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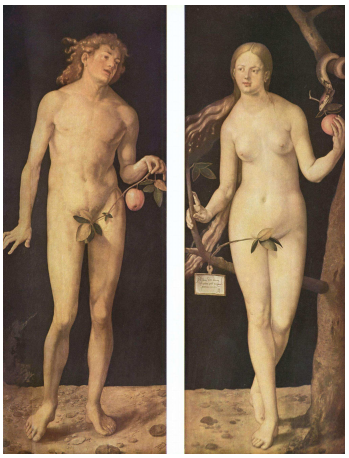
♂ or ♀: that is the question



We all know what a boy or a girl looks like. The differences between a man's body and a woman's are a mystery to no one – be it penis or vagina, we are either male or female, respectively. But why are we blessed with one or the other? Such a question has intrigued many a scholar from time immemorial… In the past decades, the human body has unveiled a few of its secrets. Whether our destiny is to be male or female, the process starts at the embryonic stage to end with puberty, and is orchestrated by a succession of molecules, amongst which proteins known to us since the 1980s.

On the tracks of X and Y

The human population is divided into two main categories: man and woman. Today, this difference lies essentially in the nature of our sexual chromosomes. Our genetic heritage is shared by exactly 23 pairs of chromosomes[1], one of which determines our sex. Women carry the XX pair, while men carry the XY pair.

In Antiquity, scholars were far from imagining such a thing as chromosomes, and various theories flourished. In particular, Aristotle believed that the summer season was propitious for conceiving a male descendant and declared that the more passionate the embrace, the more chance there was of giving birth to a son. Thus, according to him, the difference between the two sexes lies in the antithesis of heat and cold. He contended that a woman is an "unfinished" man precisely because of her natural coolness which he considered to be an impediment to the development of male attributes. The notion of "temperature" persisted through the centuries and was widely adopted during the Renaissance when man and woman were defined by a succession of opposites, such as cold/heat, imperfect/perfect, damp/dry, inside/outside, and so on. According to this concept, a woman's sex is by nature a man's inverted sex and is withheld inside the body by her frigidity. Such a view lasted until the Enlightenment when the male and female body were described on a purely anatomical basis.

In 1890, Hermann Henking brought a new light on the subject when he isolated chromosome X. Some ten years later, Clarence E. McClung suggested that this "accessory" element – the

[1] Chromosomes contain genetic information. A chromosome is made up of proteins and a very long molecule of DNA compressed up to 10'000 times. The chromosomes are held within the cell's nucleus.
Except for the gametes, a human being carries 46 chromosomes in each of his cells, i.e. 23 given by the father and 23 given by the mother.

function of which was poorly understood – was linked to the male sex. Shortly after, two Americans – the biologist Nettie M. Stevens and Prof. Edmund B. Wilson – undertook the task of elucidating its role. And in 1905, they independently published similar observations: in certain insects, sex is determined by the presence of a small chromosome in the male subjects, and by a large one – as it happened chromosome X – in the females. Fully aware of the uproar such a discovery would cause in opposing ranks of thought – which still subscribed to the influence of the environment – in the years that followed, they continued to search for confirmation of their first results. Ironically, it was their main opponent who exposed their theory shortly after. Thomas H. Morgan was at first very sceptical of the conclusions reached by Stevens and Wilson but he didn't adhere to the environment theory either. He was a specialist in the genetics of flies and in 1910 he adopted Steven's and Wilson's ideas to explain his own work. It was Morgan who established beyond doubt that the sex of an individual is determined by its chromosomes, which is the reason why his name is remembered in the history of science.

New discoveries were made in 1912. Hans von Winiwarter noted that women had two X chromosomes while men only carried one. As for Y, it remained unnoticed for a long time because of its small size, and its existence was only described in the 1920s by the American zoologist, Theophilus S. Painter, who found it in several animals and men.

It is hardly surprising that X and Y were not identified simultaneously. They are the most ill-assorted pair. All of our chromosomes come in twos where each chromosome is similar to the other – save for the XY pair... X is very long, whereas Y is surprisingly short. They also differ singularly by their contents: X numbers about 1'100 genes, whereas Y has a meagre 76. Yet they have the same origin and there was a time when they strongly resembled each other. 300 million years of evolution have, however, nibbled away at the length of Y in what seems a slow but inexorable degradation. Is "Y" threatened? Is the masculine sex to become extinct?... Scientists are not only worried about the future of the chromosome but are now debating on the perpetuity of the human species...

On the necessity of sexes

Nature offers more than just XY as sex chromosomes; XY is specific to mammals. Birds, for instance, have another pair whose chromosomes have been named Z and W. Fish, reptiles and amphibians are submitted to the influence of their environment, and gender frequently depends on the temperature at which an egg is incubated.

Whatever the combination may be, "sex" rhymes with "reproduction". Sexual reproduction unites two organisms of the opposite sex. In humans, any descendant is the result of the fusion of two gametes: the father's spermatozoid and the mother's ovum. However, not all living creatures resort to sexuality to reproduce. Yeast and bacteria, like certain bees or even plants at various stages of their life can waiver this rule and fall back on asexuality by dividing into two for example. Asexual reproduction does have the advantage of saving energy but sexual reproduction has the very definite advantage of mixing both the paternal and maternal genetic heritages. In this way, each individual inherits a new genetic combination that is specific to him – or her – alone.

The birth of the sexes

Besides the presence of the X and Y chromosomes in men and the two X chromosomes in women, what is it that really determines the fact that we end up being a boy or a girl? It all boils down to a group of molecules: proteins and hormones together define our gender from the first few weeks in the uterus to puberty. Although the embryo is already either XX or XY, strictly speaking, it is neither male nor female until the sixth week of pregnancy. It does not have ovaries or testicles, but genital glands that are as yet undifferentiated. However, at this very early stage, the embryo is equipped with a dual system of genital canals: the Wolffian and the Mullerian ducts that will develop to be part of the male genitalia or the female genitalia, respectively.

The birth of the ♂ sex

During the seventh week of pregnancy, a protein found in the testicles and known as the anti-Mullerian hormone (AMH) is secreted in the XY embryos. As its name suggests, its role is to eliminate the Mullerian ducts. Towards the tenth week, a steroid hormone – testosterone – is produced from the cholesterol present in the male glands. Testosterone then takes the relay and stimulates the development of the Wolffian ducts, which will ultimately become the vas deferens – the narrow tube which conveys spermatozoa to the urethra (cf.fig.1).

The birth of the ♀ sex

The sexual transformation of the XX embryo occurs a little later than its XY counterpart, i.e. from the tenth week of pregnancy. Without the masculinising hormones AMH and testosterone, the Mullerian ducts persist, whereas the Wolffian

ducts degenerate and finally disappear altogether. It is then thanks to the ovarian hormones – the oestrogens – that the Mullerian ducts form the Fallopian tubes, the uterus and then part of the vagina (cf.fig.2).

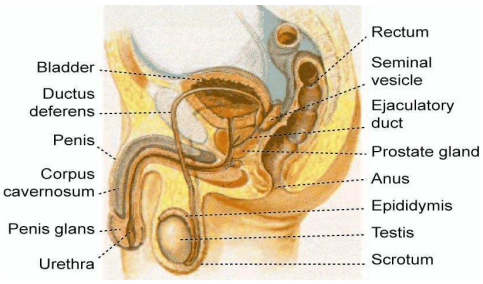


Fig.1 *Male sex organs*

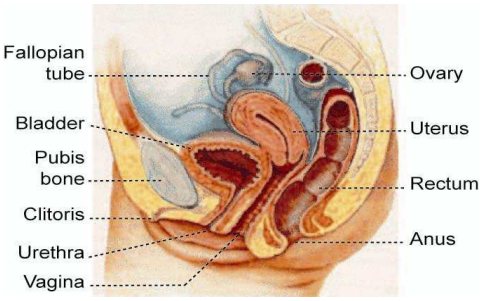


Fig.2 *Female sex organs*

The internal genital organs, such as the ovaries, the uterus, the testicles and the prostate, like the external ones, such as the mammary glands and the penis, continue to develop inside the mother's uterus under the control of hormones produced by the genital glands. And they only reach full maturity at puberty when they define unambiguously the sexual appearance of an adult.

Female by default?

Embryos become boys thanks to the precocious action of a "male" protein: AMH. Without AMH, you become a girl… Consequently, could the female sex be a sex by default? Such a concept was put forward in the 1950s and was steadfastly adhered to for decades.

It all began with Alfred Jost's work in 1947. The French biologist showed that the removal of the undifferentiated genital glands in a rabbit embryo always leads to the development of a female subject in both the internal and external organs, supposedly under the influence of maternal hormones. The idea was launched. The XX and XY foetuses have a "feminine" program common to both but which is obstructed in the male foetus by certain hormones. The ulterior observation that some men carry the peculiar XXY combination, and some women carry only one single X, gave the Y chromosome a pivotal position in the matter and the hypothesis that it held the key in determining the male or female gender.

From then on, researchers tackled the mystery of Y and it was only in 1990 that they were able to isolate SRY, "the protein that appears to determine" our sexual profile. Adding an SRY gene to a mouse XX embryo produces amazing results. All the mice, without exception, become male. How does the protein work? SRY is a transcription factor[2] and has a very early effect on the development of the embryo by influencing the production of proteins, most of which are still to be discovered. However, SRY is known to stimulate indirectly the production of AMH which sets the embryo on its male course.

It does seem then that the male profile is dependent on the presence of the SRY protein. Conversely, could there be an equivalent protein whose gene, carried by X, would change an XY embryo into a female? This question preoccupied some researchers long before SRY was identified. Yet studies were at a standstill. Why? Because of a lack of conclusive results? Or because of technical difficulties? Or the social image given to women by science? Whatever it was, the year 1994 heralded a positive turning point. Researchers believed they had, at last, tracked down the key to female genetic determination thanks to the discovery of the DAX1 protein. DAX1 is also a transcription factor that acts under the impulse of a molecule – unknown – which binds to it. To this day, its mechanism has only been partially decoded. DAX1 certainly seems to play an important part in ovarian development, yet it does not seem to be as decisive as SRY in testicular development. What is more, adding DAX1 to a mouse XY embryo impedes but does not completely suppress the development of its testicles. The concept of the female sex by default has been flawed but not definitely confounded.

October 2006: the debate is revived. Giovanna Camerino, an Italian scientist, made some surprising observations in the course of her research. She came across an Italian family in which several brothers were XX. Although sterile, they had all the male attributes. Other XX males had already attracted attention and been found to have inherited the paternal SRY following its transfer from Y to X. The Italian brothers,

[2] A protein that interacts with genes. It decides when a gene is expressed or not, i.e. when the information contained in a gene is used to produce proteins. Certain genes are expressed under specific circumstances (infection...), others only when the embryo is developing or in adulthood, for example.

however, were not carriers of SRY. So how was it that they were "male"? An extensive study led the researchers to a double discovery: they lacked a protein known as RSPO1 as a result of a modification in its gene, and RSPO1 seems essential in determining the female sex. It's a scoop. Could RSPO1 be SRY's long sought for "homologue"?

The researchers' amazement must have equalled their astonishing discovery. Until then nothing had led to suppose that RSPO1 was implied in sexual determination. Indeed, it had been known to be a member of a family of growth factors[3] – the R-spondins – that play a part in the formation of skin cells. Today, researchers know that RSPO1 is produced by cells in the ovaries/testicles and seems to be indispensable for the development of a female subject since, in its absence, any female development recedes and the embryo acquires the male organs.

The hermaphrodite myth

The Italian brothers are not the only ones to suffer from Nature's quirks. Anomalies that affect the sexual chromosomes are very rare but varied, and individuals which suffer from them are generally sterile. Similar to XX men, certain XY individuals turn out to be women because they lack the SRY gene. Even more disconcerting are individuals who inherit an abnormally high number of sexual chromosomes, amongst whom those inflicted with Turner's or Klinefelter's syndrome. X0 women suffer from Turner's syndrome, i.e. they carry only one X and no Y. They have a small uterus and diminished ovaries, are frequently small, and their breasts and capillary system remain undeveloped. At the other extreme, XXY men are affected by Klinefelter's syndrome. As children, they are taller than the norm. At puberty, their testicles are atrophied and sterile, and their mammary glands may begin to swell. Fortunately, if detected in childhood, such abnormalities can be treated at puberty by the administration of hormones.

The myth of Hermaphrodite illustrates beautifully the idea of being man and woman simultaneously. Hermaphrodite was the son of Hermes and Aphrodite and was deeply in love with the nymph Salamacis. So as not to be separated from her beloved, she requested to be one with him. Echoing the mythological character, the very rare individuals that are called hermaphrodites carry both XX and XY cells. As a result, they have both testicles and ovaries, accompanied by abnormalities of the genital organs (cf.fig.3).

[3] Proteins secreted by cells and which are necessary for cellular growth (multiplication, specialisation...).

Fig.3 *Hermaphroditus asleep, Roman copy of a Greek original, ca. 2nd century CE. The feminine silhouette is hiding a male sex.*

Another peculiarity of Nature: Middlesex. *Middlesex* is the story of a pseudo-hermaphrodite – Cal Stephanides – written by the novelist Jeffrey Eugenides after a true story. Born a little girl, Cal Stephanides goes through a second birth at puberty when she becomes a young man. Cal Stephanides is an example of male pseudo-hermaphrodism, brought about by the modification of a protein: Type 2 5-alpha reductase. Type 2 5-alpha reductase transforms testosterone, a necessary stage for the formation of the external masculine genital structures in the embryo. XY individuals who carry the faulty protein are born with a more or less well developed vagina and clitoris but without a uterus or a scrotum. As a consequence, they are raised as girls. However, at the age of puberty when the testicles descend, their sexual appearance and identity become male.

In reality, various proteic anomalies usually expressed by a hormonal dysfunction lead to pseudo-hermaphrodism both in XX and XY individuals. The ambiguity of genital organs can be such that some pseudo-hermaphrodites are faced with having to "choose their gender" by way of surgery, so as to assert their identity.

♂ or ♀?

All these oddities and paradoxes offered to us by Nature raise a fundamental question. How do you define an individual's sex? By determining the chromosomal formula – XX or XY – as they do for the Olympic Games? By detecting the presence of ovaries or testicles, a method much favoured in the second half of the 19th century? Or by examining the external genital organs as it is customary to do at birth? After all, it is in the first few moments of life that our social sex is "set": you are either a boy or a girl.

And what about our cerebral sex? Yet another aspect that is just as important in the construction of an individual's personality. The brain also appears to assert its sex at the time of puberty under hormonal influence, further accentuating certain differences between men and women. But the personal history of each individual

is also paramount in the full burgeoning of sexual identity, i.e. of an individual's psychological sex. We are born either girl or boy. But do we feel woman or man? Can we become what we believe to be? Gender is a huge debate that affects our society today – a society that questions the social recognition of homosexuals and transsexuals. Sex, most obviously, has not said its last word...

Séverine Altairac*

*Translation: Geneviève Baillie

For further information

On the Internet:

- In French, Institut national de Recherche Pédagogique, Les déterminismes du sexe : http://www.inrp.fr/biotic/procreat/determin/html/synthese.htm
- Klinefelter and Turner's Syndromes: http://www.aaa.dk/TURNER/French/index.HTM

A little more advanced:

- In French, La recherche Hors-série, "Sexes, comment on devient homme ou femme", numéro 6, novembre- décembre 2001/janvier 2002
- Sex determination: http://www.embryology.ch/anglais/ugenital/planmodgenital.html
- Article about the SRY protein, Protein Spotlight, "The tenuous nature of sex" : http://www.expasy.org/spotlight/back_issues/sptlt080.shtml

Illustrations:

- Heading illustration (Adam und Eva by Albrecht Dürer), Source: http://de.wikipedia.org/wiki/Bild:Albrecht_D%C3%BCrer_002.jpg
- Fig.1, Adaptation: http://fr.wikipedia.org/wiki/Appareil_reproducteur
- Fig.2, Adaptation : http://de.wikipedia.org/wiki/Geschlechtsorgan
- Fig.3, Source: http://fr.wikipedia.org/wiki/Hermaphrodite

At UniProtKB/Swiss-Prot:

- Anti-Muellerian hormone (AMH), Homo sapiens (human): P03971
- Sex-determining region Y protein (SRY), Homo sapiens (human): Q05066
- Steroid 5-alpha-reductase 2, Homo sapiens (human): P31213
- Nuclear receptor DAX-1, Homo sapiens (human): P51843
- R-spondin-1 (RSPO1), Homo sapiens (human): Q2MKA7

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