

# Epigenetics and Cellular Memory

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## Plan détaillé

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## Texte intégral

Mr Administrator,  
My dear colleagues,  
My friends, *agapimeni mou fili*,

The creation of a Chair of Epigenetics and Cellular Memory is the beginning of a new adventure, both for the Collège de France and for myself. I firstly feel highly honoured to have been deemed worthy of it, and I would like to thank all the professors for welcoming me into their prestigious faculty. I am especially grateful to Christine Petit, who put her trust in me and gave me her constant support. I am also deeply and sincerely grateful to Pierre Corvol, who supported the creation of this Chair, and to Alain Prochiantz for his advice, his help, and his friendship. I will be mentioning many of my teachers and close colleagues over the course of this lecture.

I would most particularly like to thank Vincent Colot for being a constant source of inspiration and support through this complex journey that has brought me here before you today.

“Epigenetics and cellular memory”: as we will see in a moment, this title refers to a new and rich field of research in biology, relating to the development of organisms and the normal or pathological variations thereof, particularly in connection with the environment, as well as to heredity and evolution. This new Chair is therefore at the interface between the different disciplines of genetics, developmental biology, ecology, and evolution.

Before describing this new research field, let me first highlight the discovery of an extraordinary mid-twentieth century reality: genes are carried by the DNA molecule, and by that molecule only. They form information units embedded in the sequence of the four nucleotides A, T, G and C that make up DNA. This information determines, “encodes”, proteins, which are molecules crucial to the functions necessary for life.

The DNA structure with two complementary strands is key to the extremely accurate transmission of genetic information through cellular division. Thus, the myriad of cells that make up the human body all have the same 3-billion nucleotide sequence, save a few rare exceptions. This raises the question of what distinguishes a lymphocyte from a neuron, to take but two examples.

While we are far from having a clear answer, we have known since the advent of molecular biology that the regulation of gene expression – that is, the regulation of the transcription of genes into messenger RNA, and the latter’s translation into proteins – is crucial to cellular differentiation. The question then becomes how, within a cell, only certain genes are expressed, and through what

mechanisms the cell makes this choice. Does the cell perhaps keep a memory of this gene expression pattern even after the conditions that gave rise to it have disappeared, sometimes a very long time ago? Or does it transmit the pattern clonally, through multiple cell divisions? To which we can add the equally important question: how can the differentiation process be reversed? Fertilization, for example, involves two highly differentiated cells – the spermatozoid and the ovule – and almost immediately produces a totipotent cell, the zygote, triggering a spectacular string of cell divisions and differentiations to form the new person. The counterpart of this programming necessary for life is a potentially deadly kind, as in cancer, against which we are still largely helpless. The work of the Japanese researcher Shinya Yamanaka, recently honoured by the Nobel committee, shows that it is possible to “reprogramme” differentiated cells into pluripotent stem cells (induced pluripotent or iPS cells), through the expression of just four genes encoding for transcription factors! However, while this forced reprogramming opens up fantastic therapeutic potential through regenerative medicine, it is not very effective since it requires a huge number of cellular divisions, and rarely comes to fruition. Why? What stalls it?

The answers to all these questions lie in the organization of the genome within the nucleus of our cells, which is associated with a myriad of proteins and RNA molecules, as in all eukaryote organisms. This complex nucleoprotein structure, called *chromatine*, was described in 1928 by Emil Heitz as existing in two states, *heterochromatin*, or condensed chromatin, which is gene-poor, and *euchromatin*, or decondensed chromatin, which is gene-rich (Heitz, 1928). Despite this distinction, chromatin was long perceived essentially as a structure packing the genome into the confined space of the nucleus. Indeed, the DNA of the human genome is almost two metres long, whilst the nucleus is only ten microns in diameter. However, we now know that chromatin plays a decisive role in the modulation of accessibility to genetic information and the memorizing of gene expression patterns through cell divisions.

## From epigenesis to epigenetics

I will now define the word *epigenetics*. The use of both the adjective (epigenetic) and the noun (epigenetics) are recent. The former seems to have appeared in the nineteenth century, most certainly with reference to epigenesis, which I will define in a moment. The origin of the latter dates back to 1942.

Conrad Hal Waddington, a British scientist and philosopher, was the first to formulate explicitly the need to draw causal links between genotype and phenotype so as to grasp developmental processes. In other words, it was important for him to build bridges between genetics – the science of the heredity of characters, created with the rediscovery of Gregor Mendel’s work at the turn of the twentieth century –, and epigenesis – the theory of development through the gradual elaboration of forms. This theory was initially formulated by Aristotle, four centuries BCE, in opposition to that of preformation and the term epigenesis was coined by William Harvey in the 17<sup>th</sup> century. The theory of epigenesis was only validated experimentally centuries later, in the eighteenth and nineteenth centuries, particularly owing to progress in microscopy that finally allowed for the inside of an egg and cell divisions to be observed. In a short article, Waddington therefore proposed merging the terms *epigenesis* and genetics to refer to the study of the mechanisms whereby genes determine characters (Waddington, 1942).

What did we know about the action of genes back in 1942? Nothing, or virtually nothing. But that did not stop Waddington from positing that embryonic development mobilizes changing networks of interaction between genes, and in this respect he can be considered as one of the pioneers of systems biology. With some artistic talent, he illustrated his concepts with sketches: his drawing of

the “epigenetic landscape”, a metaphor for the role of gene regulation in development, is still famous in the field of biology (Waddington, 1957). Waddington published abundantly on the subject, based on his observations of embryonic development and his genetic analyses of birds, flies and other species. He also studied and described the consequences of environmental or genetic changes on development. In my lectures over the next few years, I will return many times to Waddington’s work and ideas, the importance of which, in my opinion, is vital and insufficiently recognized.

In the early 1950s, several major discoveries paved the way for the molecular analysis of the causal relationships between genotype and phenotype – therefore the approach advocated by Waddington. As I have already mentioned, it was in particular the revelation that genes are carried by DNA molecules, followed by the elucidation of the double helix structure of DNA, which marked the advent of molecular biology in the 1960s. A crucial conceptual step forward was made with the work of François Jacob and Jacques Monod on the *Escherichia coli* bacteria and the phage lambda, in André Lwoff’s laboratory at the Institut Pasteur.

Jacob and Monod developed the first model of gene expression regulation through the interaction of a specific, nearby region of the DNA with a regulatory RNA or protein molecule (Jacob and Monod, 1961), and suggested that this model could explain differential gene expression during development. How? As early as 1934, geneticist Thomas Hunt Morgan had put forward the idea that “different batteries of genes come into action one after the other, as the embryo passes through its stages of development” (Morgan, 1934). In the late forties and early fifties, based on her observations on maize, Barbara McClintock argued that gene activity is regulated, during development, by “controlling elements” which she also called *transposons*, because they can move along the genome. She argued that the mobilization of transposons – which we now know are the main component, with their relics, of the genome of maize, humans and many other eukaryote organisms – is an integral part of the mechanism regulating gene expression. While this hypothesis has been proved wrong – though not entirely, as recent work on the brain suggests –, what Barbara McClintock proposed about the “controlling elements” whose existence she had actually just revealed was incredibly accurate. According to her, these controlling elements can modify gene activity in many different ways. They can decide on the period when the gene will be active in a tissue’s development. But they can also determine in what cells this gene will be active.

Based on these different studies, in 1969 Roy J. Britten and Eric H. Davidson proposed a theoretical model of the regulation of networked genes. According to this model, regulatory genes code factors which, through various combinations, activate in concert several sets of non-contiguous genes during cell differentiation (Britten and Davidson, 1969).

During this period, experimental embryology was itself making strides. Among the many major breakthroughs, I will mention the work of Robert Briggs and Thomas King (Briggs and King, 1952), and that of John Gurdon (Gurdon, 1962) for which he was honoured by the Nobel committee this year, alongside Shinya Yamanaka. Through a series of landmark experiments, these researchers demonstrated that it is possible, in enucleated frog oocytes, to transplant the nuclei of differentiated cells and thereby successfully develop new frogs. These results prove that genetic material is preserved during cellular differentiation, and that embryonic development stems from changes in the genome’s expression rather than its content.

On an entirely different level, I will also mention the pioneering work of Nicole Le Douarin, who was previously Chair of Cellular and Molecular Embryology at the Collège de France. She developed the quail-chick chimera system, an experimental exploit making it possible for the first time to follow the fate of specific cells during development, particularly cells of the neural crest, the

transitory structure of vertebrate embryos. With her team, she showed that these cells migrate to give birth to a wide variety of cell types in adults.

Despite the many advances in molecular biology and experimental embryology, the links between gene activity and development were however still hopelessly obscure. In fact, these two disciplines were developing in parallel, without any real communication. Epigenetics was stagnant and very few researchers used this term. Perhaps rightly so at the time, for the causal relationships between genotype and phenotype only started to be truly grasped in the early eighties, particularly with the identification by Christiana Nüsslein-Volhard and Eric Wieschaus of a series of mutants affecting the viability of the drosophila embryo. This work followed that of geneticist Edward Lewis who, from the forties to the eighties, performed a systematic genetic analysis of homeotic mutants, named as such because they trigger the substitution of an organ by another in adult flies. Two striking examples are *Antennapedia* mutants, whose antennae are replaced by legs, and *Ultrabithorax* mutants, which have a second pair of wings instead of halteres. Focusing on the study of the early stages of the fly's development, Nüsslein-Volhard and Weischaus were the first to investigate directly the logic of the genes controlling embryogenesis. This work marked the birth of developmental genetics, and was soon followed by the discovery that many genes identified in drosophila still exist in vertebrates and fulfil similar functions. Thus emerged another extremely prolific discipline: evolutionary developmental genetics, Evo-Devo.

## Epigenetics and the notion of states of activity of transmissible or inheritable genes

At the turn of the eighties/nineties, the term *epigenetics* was revived and it took on a new meaning. This semantic change followed the discovery that certain changes in gene expression are transmitted through cellular divisions, across generations in some cases, and without the DNA sequence itself changing. In 1971, Eduardo Scarano argued that processes of DNA sequence modification rather than mutation explained this stable transmission of particular states of gene activity (Scarano, 1971). Such modifications were evidenced soon after, particularly the methylation of one of the four DNA bases – cytosine. Moreover, it soon became clear that this methylation is transmitted through cellular division and, significantly, that it is often associated with a stable repression of genes' activity. It was the geneticist Robin Holliday who, in 1994, proposed that epigenetics be redefined as the study of the changes in gene expression transmissible through cellular division, or even across generations, without there being any change in the DNA sequence (Holliday, 1994).

The idea therefore emerged that “epigenetic” modifications constitute inheritable regulatory signals, supplementing the information carried by the DNA sequence. This concept experienced rapid success. In fact, it also offered a welcome explanation for several disparate observations, which had left scientists perplexed for years as they defied the classical laws of Mendelian genetics. Examples of this “heterodox heredity” abounded.

Let me start with variegation in plants. Barbara McClintock had shown that some variegations are linked to the state of activity of the transposable elements she had just identified. She spoke of “changes in phase”, which modify the frequency at which transposons move and the spatiotemporal patterns of expression of the genes situated in the vicinity of these mobile elements. Often regulated during development, these changes in phase are transmitted clonally and can also be inherited over several generations. We now know, thanks to the work of Nina Fedoroff and Rob Martienssen, that

they correspond to the shift in the cytosines of the transposons in question, from a methylated state to a non-methylated one and vice versa.

Another example, in mammals this time: the inactivation of the X chromosome. In 1961, mouse geneticist Mary Lyon made a surprising observation: female mammals with two X chromosomes show unusual “mosaic” coat-colour phenotypes – unlike the males, which carry only one X chromosome and one Y chromosome. A century earlier, Charles Darwin had already noted this strange phenomenon in his book *On the Origin of Species* (1859): “What can be more singular than the relation in cats between [...] the tortoise-shell colour and the female sex?”

Where do these mosaic phenotypes specific to female mammals come from? Mary Lyon suggested that they result from the random inactivation of one of the two X chromosomes in each of the early embryo cells followed by the stable transmission – in other words the memorization – of this silent state during the successive cellular divisions. Since the two X chromosomes carry different forms of the same gene, for example controlling coat-colour, in adults the random inactivation of one of these two chromosomes and the ensuing clonal expansion of the cells results in a juxtaposition of cells expressing either one of the X chromosomes, each with a distinct phenotype. Mary Lyon also made the hypothesis, which has since been confirmed, that the “sexual chromatin” identified by the cytologist Barr in the nucleus of female cells corresponds to the inactive X (Lyon, 1961).

In 1975, Art Riggs proposed that X-inactivation and its stable transmission through cellular divisions rely on DNA methylation (Riggs, 1975). This hypothesis was validated by the observation that the regulatory regions situated near the genes are indeed methylated on the inactive X. Other researchers subsequently discovered that this methylation is accurately reproduced after DNA replication, using specific enzymes, so-called “maintenance” DNA methyltransferase. Better yet: these enzymatic activities can be blocked with drugs, leading to the reactivation of certain genes carried by the inactive X chromosome.

A second example of unexpected heredity in mammals is “parental genomic imprinting”. In 1986, two embryologists, Davor Solter and Azim Surani, each carried out pioneering experiments with nuclear transplantation in mouse oocytes with the aim of obtaining embryos carrying two maternal genomes – gynogenotes – or two paternal genomes – androgenotes (McGrath and Solter, 1984; Surani *et al.*, 1984). In both cases they observed early embryonic lethality, even when the maternal and paternal genomes carried the same genetic information. There again, it was rapidly established that DNA methylation plays a crucial role in the functional non-equivalence of maternal and paternal genomes. This differential methylation is established in the parental germline and transmitted to the progeny, which maintains it.

These two striking examples illustrate another crucial point about epigenetic memorization processes in connection with development: X-inactivation and parental genomic imprinting are erased at every generation. This reprogramming, or *tabula rasa*, is necessary for a new cycle of life to start.

While DNA methylation was the first molecular mechanism associated with the new definition of epigenetics proposed by Robin Holliday, another system of stable transmission of gene activity differential states was beginning to be revealed in fruit flies, which do not have DNA methylation. It involves two groups of regulatory proteins, Polycomb and Trithorax, the names of which relate to the aberrant phenotypes observed in mutant embryos for the corresponding genes. The genetic and then molecular approaches have shown that these two groups of protein act antagonistically on the chromatin, not to establish the patterns of homeotic gene expression along the fly’s antero-posterior axis in the earliest embryonic developmental stages, but to maintain these patterns until adulthood.

In other words, the complexes formed by the proteins of the Polycomb and Trithorax groups “memorize” states of gene activity throughout cellular divisions. Remarkably, these protein complexes are largely preserved in most multicellular organisms, including plants that have evolved from single cells independently of animals. This epigenetic regulatory mode is thought to be at the very origin of multicellularity.

## **X-chromosome inactivation: a paradigm for the study of epigenetic mechanisms**

In 1990, I joined Philip Avner’s team at the Institut Pasteur as a post-doc. Avner, an eminent mouse geneticist, and Jean-Louis Mandel, Chair of Human Genetics, had just identified the mouse equivalent of the gene responsible for Duchenne muscular dystrophy. Philip Avner was also fascinated with the process of X-inactivation. As a geneticist, he was particularly interested in the fact that the initiation of this process is controlled by a specific region of the chromosome. This region, called the “inactivation centre”, had been genetically identified in the sixties in mice and humans, through translocations and deletions involving the X chromosome. Most importantly, this work established that the inactivation centre operates on chromosome portions other than the X present in translocations, and that two inactivation centres are needed in the nucleus of the cells to trigger the process. Furthermore, in the mid-eighties Martin Evans and his colleagues isolated the first embryonic stem (ES) cells.

Sohaila Rastan, a former student of Mary Lyon, subsequently showed that the differentiation of female ES cells induces the inactivation of one of their two X chromosomes (Rastan and Robertson, 1985). This discovery thus made it possible to study this process *in vitro* rather than *in vivo*, which offered a considerable advantage since the random inactivation of one of the two X chromosomes occurs at the time of the implantation of the embryo – the most difficult stage to access for experimentation.

Based on these different advances, Philip Avner proposed that I use embryonic stem cells to try to identify the region of the X chromosome responsible for the initiation of inactivation, through transgenesis approaches. We were specifically concerned with determining whether the insertion of transgenes on chromosomes other than X could lead to their inactivation and whether it was possible to “deceive” male ES cells, that is, to inactivate their only X chromosome. In other words, we wanted to know whether transgenes could function as ectopic inactivation centres.

The challenge was the size of the candidate region: several million nucleotides, which amounted to looking for a needle in a haystack. But as fate would have it, the needle was found before the haystack was completely disintegrated! Just a few months after my arrival at the Institut Pasteur, a highly unusual gene was discovered by pure chance by Huntington Willard and Andrea Ballabio on the human male X chromosome, in the very region of the inactivation centre (Brown *et al.*, 1991).

This gene was named Xist (X-inactive specific transcript) because of its expression exclusively from the inactive X chromosome as well as the fact that it was non-protein coding. Shortly afterwards, the teams of Philip Avner and Sohaila Rastan isolated the mouse homologue.

In addition to its unexpected expression profile, Xist presented another remarkable property: its RNA seemed to “coat” the inactive X chromosome, which suggested that the Xist gene and its transcript play a key role in the inactivation process. I therefore decided to focus my research on this gene. Xist-deletion experiments carried out by other teams established that it is necessary to the

inactivation of the X chromosome carrying it. I chose to determine whether it was sufficient. To do so, I inserted, in the genome of ES cells and of mice, transgenes containing Xist, as well as large areas surrounding the gene. I showed that despite their large size (several hundreds of kilobases), these transgenes were not able to trigger the inactivation process properly and therefore did not contain all the information required by the inactivation centre or for the regulation of the Xist gene. This negative finding, which Philip Avner fiercely defended with me, was met with resistance from the scientific community. It went against the dogma according to which Xist was enough to induce X-inactivation! It therefore forced me to explore new hypotheses.

I drew inspiration from a series of studies published in the nineties, especially by John Sedat, Steven Henikoff and Amanda Fischer, which showed how genes' chromatin context and their position in the nucleus can significantly influence their expression. Thus it was becoming increasingly clear that the genome is not a simple linear sequence of information randomly packed into the nucleus. This realization was particularly relevant in the context of X-inactivation, since this process involves the differential treatment of two identically-sequenced chromosomes within a single nucleus.

In fact, in 1968 Comings had already proposed that the tethering of one of the two X chromosomes to the nuclear periphery could explain the differential treatment of the two homologues (Comings, 1968). He was inspired by the "bacterial replicon" of François Jacob, Sydney Brenner and François Cuzin, discovered in 1963, whereby replication of the bacterial chromosome is initiated at a specific site – the "replicator" – attached to a specific location on the cell membrane.

Based on these considerations, I wished to explore the possibility that, at some distance from the Xist gene, the inactivation centre has sequences involved in the formation of particular chromatin structures or in tethering the locus to a specific location of the nucleus. Microscopy and cell biology approaches had made fantastic strides in the nineties. Fluorescent probes could now be used with unprecedented precision to visualize the expression and position of genes in the nucleus. In order to apply these new methods to X-inactivation, I decided to take a sabbatical between 2000 and 2001 at the Cold Spring Harbor Laboratory near New York, in the laboratory of David Spector, one of the leading specialists of the study of nuclear organization. I was fortunate to find myself in this mecca of molecular biology at a turning point in epigenetics research.

First there was the work of Rob Martienssen and his colleagues, at the Cold Spring Harbor Laboratory in fact, who had just discovered the importance of the link between chromatin and small RNA molecules – a process known as "RNA interference" – in the establishment and maintenance of heterochromatin (2002). A major discovery if ever there was one, since this interaction between small RNAs and chromatin proved to be a key element of the reprogramming of the genome during fertilization in most species.

Then there was the possibility of using extraordinarily specific antibodies to recognize a whole set of subtle chemical modifications of histones, the major protein components of chromatin. This approach was a turning point insofar as it allowed for different states of chromatin to be identified, and revealed just how rich with potential information the latter is. In 2000, the "histone code" hypothesis, analogous with genetic code, was proposed by David Allis (Strahl and Allis, 2000) and Bryan Turner (Turner, 2002) independently. According to these researchers, combinations of modified histones form signatures that recruit specific factors. By linking themselves to chromatin, these factors modulate gene activity and other functions of the genome. Moreover, these modified histones could constitute "epigenetic marks" transmitting inheritable character changes – a hypothesis that is still currently being debated!

The stay at the Cold Spring Harbor Laboratory thus allowed me, in collaboration with David Allis, to show that several histone modifications distinguish active X chromosomes from inactive ones, effectively suggesting the possibility of an epigenetic memory consisting not only of DNA methylations, but also of chemical modifications of histones and of other associated proteins. Researchers are at present still searching for the “writer” proteins that lay down these histone chemical modifications and the “reader” proteins that bind themselves to the modified histones. Incidentally, in 2001 researchers had already discovered that Polycomb group proteins form a system of writer/reader proteins that contribute to maintaining the silent state of the X chromosome (Wang *et al.*, 2001).

Note that this new era, characterized by the exploration of the innermost variations of chromatin, led to a new use of the term *epigenetics*. In 2007, Adam Bird formalized this second semantic change since Waddington: epigenetics can be considered as the study of “the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states” (Bird, 2007). This definition therefore goes beyond the stricter concept, proposed by Holliday, of inheritable change in gene functions.

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Armed with the tools and approaches I had developed in Cold Spring Harbor, in 2001 I set up my own team at the Institut Curie in Paris to carry on exploring the X-chromosome inactivation process. The Institut Curie afforded an ideal environment for this new stage. I joined the Nuclear Dynamics and Genome Plasticity unit headed by Geneviève Almouzni, certainly one of the top chromatin specialists. I would like to take a moment to say just how much Geneviève Almouzni has done for me, and to thank her, both for her contagious energy and for her unfailing support. Thanks to the visionary direction of Daniel Louvard, director of the Research section, the Institut Curie is also one of the rare places in France to bring together biology, chemistry, physics and medicine so successfully.

My arrival at the Institut Curie coincided with the development of microscopy techniques allowing us to explore gene activity in connection with chromatin status and nuclear localization at the single cell level.

With these techniques, we could now follow the different stages of X-inactivation with formidable precision, in living cells. We were thus able to decipher the mechanisms of differential treatment of the two X chromosomes in the same nucleus and analyse the dynamics of chromatin changes over different timescales: within a cell cycle, during embryogenesis and throughout an individual’s life, in both normal and pathological situations (like cancer), and in mice and other species.

Our aim was primarily to determine whether the nuclear localization of the inactivation centre played any role in its function. We first showed that the two inactivation centres are situated close to the nuclear envelope, which contradicted Comings’ hypothesis of differential localization.

However, our results also indicated that just before the inactivation of one of the X chromosomes, the two centres briefly come closer to each other – an entirely new observation since nothing similar had ever been described previously.

We also started a particularly fruitful collaboration with Job Dekker’s group in the United States that had just developed chromosome conformation capture, a technique to quantifiably observe long-distance interactions within a chromosome. We showed that the inactivation centre is organized into a succession of interaction domains adjacent to each other, each covering several

hundred thousand nucleotides. The expanse of the interaction domain containing Xist indicated that some of this gene's controlling elements are situated very far away from it. This finding finally provided the molecular explanation for the lack of function of our Xist transgenes: they were all missing a part of the interaction domain! More generally speaking, this study shows that the organization of chromosomes into a succession of interaction domains could underpin a significant part of gene regulation.

We were also highly intrigued by the role of the elusive RNA molecule produced by the Xist gene. Still thanks to our microscopy approaches, we showed that in all early phases of X inactivation, Xist RNA creates a "compartment" in the nucleus, in which genes of the X chromosome are found as soon as they are inactivated. Carefully analysing this compartment, we discovered that it is mainly comprised of repeat DNA, especially a class of particular transposons, L1 retroelements. In 1998, Mary Lyon had proposed that these L1 sequences allow inactivation to spread along the X chromosome (Lyon, 1998). Our studies confirmed this hypothesis and also suggested that the L1 elements "help" the genes of the X chromosome enter the silent compartment, thereby encouraging their inactivation. Our observations therefore provide a new illustration of Barbara McClintock's visionary work on the role of transposons in the regulation of gene activity.

Carefully examining the different stages of X-inactivation in early embryos, we also had a few big surprises. With others, we discovered a completely unexpected dynamic in mice: inactivation comes very early and is subject to parental genomic imprinting, since it concerns the paternal X chromosome exclusively. While this inactivation is lasting in extraembryonic tissues, the same does not apply to the inner cell mass, which will give rise to the embryo as such. In fact, the paternal X is reactivated and inactivation, random this time, of either one of the two X chromosomes immediately ensues.

This extraordinarily dynamic process provided a first glimpse of genome-wide reprogramming events in the early stages of embryogenesis. These stages are equivalent to embryonic stem cells or induced pluripotent stem (iPS) cells. These results also revealed for the first time the plasticity of epigenetic processes themselves during development.

We obviously wondered about the *raison d'être* of this dynamic, whereby inactivation is first established and then erased, before being re-established a few cell divisions later. Following the precept of Theodosius Dobzhansky, the great theorist of evolutionary genetics for whom "nothing in biology makes sense except in light of Evolution", we began a comparative study of X-inactivation in mice, rabbits and humans.

This work revealed marked differences in pace between these three species, particularly a later induction that is not subject to parental genomic imprinting in rabbits and humans, and the lack of reactivation in the inner cell mass. Why such diversity? Mice have the particularity that in this species, the embryo's genome is activated very early – from the stage of one or two cells. Mice also develop very rapidly in comparison to other mammals, which could require very precise gene regulation systems, like parental genomic imprinting and the need for reprogramming, before the embryo-proper can develop from the inner cell mass. Once established in the embryo, inactivation is in any case remarkably stable on the scale of the chromosome. To what is this stability owed? Probably to the accumulation of different epigenetic "layers": DNA methylation, Polycomb group proteins, nuclear compartmentalization and many others which all together "padlock" inactivation. But X-inactivation does sometimes have its flaws. Our work, carried out in collaboration with Dr Anne Salomon from the Institut Curie, shows that some breast cancers are associated with X-inactivation instability.

Other questions are still unanswered, particularly concerning the reactivation of the inactive X chromosome in the germ line. How does this reversal take place? Do all epigenetic marks disappear passively during DNA replication? Or are they erased by specific enzymes remodelling chromatin?

I would have liked to present other research carried out by the members of my team, but I am short for time. Still, allow me to take a moment to tell them how deeply grateful I am. The science we are doing is inherently teamwork and I have the immense privilege of being surrounded daily with brilliant researchers. To name but a few: Julie Chaumeil, Sandrine Augio and Elphège Nora – my first students, who taught me so much; Ikuhiro Okamoto, Osamu Masui and Jen Chow – my first post-docs, and formidable drivers of my laboratory; and Macha Guggiari, Patricia Diabangouaya and Christel Picard – the pillars of the team.

To conclude on X-inactivation, I would like to say a few words on the crucial question of the role of this process in mammals.

Analogous processes can be observed in a great number of other species, like the drosophila, which doubles the expression of the genes carried by the X chromosome in males, and the nematode, which halves gene expression on each of the two X chromosomes in hermaphrodites. All these observations suggest that these processes play a crucial role in X chromosome dosage compensation between males and females. In fact, we know that dosage imbalances can have serious consequences, dramatically illustrated by Down syndrome in humans.

This dosage compensation offers food for thought. The X chromosome carries over 1,000 genes, whereas chromosome 21 contains only about 250. For which genes is compensation essential? Is it essential at all stages of development, and in which cells? Clearly some genes must be fully “silenced”. This is the case of the *Mecp2* gene, for example. In females, its absence leads to Rett syndrome, a severe type of autism. In males, its absence is lethal at the embryonic stage, whereas its duplication leads to severe mental retardation. For other genes, however, dosage does not seem to be so critical. Depending on the mammal species, up to 15% of X-linked genes can thus escape inactivation. Finally, for some genes, a double dose confers a major advantage. This is the case of the *Atrx* gene which escapes inactivation, perhaps precisely because it codes a protein involved in the remodelling of chromatin, and that this remodelling in turn contributes to X-inactivation in some cell lineages.

As all this research shows, the X chromosome is not a homogenous entity. On the contrary, like other chromosomes it is composed of a diverse set of genes, each with a precise role and a specific expression pattern during development and in each cell lineage. Thus female mammals are mosaics not only in terms of the respective expression of their two X chromosomes, but also in terms of the patterns of expression of X-linked genes! Does this provide them with an advantage over males that only have one X? Males obviously present an immediate vulnerability to X-linked gene mutation. Jean-Louis Mandel’s work on Fragile X syndrome and other “X-linked” diseases offer a striking illustration. But beyond this, does females’ wider range of cell phenotypes provided by X-linked genes provide them with any kind of advantage? While this question is in itself neutral, I cannot resist the slightly provocative pleasure of recommending the reading of Rudyard Kipling’s poem, *The Female of the Species*.

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It is now time for me to conclude. This Chair has a double title and includes the expression “cellular memory”. The notion of maintenance of a cellular state applies both through cell divisions and over

time. Moreover, cellular memory concerns gene activity as much as protein folding or the cellular structure. But where is epigenetics at today?

There has been growing public fascination with epigenetics over the last ten years, catalysed by the completion of the human genome sequencing in the early noughties. This feat sounded the start of an exciting yet terrifying stage for humanity, confronting us with the reality, and perhaps fatality of our genetic heritage. It opens up the possibility for all our characteristics – our strengths, weaknesses and predispositions to diseases – to be read in the book of our genome, with all the ethical and philosophical questions this raises. Ultimately, epigenetics creates hope that we are “more” than the sequence of our genes. This idea probably underlies the formidable explosion of interest in this field of study by the media and the general public. Can we escape the fatality of our genetic destiny? Can the food we eat, the air we breathe and even the emotions we feel influence not only the expression of our genes but also that of our children and grandchildren?

It is certain that our environment modifies the expression of our genes, and that it can sometimes lead to stable changes in our characters – and even, in some cases, to the appearance of diseases. But to what extent can these changes be transmitted from one generation to the next? That is another story. The last few years have seen the discovery of a heredity of certain characteristics that is not based on changes in the DNA sequence. This heredity, transmitted over generations, seems to be particularly important in the plant kingdom, and has been proposed as a possible explanation for the missing heredity in human genome-wide association studies (GWAS), which have recently exploded. Projects have even already been launched to identify this potential epigenetic component of susceptibility to various diseases on the scale of the genome.

But how important is the environment’s role in this epigenetic heredity? This question is far from being resolved, despite the hype, which has led to a surprising revival of interest in Lamarck’s theory of the inheritance of acquired characteristics – in other words, the idea that “beneficial” characteristics acquired during a person’s life under the effect of environmental changes can be transmitted to the following generations. The ultimate question is that of the importance of epigenetic processes in evolution. “Heredity is nothing but stored environment”, considered Luther Burbank. What role then do sporadic or environmentally-induced epigenetic changes transmitted over several generations play in evolutionary processes? The scientific community is still largely divided and a core question remains: are epigenetic states transmitted over a sufficient number of generations to give rise to natural selection?

The “popularity” of epigenetics, in the wake of growing media interest, can be dangerous. It mixes recognized scientific data with the most fanciful information. This confusion is highly detrimental to the progress of knowledge. Most fortunately, research in this field is advancing at a startling pace. It should provide clarity on these notions in the years to come.

This Chair bestows upon me the responsibility to communicate clearly and precisely about the research and concepts underpinning this emerging scientific field – a huge challenge in view of the fact that, as we have seen, the meaning of the term *epigenetics* is constantly evolving.

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