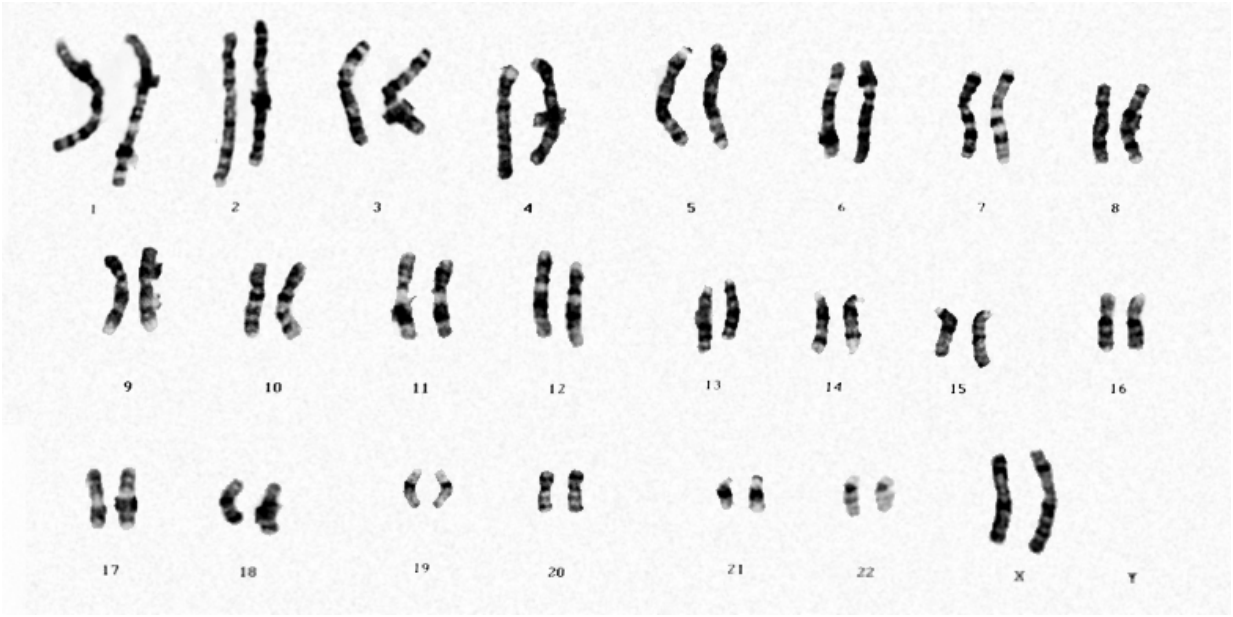
How a female X chromosome is inactivated

August 10 2015, by Fabio Bergamin



A full chromosome set from a woman's somatic cell. Credit: Serpil Borlu

In female mammals, one of the two X chromosomes is inactivated. Thanks to research using special stem cells, geneticists at ETH Zurich have been able to provide detailed insight into the molecular mechanism behind this inactivation process.

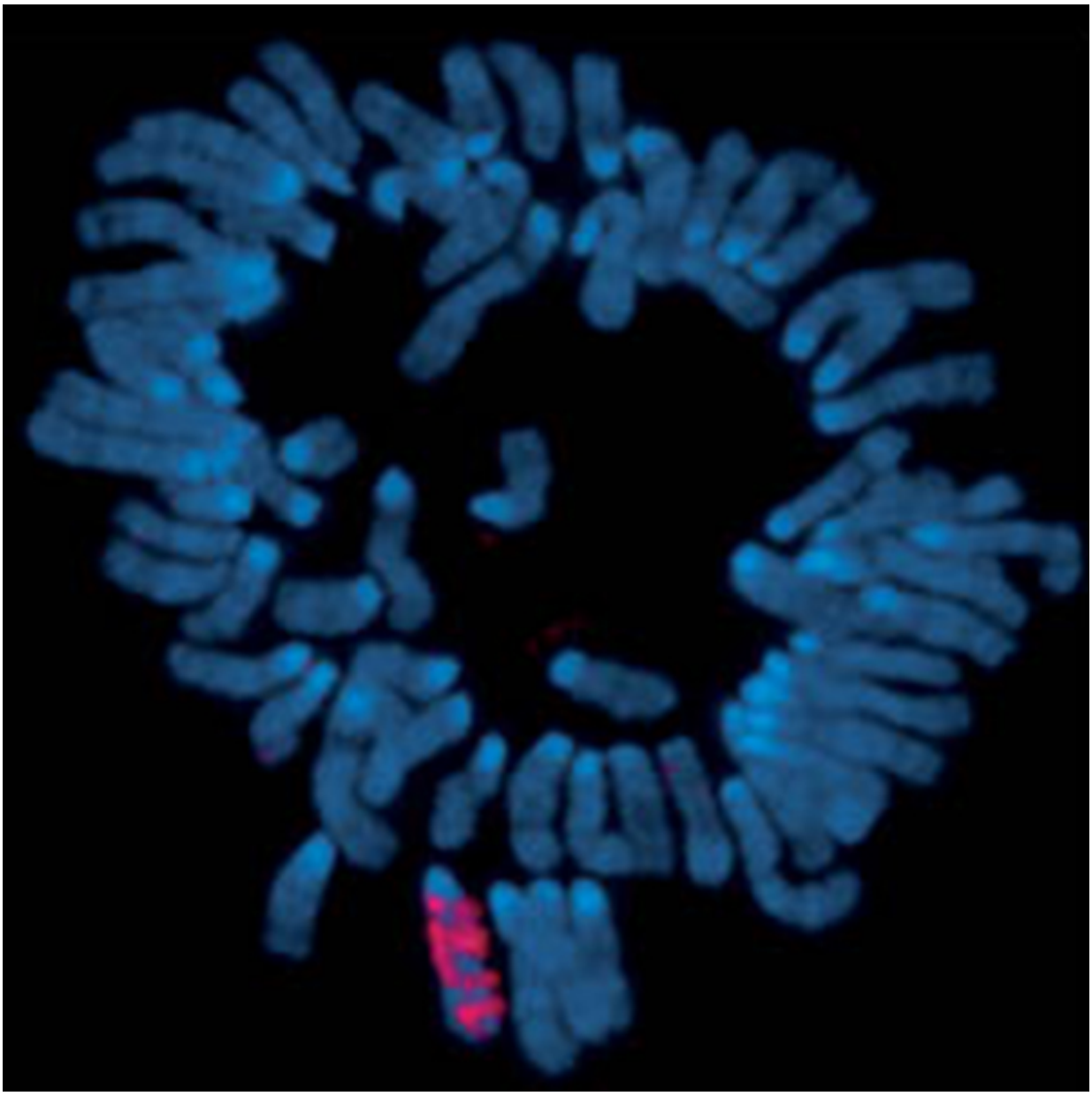
Chromosomes differentiate men from women. A woman's somatic cells have two X chromosomes, while a man's carry only one. If both X chromosomes and all of their genes were to be active in women, they would have twice as many copies of the proteins that they produce in

men. This would consequently result in a disequilibrium that would disrupt the finely balanced biochemistry of the human body.

Nature ensures this does not happen: one of the X chromosomes is completely and permanently inactivated during a female's early development in the womb. The mechanism responsible for this inactivation is not yet fully understood. However, research into mice has shown that a ribonucleic acid (RNA) molecule called Xist plays a pivotal role in the process. Several hundred copies of this molecule attach themselves to one of the two X chromosomes. Scientists believe that these RNA molecules dock onto other molecules which then inactivate the chromosome. A team of researchers lead by Anton Wutz, Professor of Genetics at ETH Zurich, have now discovered several of these inactivation molecules.

Screening to rescue cells

To this end, scientists used mouse stem cells, which exhibited two particular characteristics. Firstly, like unfertilised egg cells (and in contrast to somatic cells), they had just one instance of each chromosome. Secondly, they were modified to a degree that allowed the scientists to continuously produce the Xist RNA. This led to the inactivation of the single X chromosome and the death of the cells, since the genes needed for their continued survival could no longer be read.



Chromosomes from a mouse cell. Red indicates the visible Xist RNA on an inactivated X chromosome (micrograph). Credit: Ng K et al. *EMBO Reports* 2007, 8: 34

In a large-scale screening experiment using these stem cells, scientists were able to identify which genes were important for X inactivation. It is

possible to think of the experiment as a sort of rescue operation for the stem cells that would otherwise have died. Specifically, researchers used a virus to randomly damage individual genes in the genetic material of a large number of stem cells . Virus insertions that destroyed a gene, which was required for Xist RNA to inactivate the X chromosome, the X chromosome was not inactivated, and the corresponding cells survived.

The scientists were thus able to isolate surviving stem cells and identify seven genes that are central to X inactivation. One of them is called Spen. Scientists were already aware that Spen produces a protein which allows it to bind with RNA and essentially prevents the genes from being read. In other experiments, ETH researchers were able to show that if a mouse cell lacks the Spen gene, the proteins responsible for altering chromosome structure are not able to accumulate as efficiently at the X chromosome. ETH Professor Wutz explains that further research is required to understand exactly how this mechanism works and what role the other recently discovered genes play in it.

Research made possible thanks to earlier advances

"Genetic research such as this is extraordinarily complex," says Wutz. For example, a significant body of knowledge about mammalian genetics comes from conclusions yielded by research into drosophilidae (fruit flies), which are a model organism for biology and, in particular, for genetic research. Unlike mammals, however, fruit flies have a different chromosome system that does not include X inactivation. You cannot therefore draw on fruit-fly genetics to find gene candidates in mammals.

According to the professor, methodological advances made in recent years have made his research possible. Research of this type is now possible thanks to stem cells with the simple set of chromosomes,

created by Wutz five years ago while he was still at the University of Cambridge.

The ETH researchers published their work in the latest issue of the scientific journal *Cell Reports*. A British research team also published its findings in the same issue. Using a different method - RNA interference - they discovered several of the genes involved in X inactivation. One of them is Spen.

Slight differences in humans

The genes for Xist and Spen are found in humans as well. Thus, as Wutz points out, this research offers us some insight into the human system - at least at the theoretical level, as mouse genetics cannot be mapped directly to humans.

A few years ago, a team of French researchers postulated that, in addition to Xist, humans also have another system which ensures that the single X chromosome in men and one of the two X chromosomes in women remain active. This activating system does not exist in mice. Due to the interplay of activating and inactivating factors, regulation of X chromosomes in humans might therefore be more complicated than originally thought. Geneticists wanting to understand these processes in detail still have plenty of work ahead of them.

More information: Monfort A, Di Minin G, Postlmayr A, Freimann R, Arieti F, Thore S, Wutz A: Identification of Spen as a crucial factor for Xist function through forward genetic screening in haploid embryonic stem cells. *Cell Reports* 2015, 12: 554-561, DOI: 10.1016/j.celrep.2015.06.067

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