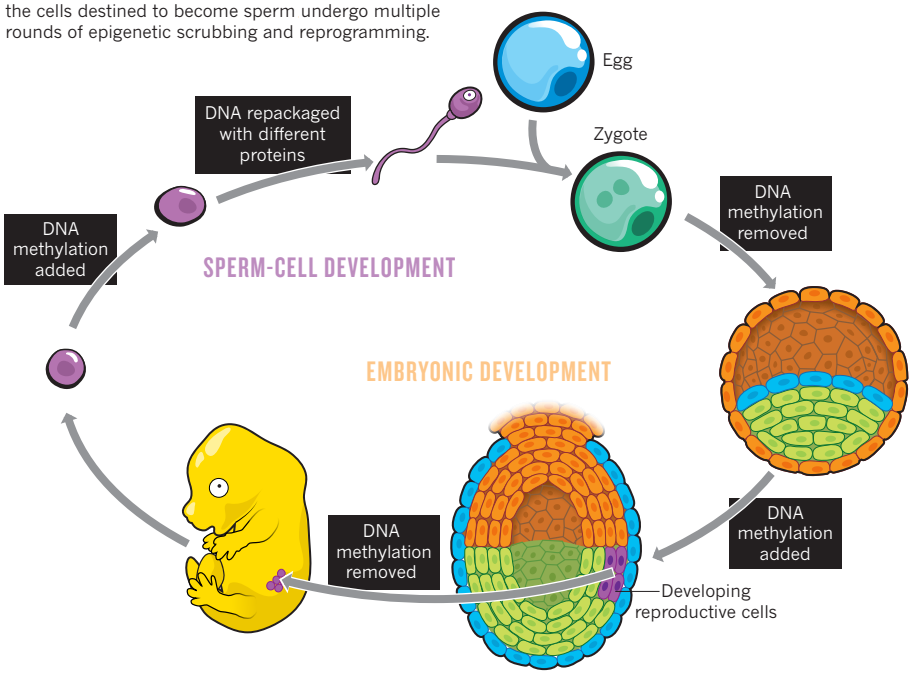
Some genes manage to escape these periods of major reprogramming. The best example is genes that are imprinted — whereby one copy from the mother or father is robustly methylated and effectively silenced. These silencing marks crop up in the egg or sperm and are retained in the embryo.

About 100 genes are known to be imprinted, but some non-imprinted genes may also escape the scrubbing through a similar mechanism. "There is a growing consensus that there are more regions than previously thought that escape reprogramming in sperm," says Sarah Kimmins, an epigeneticist at McGill University in Montreal, Canada. "Why this is, and how, is not yet known, although studying imprinted genes may reveal clues."

Then again, even if *Olfr151* does escape reprogramming, it is hard to explain how that could lead to a noticeable difference in the behaviour of fully formed offspring. Dias and Ressler reported that in sperm samples from mice trained to fear acetophenone, about 86 out of every 100 sperm show *Olfr151* methylation, whereas in mice trained to fear a different odour it is about 95 out of every 100. This difference is statistically significant, but fairly small. And yet the behavioural effects in the second generation were robust: about half of the acetophenone-trained animals' offspring showed increased sensitivity to the odour.

WITHOUT A TRACE

Researchers are struggling to understand how epigenetic marks, such as DNA methylation, could pass from one generation to the next in mammals. During development, the cells destined to become sperm undergo multiple rounds of epigenetic scrubbing and reprogramming.



'SOMETHING GOOFBALL'?

Although many are scratching their heads over the holes in the proposed mechanism, few are suggesting that the underlying phenomenon is a fairy tale. "Impossible things are happening every day," says Kelly, quoting a line from Rodgers and Hammerstein's *Cinderella*.

It is possible, for example, that the DNA-methylation tweaks reported in the odour study are simply a by-product of an altogether different mechanism.

One route might be chemical marks on histones, the proteins around which DNA wraps. Acetyl and methyl groups can attach to histones and affect the expression of nearby DNA. But during sperm-cell formation, DNA is stripped of most of its histones (and their attendant marks) and wraps instead around protamines, which pack it more tightly.

Nevertheless, about 10% of human histones — and about 1% of mouse ones — are retained. These sites might carry information from one generation to the next. In 2011, researchers reported that, in nematode worms, certain histone marks correlate with long life and can be passed down through several generations[13]. And last December, Kimmins and her colleagues showed that feeding male mice a diet low in folate — a nutrient that provides the raw materials for methylation — led to significantly reduced methylation of histone proteins in the animals' sperm and more birth defects in their offspring[14].

Still other studies point to a mechanism involving short RNA molecules latching on to DNA and affecting gene expression. Twenty-eight microRNAs are expressed differently in the sperm of men who do and do not smoke, according to a study reported in 2012 (ref. 15). And these RNA patterns may persist through multiple generations. Last year, Lane's group found that obese male mice show abnormal expression of 11 microRNAs in their sperm — and that they pass on insulin resistance to the next two generations[16].

Then there is the possibility that the mechanism is, as Rando puts it, "something goofball". That might be prions — misfolded proteins that act as infectious agents — which have been shown to transmit heritable traits in budding yeast (see *Nature* **482,** 294–296; 2012). Or it could be something in semen besides sperm. Researchers reported in January[17] that mice born of fathers lacking seminal vesicles are fatter and have more metabolic problems than controls, suggesting that molecules in seminal fluid influence gene expression in sperm and the female reproductive tract.

If the mechanism involves DNA methylation, histones or RNA, the field is likely to make great progress in the next few years, Rando predicts. "But if it's something completely novel," he says, "Maybe it will take decades to figure out."

Dias has his fingers crossed for the former. He is going to Boston, Massachusetts, in April for a Keystone meeting on epigenetic inheritance, to get a sense of the most promising mechanistic avenues to follow. "If science has taught me anything," he says, "it is to not discount the myriad ways of becoming and being." ■

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