

DEVELOPMENTAL BIOLOGY

The X-inactivation yo-yo

Wolf Reik and Anne C. Ferguson-Smith

In female mammals, one of two X chromosomes has to be shut down during early development. To what extent does this 'imprinted X-chromosome inactivation' involve the history of the chromosome?

In most mammals, males have the male sex-determining Y chromosome and a single X chromosome, whereas females have two X chromosomes. In females, the resulting imbalance in the 'dosage' of genes on the X chromosomes needs to be compensated so that gene expression from the X chromosome is equivalent in males and females. Mammals have evolved a unique form of dosage compensation, called X-chromosome inactivation, in which one of the two X chromosomes in female cells is silenced epigenetically¹ — that is, by factors such as chemical modification of the DNA, or of the histone proteins that package DNA into chromosomes, often involving non-coding RNAs. Many aspects of mammalian X inactivation remain mysterious. But through elegant studies in the mouse, Okamoto and colleagues (page 369 of this issue)² have unravelled some of the earliest events in the process.

During early development of female mouse embryos, and in extra-embryonic tissues such as the placenta, it is always the X chromosome derived from the father that is inactivated³. Gene expression from only one parental member of a chromosome pair is known as imprinting, and is caused by an epigenetic memory first arising in the egg or the sperm. Later on, in the embryonic tissues, X inactivation is random with respect to the parental origin of the X chromosomes⁴.

There is considerable interest in understanding the mechanisms that specifically silence the paternal X chromosome in early development, and the extent to which the history of that chromosome is involved. During male meiosis, in which sperm are produced, a process known as meiotic sex chromosome inactivation (MSCI) occurs. In developing male sperm, the sex chromosomes form a unique structure, the XY body; MSCI occurs here and leads to repression of the transcription of X- (as well as Y-) linked genes⁵. This meiotic inactivation uniquely affects the sex chromosomes and may be associated with the inability of the X and Y chromosomes to pair during male meiosis⁵.

One proposal^{6,7} is that, when an egg is fertilized, the X chromosome from the father's sperm arrives in a 'pre-inactivated' state, which is a continuation of MSCI, and which persists during the period before the early embryo implants in the uterus. Okamoto *et al.*², however, now show that genes on the paternal X chromosome are transcriptionally

active at the very earliest embryonic stages, and that subsequent inactivation of the paternal X can occur without prior MSCI. This work shows that the two processes (MSCI and *de novo* paternal X inactivation after fertilization) can be mechanistically separated, and it confirms that there is a period after fertilization when the paternal X chromosome is transcriptionally active.

Both imprinted and random X-chromosome inactivation depend on the expression of a non-coding RNA called *Xist*. As the cells differentiate, *Xist* synthesis increases on the chromosome that is to be inactivated, coating the X chromosome and leading to the acquisition of repressive epigenetic marks (including modifications to core histones and to DNA) and gene silencing⁸. Okamoto and colleagues² took advantage of transgenic mice in which large pieces of the X chromosome, encompassing the *Xist* gene, had been inserted into non-sex chromosomes. They then studied various characteristics associated with X-chromosome activity and repression on both maternally and paternally inherited transgenic X sequences, and on the normal X chromosomes themselves. In all cases, the expression and dynamic epigenetic states of the transgenes were the same as those on the respective X chromosomes.

Okamoto *et al.* found that RNA signals —

representing gene transcription — occurred on both the maternal and paternal X-chromosome sequences in embryos at the two-cell stage (the stage at which the embryonic genome begins to be transcribed). Because the authors could not look at a large number of genes, it may still be that some genes on the paternal X are repressed at the two-cell stage — but these experiments clearly show that many are not.

Among the genes expressed on the paternal X at the two-cell stage is *Xist*. Shortly afterwards, sequences on the X chromosome that is to be inactivated undergo repressive modifications in a hierarchical and dynamic manner. For example, at the 8–16-cell stage, X-linked genes show a gradient of inactivation along the chromosome, with those farther from *Xist* being less tightly silenced⁶. These observations support a model in which the expression of *Xist* triggers an epigenetic inactivation process that spreads along the X chromosome during pre-implantation development. By contrast, the maternal copy of *Xist* is kept silent in the early embryo by an unknown epigenetic mark that originates in the egg⁹, thus keeping the maternal X active.

But there is one important difference between the transgenes and the normal X chromosome. The paternally inherited X has been subject to MSCI whereas the transgenic sequences have not. The mechanism of MSCI during sperm formation does not involve *Xist*, but a different epigenetic pathway⁵. It is unclear to what extent X-linked genes inactivated by MSCI remain silent during the later stages of sperm formation¹⁰, although it has been suggested that this silencing is carried over into the fertilized egg and gives rise to imprinted X inactivation^{6,7}. But the fact that the paternally inherited X transgenes are

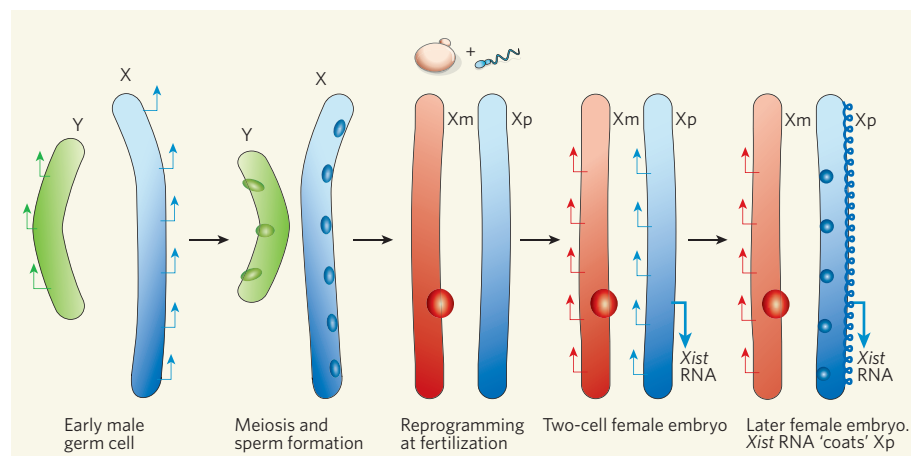


Figure 1 | Imprinted X inactivation: on again, off again? In early male germ cells, genes on the X and the Y chromosomes are transcribed (coloured arrows). During male meiosis, genes on the X and the Y become silenced and epigenetically marked (ovals). A female fertilized egg inherits the paternal Xp from the sperm and a maternal Xm from the egg. During or after fertilization of an egg, the epigenetic marks on the paternal X are reprogrammed; the *Xist* gene on the maternal X has been tagged with a repressive epigenetic mark before fertilization (circle). In the two-cell female embryo, genes on both Xs are transcribed, with the exception of the maternal *Xist* gene, which is repressed by the epigenetic mark. After the two-cell stage, *Xist* RNA begins to coat the paternal X, leading to silencing and epigenetic modification of the paternal genes. Genes on the maternal X remain active.



50 YEARS AGO

An Outline of the Cancer Problem.

— It is almost as difficult to review a popular book on cancer as to write one, and for similar reasons. The subject ranges over such a vast canvas that one is always acutely aware of numerous important omissions and of the minor distortions which inevitably appear... One is compelled to stress those aspects most dominant in one's own experience, and Dr. I. Hieger naturally stresses the important role of the chemical carcinogens in etiology. He was in the team which first isolated from coal tar a pure chemical carcinogen, and he gives us the most interesting story of this pioneer discovery. Moreover, we have seen lately the firm linkage of exposure to tobacco and industrial smoke to the tremendously rising incidence of lung cancer... If it is shown that lung cancer is in fact due to exposure to chemical carcinogens in smoke, their practical significance in human cancer will become of greatly enhanced importance.

From *Nature* 19 November 1955.

100 YEARS AGO

American palaeontologists are becoming more and more strongly convinced of the decisive character of the evidence afforded by extinct faunas of a comparatively recent connection between South America, South Africa, and Australia. A short time ago, Dr. W. B. Scott... announced his opinion that the fossil Santa Cruz insectivore *Necrolestes* is closely allied to the South African *Chrysochloris*, and that this relationship indicated a connection between South Africa and South America. Now Mr. W. J. Sinclair... states unequivocally that *Prothylacinus* and the other marsupial-like carnivores of the Santa Cruz beds are true marsupials closely related to the Australian thylacine... Mr. Sinclair considers himself justified in stating that... "a land connection between Patagonia and the Australian region existed not later than the close of the Cretaceous or the beginning of the Tertiary".

From *Nature* 16 November 1905.

inactivated after fertilization in the absence of prior MSCI clearly shows that key aspects of imprinted X inactivation can take place without it.

One interesting outcome of these studies^{2,6,7} is that the pre-inactivation and the *de novo* inactivation hypotheses can both be correct, as summarized in Fig. 1. X-linked genes in the sperm could arrive in the egg in an epigenetically inactive form. The egg cytoplasm could then exert an immediate response to reverse this state — perhaps as part of the genome-wide reprogramming events that affect the paternal genome in the newly fertilized egg¹¹. It is likely, given the results of Okamoto *et al.*, that most genes on the paternal X, including the *Xist* gene, are activated at the two-cell stage. But it seems that over the next few cell divisions they become inactivated again through the action of *Xist*, without necessarily requiring prior MSCI.

The latest work illustrates the amazing plasticity and dynamic nature of epigenetic programming and reprogramming in germ

cells and early embryos. Insight into these events will give us clues about how the genome functions in normal development and in disease. It may also eventually provide tools for treating diseases of genome malfunction. ■

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COMMUNICATIONS TECHNOLOGY

Chaos down the line

Rajarshi Roy

Chaos, goes conventional wisdom, can only be a malign influence in telecommunications. But a technique that uses chaotically varying signals to transmit information more privately may help it shed that bad-boy image.

Synchronization leads to communication — even when the signals used are chaotic. That is the lesson of a study by Argyris and colleagues on page 343 of this issue¹. It reports the successful transfer of digital information at gigabit rates by chaotically fluctuating laser light travelling through more than 100 kilometres of a commercial fibre-optic link around Athens, Greece (Fig. 1). The transmitter and receiver become harmonized in chaotic synchrony, allowing information to be reliably extracted at the other end — a result that brings us closer to exploiting the inherent advantages of chaos, rather than trying to eliminate it whenever it appears.

The phenomenon of synchronization in

periodic systems has been known since at least 1665, when Christiaan Huygens observed that pendulum clocks become synchronized when placed close to each other on a common support. Asian fireflies flashing together, flocks of geese flying in remarkable formations and pedestrians in lock-step on London's Millennium Bridge are illustrations of synchronization when large numbers of living creatures get together². But synchrony also arises in inert matter: lasers and masers both exploit the ability of large ensembles of atoms and molecules to harmonize their oscillations and emit light in coherence. The key to synchrony in such systems is that the individual elements



Figure 1 | Attic experiment. Argyris and colleagues¹ successfully used chaotic waveforms to transmit information over a distance of more than 100 kilometres in the telecommunications network of Athens (seen here from the Acropolis).

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