GENETICS

Sex and the single cell

Sex chromosomes in every cell of the body exert widespread and sometimes unexpected effects.

BY CLAIRE AINSWORTH

It was the mouse equivalent of the midnight munchies. Instead of sleeping normally, Karen Reue's lab mice were waking early and nibbling on extra snacks, which was making them obese. On investigation, she was surprised to find that the probable reason for this out-of-hours feeding was the genetic sex of their cells — the number and kind of sex chromosomes they contain. "It wasn't at all what we expected," says Reue, a geneticist at the University of California, Los Angeles (UCLA).

The idea that our body cells have a 'sex', and that this property has consequences for our health, has taken biologists by surprise. Experiments performed in the mid-twentieth century had implied that the hormones produced by the ovaries or testes were the source of physiological differences between males and females. But Reue's findings are part of a growing body of evidence showing that hormones are only part of the story. It now seems that the genetic sex of cells is crucial too. Cellular sex

may also help to explain why women and men have different susceptibilities to conditions such as obesity, heart disease, neurodegeneration, autoimmunity and cancer, and why such conditions can behave differently in the two sexes. Certainly, when it comes to metabolism, "there is a huge consequence to having two X chromosomes versus an X and a Y throughout your whole body," says Reue.

Researchers are using state-of-the-art molecular biology to delve into the sex chromosomes, particularly the X. Their findings are feeding into a new way of thinking about how the X and Y chromosomes make the two sexes different. Rather than being the result of one or two genes acting early in the development of the ovaries and testes, sex seems to emerge from an ongoing, complex balance of interactions between many genes throughout the body. Some of these genes counterbalance each other, with those tipping the balance towards traits typical of males being offset by genes that favour female-like physiology. This view is informing new approaches to

understanding sex differences in disease. "In disease, one of the sex-biasing factors may be suppressed, leaving the others to have a bigger effect," explains Art Arnold, a biologist at UCLA who has pioneered much of the work into the effects of cellular sex.

X MARKS THE SPOT

Differences in the susceptibility of the sexes to disease can be dramatic. About 80% of people with autoimmune disorders in the United States are female. Women tend to have a stronger immune response to infection, and often produce more antibodies in response to vaccination than men. Men are more cancer prone, are twice as likely to die of malignant disease, and respond differently to cancer therapy. But more women than men die from cardiovascular disease, and become obese.

Sex hormones undoubtedly have a role in these differences. Experiments carried out in the first half of the twentieth century showed that the X and Y chromosomes dictate whether the developing gonads in a mammalian embryo

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become ovaries or testes. Once the sex of the gonads was set, it seemed that the sex chromosomes' job was done. Everything that followed, including development of the genitals, puberty and sex-specific adult physiology, was apparently due to hormones secreted by the gonads.

But hints began to emerge that this scheme was too simplistic. Researchers noticed, for example, that male and female wallaby embryos began to develop genitalia before their gonads started to make hormones. Others observed differences in gene activity between XX and XY rat neurons growing in lab dishes, free from the effect of gonadal hormones.

Striking evidence came in the form of a peculiar zebra finch that arrived in Arnold's lab in the early 2000s while he was studying the neurobiology of birdsong. Only male finches chirp a courtship song, and the neural circuits that control this behaviour are much bigger in males than in females. In that particular finch, however, a rare quirk of development meant the right side of the bird was genetically male, whereas the left side was genetically female (see image on page S8). In its brain, the neural circuits controlling singing were larger on the male side, and smaller and less 'masculine' on the female one. Yet both sides of the brain had been bathed in the same cocktail of gonadal hormones during development. The reason for the differences had to lie in the chromosomes.

It is difficult to do genetics experiments in birds, and their sex chromosomes are quite

different to those of mammals. To investigate further, Arnold and his team turned to the more tractable laboratory mouse. Mice, similar to humans, are female if they have two X chromosomes (XX) and male if they have an X and a Y (XY). Arnold and his colleagues used a strain of mouse that could exist as typical XX females and XY males, but could also be XX with testes (and develop as a male) or XY with ovaries (and develop as a female). By comparing mice with the same chromosome set but different gonads, the team could start to distinguish which physiological differences were caused by hormones and which were caused by chromosomes. What's more, removing the gonads of the adult mice allowed researchers to see the chromosomal effects after withdrawing the influence of gonadal hormones.

Arnold's initial studies suggested that the X and Y chromosomes can indeed generate sex differences in their own right. His team found that the chromosomes can help to explain, for example, sex differences in specific brain structures and, in collaboration with UCLA colleague Rhonda Voskuhl, the greater incidence of autoimmune disorders in females.

A chance meeting between Arnold and Reue, who studies fat tissue, made the two scientists wonder whether the sex chromosomes might also affect metabolism. Working with Arnold's mice, Reue found that having two X chromosomes caused mice to get fatter than XY mice did after the gonads were removed. They determined¹ that the difference stemmed from the 'dosage' of the X chromosome — the presence or absence of a Y chromosome had little effect. The weight gain did not seem to be related, as Reue first suspected, purely to metabolic activity, but was also influenced by the intake of food — snacking during the usual hours of sleep.

If having two X chromosomes also has non-hormonal effects in humans, the finding could help to explain why women are more prone to obesity and often put on weight after the menopause, when levels of their sex hormones drop. Arnold speculates that this drop might expose the fat-promoting effects of the X chromosome, in a similar way to how removing the gonads uncovered X-chromosome-related obesity in the experimental mice.

Reue's team has also teased apart the effects of gonadal hormones and sex chromosomes on the kinds of cholesterol in mouse blood. "The X-chromosome dose is underlying a tremendous amount of metabolic differences between males and females," says Reue.

SEX WITH AN X

Beyond creating the initial differences in the gonads, the number of sex chromosomes can affect the behaviour of cells and tissues throughout life. This revelation has coincided with the refinement of another long-standing idea: that only one of the X chromosomes is active in each cell of a female's body. This

idea, termed X-inactivation, was developed to explain how males manage to survive with only one X chromosome. Unravelling the complexities of this process has yielded surprises that are helping to explain how cellular sex exerts its effects.

Unlike the sex chromosomes, the body's other chromosomes come in pairs, and each partner in a pair (one from each parent) contains the same set of genes. Cells need only one

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copy of certain genes — having a copy on each chromosome means that there is a spare in case one copy is faulty. But for other genes, the cell requires both copies to be working. Both need to produce RNA

for the cell to function normally — the 'dose' of gene activity is crucial. So vital is this balance that if a developing embryo is missing one of a chromosome pair, it rarely survives. Gaining extra copies can be lethal or result in developmental conditions such as Down's syndrome.

If the dose is important, how do males survive with just one X chromosome? It turns out that genes on the X chromosome compensate by making more RNA than do those on a typical paired chromosome, so males get the right dose of the resulting proteins that they need. This process happens in both sexes. To avoid overdosing on X genes, females shut down the genes on one of their X chromosomes. Biologists originally thought that this shutdown was complete, so that females got the same dose of X genes as males. But this idea left some puzzling things to explain.

One such puzzle is presented by people who have too many, or too few, sex chromosomes. If XXY men inactivate one X chromosome in each cell, leaving one active X and one Y, they should be indistinguishable from stereotypical XY males. But they are not: XXY men have a condition called Klinefelter's syndrome. For some, the symptoms are so mild that they are

The X (left) and the Y chromosomes have more influence on cells than first thought.

The right side of Art Arnold's unusual zebra finch was genetically male but the left was genetically female.

never diagnosed, but others experience infertility and varying degrees of cognitive impairment. Men with Klinefelter's usually have more body fat and are more likely to have metabolic problems such as type 2 diabetes; they are also more prone to developing conditions such as osteoporosis and autoimmune disorders that most commonly affect women.

Some people have only one X chromosome (XO). Almost all human XO embryos are miscarried — only an estimated 2–3% come to term. Such individuals are women with Turner's syndrome, a condition that includes infertility and characteristic physical features such as a webbed neck and short stature.

Both Klinefelter's and Turner's syndromes suggest that there is something about the supposedly inactive X that is essential for normal development. Perhaps it is not as quiescent as was once supposed.

THE GREAT ESCAPE

Mary Lyon, the UK geneticist who first proposed the idea of X-inactivation, suggested in 1961 that some genes on the second X chromosome might not be shut down. It was known that small sections of the X and Y chromosomes harboured the same genes, and she predicted that these shared genes would be left active. She was correct: examples were found in the late 1970s and early 1980s. But by the 1990s, there were accounts of genes from outside these regions that also escaped inactivation. "Right from the beginning, the escapees were there," says Carolyn Brown, a geneticist at the University of British Columbia in Vancouver. "But what we now realize is that, in addition to those common genes, there are more genes that are expressed from the inactive X."

In 2005, scientists surveyed the whole of the inactive X and found that up to a quarter of the X genes were active to some extent, including 15% that were routinely escaping inactivation. "It was a surprise that these weren't really rare exceptions," says Brown. Researchers have performed a number of subsequent studies

using different techniques and, in 2015, Brown and her team pooled these studies to show the inactivation status² of 639 genes on the muted X chromosome — approximately half of them escape to some degree. These vary in the amount of their activity but never reach the level of their counterparts on the active X. "This is probably a continuum," says Brown.

It is clear that females are getting a larger dose of certain X-chromosome genes than previously thought. Researchers are now trying to work out what these escapee genes are doing. A surprising number control the structure of chromosomal DNA and so increase or decrease the activity of genes across the genome. Two of these structural control genes are already implicated in intellectual-disability syndromes affecting women. Earlier this year, a team at Harvard Medical School found³ that six X-escape genes were known to code for potential tumour suppressors that act as a brake on cancer development. Reue, meanwhile, has unpublished data identifying a single X-escape gene that seems to be largely responsible for the obesity of her mice.

State-of-the-art genomics technologies are now letting researchers explore how X inactivation varies between tissues and between individuals. Bioinformatician Taru Tukiainen at the University of Helsinki, Finland, and her colleagues have used a technique called single-cell RNA-sequencing to look for differences in gene expression between the two X chromosomes in individual human cells. By cross-referencing these findings with datasets describing when and where genes are active in human tissues, she hopes to understand how these variations translate into sex differences. "We are trying to capture the whole spectrum, from cells to population, by using these different data types," says Tukiainen, who plans to publish the findings later this year.

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These molecular approaches are set to link up with large-scale population studies looking for genes involved in physical traits and complex conditions such as obesity. For many years, studies tended to overlook the contribution of the sex chromosomes to such conditions. That is now changing, and X-escape genes are starting to turn up. In 2014, for example, Tukiainen and colleagues showed⁴ how an X-escape gene called *ITM2A* contributed to a person's stature, pointing to a possible role in generating sex differences in height.

The process of X-inactivation is also a focus of interest. The inactive X frequently reactivates in cancers, especially breast cancer, and there are signs that some of its sleeping genes reawaken as women age. Another intriguing phenomenon is mosaicism: the X destined for inactivation is picked at random early in embryonic development, making females a patchwork, or mosaic, of different active X chromosomes. Sometimes, however, the balance of X-inactivation in women can favour one X chromosome over the other, an effect known as skewing. It is thought that the inactivation process is random, but the subsequent survival of the cells is affected by which X copy is active, says Brown. This means that some women carrying a faulty X gene that would normally cause disease seem to be unaffected.

The closer researchers look at the sex chromosomes, the more complex the picture becomes. And the sex differences they create is something that preclinical researchers will need to bear in mind, says Reue. Until recently, most studies focused only on male animals, as researchers either overlooked sex differences in disease symptoms or saw them as unwelcome complications. Now that scientists are more aware of the issue, and funding bodies often require female animals to be included in preclinical research, Reue hopes that researchers will come to see these differences as an advantage, pointing to fresh insights into many biological traits (see page S18).

Nevertheless, in the flurry of excitement over the X, it is important not to overlook its diminutive partner, the Y, says Arnold. Once thought to be an enclave of genes that make males more masculine, the Y has been found to contain active genes that are similar to some of the X's escapees. This suggests that it gives males two doses of similar genes, one on the X and one on the Y, which perhaps makes males more like females in some ways. Looking at what makes the two sexes similar, as well as what sets them apart, will be important for understanding this dynamic genetic balancing act. ■

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