Épigénétique et mémoire cellulaire

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ENSEIGNEMENT

Cours : *Chromatin inheritance* (Chromatine et mémoire cellulaire [a])

This report is comprised of two parts: the first is a summary of the teaching and lectures delivered and the second is a research activity report. The topic chosen for my lectures this year, namely chromatin inheritance, is at central to the theme of my chair of epigenetics and cellular memory. Chromatin is the physiological template of the genome. It serves to package the genome in the nucleus, but it is also a purveyor of information, in addition to the DNA sequence. It acts to integrate signals that enable differential gene expression, DNA replication or repair, but it also acts as a buffer against untimely or inappropriate changes in gene activity. The mechanisms that enable specific chromatin states to be transmitted across cell divisions, or across generations, lie at the heart of epigenetics. The molecular building block of chromatin is the nucleosome – consisting of an octamer of histones around which 146 base pairs of DNA is wrapped. Initially, nucleosomes were regarded as rather inert scaffolds that could be packaged into more open euchromatin or more closed heterochromatin. The discovery that histones can exist in multiple states - different variants, different post-translational modifications as well as the proteins that can associate with them, led to the realisation that chromatin carries a huge potential of information to differentially mark the genome. Furthermore, it became increasingly clear that far from being inert, chromatin states are highly dynamic. This came as a surprise given the apparently stable states that can be found in some types of heterochromatin and euchromatin. In this series of lectures, I traced the history of chromatin biology, and its different constituents – as well as its diversity of states under different circumstances. I also explored the notion of heritability through the cell cycle and across generations; the mechanisms

a. Les cours sont disponibles en audio et en vidéo sur le site internet du Collège de France : http://www.college-de-france.fr/site/edith-heard/course-2014-2015.htm [NdÉ].

and timing when memory is challenged: namely during DNA replication when chromatin states must somehow be propagated, as the genome is being copied; and in the germ line, where chromatin states must be erased and then reset in order to prepare for the gametes, that will provide the next generation. Finally I discussed the emerging models for chromatin memory – how different combinations of writers, readers and erasers of histone modifications are balanced to ensure maintenance or reprogramming. This year's series of lectures form an important basis for many of the topics that I will cover in coming years, including cancer where chromatin memory can be aberrantly lost or acquired, and thus contribute to the gene misregulation that participates in tumorigenesis.

1. Chromatin and its multiple variations (La chromatine et ses multiples variations)

This first lecture consisted of a historical overview of chromatin research. Friedrich Miescher and Albrecht Kossel isolated and described the constituents of chromatin (« nuclein »), as non-protein nucleic acids and « histons » towards the end of the 19th century, it was not yet clear which of these (proteins or nucleic acids) were the carriers of the genetic information. Miescher stated in 1874 « If one…. wants to assume that a single substance is the specific cause of fertilisation, then one should undoubtedly first and foremost consider nuclein ». Although chromatin and chromosomes were accepted to form the structural basis of heredity, even in 1941, proteins (histones) were still postulated to be the site of genetic information (Schultz, J. "The evidence of the nucleoprotein mixture of the gene". CSH Symp Quant Biol, 1941). However, in 1944, Avery, MacLeod and McCarty demonstrated that DNA was the major carrier of genetic information. Nevertheless, more than a century after Miescher, the question of whether chromatin proteins might also be carried from one generation to the next is still debated. Continuing on the theme of chromatin as the physiological template of the genome, I went on to describe the discovery of the links between chromatin states and gene expression, in multiple organisms (plants, Drosophila, yeast, mouse) as well as the changes observed in chromatin states upon transcription or during the cell cycle, using cell staining methods. Importantly, studies in these organisms led to the realisation that differential chromatin states were linked to differential gene activity states. For example coat colour mosiacism due to X-chromosome inactivation in mammals, and variegated eye colors in Drosophila due to position effect variegation, demonstrated that gene expression could vary within an individual tissue, that heterochromatin could variably influence gene expression within cells of the same tissue and that differentially expressed states could be stably propagated across cell divisions. Pioneering biochemical and structural biology studies enabled the characterisation of chromatin, culminating in the structure of the nucleosome, the unit of chromatin consisting of an octamer of histones around which 146bp of DNA are wrapped. In recent decades, exciting links were made between histone post-translational modifications, histone variant proteins and gene expression states. Thanks to *in vitro* transcription with DNA and chromatin templates, as well as *in vivo* genetic and biochemical studies, it was realised that chromatin was both a barrier to, and a scaffold for gene expression. It could prevent inappropriate binding of transcription factors, but at the same time chromatin factors were required to potentiate transcription. Furthermore, the inefficiency with which somatic cells can be reprogrammed (by nuclear transfer or induced pluripotency – as discussed in my 2014 lecture series)

suggested that chromatin states underlie the stable identity of cell types. This concept of chromatin memory therefore became connected with developmental biology, as Robin Holliday and Art Riggs had predicted in the 1970's when discussing DNA methylation. In summary, chromatin was recognised to be the physiological template of the genome that could act as a default barrier to gene expression on the one hand, or as a facilitator of gene expression in the context of dedicated chromatin binding proteins and remodeling factors. Indeed, elaborate mechanisms have evolved to introduce meaningful variation into chromatin to enable and alter gene expression and other important biological processes, such as DNA replication and repair. Chromatin has also been recognised as a major carrier of epigenetic marks (cellular memory) that can propagate active and silent gene activity states during cell division.

2. Cellular Memory and Chromatin (Part 1)

The second course dealt with the mechanisms that enable chromatin marks such as DNA methylation and histone modifications, to be propagated through the cell cycle and inherited during cell division. A major challenge to the idea that chromatin could be a carrier of memory marks has been its dynamic nature. Both during DNA replication, but also throughout the cell cycle and even in resting cells. Histone proteins, and chromatin associated proteins can be removed and recycled, thus raising the question of how they are ever propagated at any one site in the genome. In this lecture I first discussed the mechanisms by which DNA methylation is copied during DNA replication. This is one of the best understood systems of chromatin replication, being DNA template-based, with specific proteins that recognize hemi-methylated DNA to redeposit DNA methylation on newly replicated DNA. Unlike DNA methylation, histones – and their modifications – do not have a DNA template based duplication system. Deposition of parental H3 and H4 occurs (randomly) within 400bp of their pre-replication position. Although histones and their modifications might be inherited, in theory, parental and newly synthesized H3-H4 tetramers are intermixed + diluted. This raises the questions of how histone modifications can be propagated – and whether they themselves are propagators of epigenetic states, comparable to DNA methylation? Biochmical and molecular genetic studies in recent years have started to decipher the nature of the proteins that can associate with the DNA "replisome" that provides the scaffold for replicating chromatin. Different trans-acting chromatin modifiers are recruited at the replication fork and associated with the DNA replisome, depending on the chromatin state and epigenetic marks being propagated. Chromatin maturation factors, including HDAC1, DNMT1 and SMARCAD1, use the Proliferating cell nuclear antigen (PCNA) which is a DNA clamp essential for replication, as a 'landing pad'. Np95/Uhrf1 which associates with PCNA and binds hemi-methylated DNA recruiting the maintenance DNA methyltransferase enzyme DNMT1, also binds and propagates H3K9 methylation. On the other hand, the mechanisms by which polycomb associated chromatin are propagated during DNA replication are still the subject of debate. Besides the need to propagate chromatin during DNA synthesis, it is now clear that chromatin proteins can be highly dynamic throughout the cell cycle, due to chromatin remodeling, and histone exchange during transcription for example, with different rates and mechanisms of histone turnover in different parts of the genome. Active, euchromatin shows the highest turnover, but even heterochromatin displays substantial dynamics. Thus it has become clear

that to maintain some states of chromatin, and gene expression, requires constant propagation. Thanks to mathematical modelling as well as in vivo tests, with an increasing knowledge of the proteins that bind histone modifications ("readers") as well as those that apply these modifications ("writers"), an emerging theme that has emerged is the need for domains of marked chromatin, spanning several kilobases of DNA, in order to stably maintain states of silent heterochromatin marked with H3K9 methylation. In this way, new models are being proposed for the maintenance of a histone modifications through replication-independent nucleosome turnover. To prevent such domains of marked chromatin from spreading into adjacent regions, the notion of boundaries has arisen. However the exact nature of these boundaries has remained somewhat unclear. Finally, the fact that silent chromatin states must be stable yet reversible was discussed, in the light of the recent exciting discovery that there an almost constant potential removal of marks such as H3K9 methylation by « eraser » proteins (eg histone demethylases) to prevent inheritance of marks. Thus it would seem that domains of H3K9 methylated chromatin are most likely to propagate stably, while smaller regions are likely to be more rapidly erased.

3. Cellular Memory and Chromatin (Part 2)

This lecture was the second part on the topic of memory mechanisms, and focused on the importance of chromatin memory in maintaining cell identity, particularly through the Polycomb and Trithorax group proteins. Thanks to the power of genetics, the roles of these proteins in maintaining developmental decisions and ensuring transitions was discovered, starting with the seminal work of Ed Lewis, and the later disoveries of Nusslein-Volhard and Eric Wieschaus showing that maternal transcription factors first establish patterns of gene expression that define the body plan of the embryo. The memory of this positional information must be conserved up to the adult stage: mis-expression of these "homeotic" genes causes developmental defects and homeotic transformations. Studies in the 1980's showed that mutations in several regulatory genes led to improper gene expression during development, and classified these regulatory factors into two antagonistic groups: Polycomb (PcG) & trithorax (trxG). Complexes with these factors were defined as master controllers of cellular memory during development and were found to be widely conserved. Biochemistry and genetics revealed the importance of these complexes in binding and modifying chromatin (eg H3K27 methylation and H3A ubiquitination are Polycomb mediated changes, and H3K4 and H3K36 methylation are Trithorax mediated changes). Specific writer, reader and eraser proteins have now been identified for both PcG and trxG and molecular insights into their mechanisms of action have been obtained in recent years. In addition to their fundamental roles in maintaining cell identities and the body plan, these complexes were also found to have key roles in maintenance of developmentally or environmentally programmed expression states, such as X inactivation in female mammals, or cold-induced vernalization in plants.

The question of how these complexes are recruited initially was discussed in this lecture. Multiple mechanisms, from recruitment via DNA sequence targeted transcription factors to non-coding RNAs have been evoked, but in the case of PcG complexes, this still remains very much an open question. How Polycomb and Trithorax chromatin states are propagated stably, once established was then discussed. During DNA replication, unlike for H3K9me3 or DNA methylation

marked chromatin, where the protein propagators appear to associate with the replisome, so far there is no evidence for direct binding of Polycomb or Trithorax complex proteins to DNA replisome. One study has reported that in Drosophila embryos, H3K27me3 modification may be lost during replication. In mammalian cells, levels are also far lower on new histones in nascent chromatin than on old histones. Nevertheless, PRC-silenced genes do not appear to be reactivated during S-phase (DNA replication). The model for Polycomb that is currently favored involves imprecise copying/re-establishment, thanks to domains of modified chromatin rather than at single nucleosomes. Thus PcG-associated histone marks are distributed over large chromatin domains, facilitating their reestablishment, similarly to H3K9 methylation domains that were discussed during the previous lecture. In conclusion, one of the main messages from these two lectures is that although nucleosomes are the basic units of chromatin, they are not necessarily the basic units for gene repression and its propagation during cell division.

In the case of Trithorax, which is implicated in the memory of active states, the mechanisms may be rather different and the process of memorisation may be rather more dynamic, being intimately linked with the act of transcription. Here, the presence of transcription factors and the RNA polymerase clearly play a role in maintaining the active expression during the cell cycle. The question is, how are active states propagated during cell division and mitosis, where there is no transcription? Some TFs may remain asociated with mitotic chromosomes and there is also evidence that some Trithorax proteins, such as MLL can remain and act as « bookmarks » at gene promoters during M phase.

Finally, the mechanisms by which Polycomb and Trithorax associated chromatin marks can be removed was also discussed. Evidence exists for both passive loss (ie the absence of maintenance mechanisms during replication for example) and active loss (enzymatic removal of histone modifications, histone exchange, nucleosome eviction, chromatin remodeling, etc.). The reprogramming of chromatin (ie loss of marks and associated factors) is particularly critical in the germ line, where cell identities must be erased in preparation for the formation of the gametes, and the next generation. In mammals, this is an active area of research (covered in past years of my lectures, and in the next lecture). Recent studies in plants have shown that aberrant PcG reprogramming can leads to inter-generational transmission. A major question that intrigues scientists and also the public, is whether all chromatin memory is erased at every generation? And if not, which epigenetic marks can be transmitted across generations and what is their impact?

4. Chromatin memory through development and across generations (La mémoire chromatinienne au cours du développement et à travers les générations)

The fourth lecture concerned chromatin inheritance and reprogramming during development. The focus was on the mouse, where the different phases of establishment, maintenance and reprogramming of chromatin states have been studied in some depth. First of all, the chromatin states of the highly differentiated and specialised sperm and egg chromosomes were discussed, as well as how they are reprogrammed in the zygote. Spermatogenesis involves dynamic chromatin changes to package, protect and possibly mark the genome. The sperm genome end us wrapped into an almost crystalline state thanks to the removal of histones and

reitroduction of protamines to enable the extremely tight packaging of the sperm genome. Residual nucleosomes can nevertheless be found in sperm. However where these lie exactly, what histone variants they are made of, what role they might play, if any, in later development and whether they resist the massive reprogramming that occurs after fertilisation, remain largely open questions. After fertilisation, the sperm genome is remodeled and repackaged with maternal histones into chromatin. Remarkably, it has been proposed that the few paternal nucleosomes that are present in sperm may resist this dramatic remodeling and potentially carry epigenetic information, although this remains a controversial topic. Massive remodeling of the paternal chromatin that occurs after fertilisation results in a transient period of asymmetry between the paternal and maternal epigenomes for the first few cell divisions. However, dynamic changes ensue as embryogenesis progresses and new chromatin states that are set up lead to global equalisation of chromatin states between the parental epigenomes. Genetic studies involving mutants in chromatin factors reveal their critical role for reorganizing the paternal and maternal epigenomes and preparing the zygotic genome for transcription. For example, recent work has demonstrated a critical role for Mll2 (TrxG) in the acquisition and maintenance of H3K4 methylation in the zygote and for normal embryonic gene activation. Furthermore, multiple chromatin factors are required for the establishment and maintenance of the extra-embryonic lineages. Thus, although the role of transcription factors in establishing gene expression programs is clearly important, more and more evidence is emerging for a critical role of chromatin factors in facilitating these patterns and in perpetuating them once they are established. Numerous developmental phenotypes due to mutation of chromatin modifiers have been reported, suggesting that many of these proteins may have one or more roles in development. Indeed, the specific roles of different chromatin proteins (writers, readers and erasers) are only just starting to emerge thanks to the use of conditional mutants.

The final question discussed in this lecture, was whether chromatin can retain any memory (somatic, germ cell or environmental) from one generation to the next and resist developmental and germ line reprogramming. In mammals, chromatin states are reprogrammed very efficiently in the germ line (somatic marks, inactive X, imprints) and during early development (just after fertilisation and also in the inner cell mass of the blastocyst), (as discussed in my 2014 lecture series on Reprogramming). On the other hand, in plants, unlike animals, there is no early separation of germline and soma, thus some epigenetic marks acquired throughout their lifetime can be included in the gametes. Most plant developmental genes involve non-CpG DNA methylation which requires a continuous remethylation cue and as such is continually reprogrammed. On the other hand, transposable elements (CpG methylation) are probably key targets for trans-generational effects. Recent work in the worm, C. elegans, has shown that H3K27me3 can be transmitted in the absence of PRC2 through cell division and even across generations. Indeed, in worms there is now convincing evidence that a combination of RNAi and histone modifying mechanisms can lead to the transmission of phenotypes over generations and this seems to be intimately linked to defense mechanisms. Exploring the potential stability of epigenetic states across generations and the implication for phenotypes linked to such epigenetic states, if they exist, is an exciting domain and the future will undoubtedly bring new insights for many organisms, including mammals as the possibility to control for genetic variation is improved, as hidden DNA sequence variation can be an important confounding effect in apparent « epigenetic » transgenerational effects.

5. Chromatin Stability Versus Plasticity in Response to Stress (Stabilité versus plasticité chromatinienne en réponse aux stress)

The final lecture focused on the impact that different types of stress – intrinsic (eg errors during DNA Replication) or extrinsic (environmental) can have on chromatin and the inheritance of changes induced by stress. All eukaryotic organisms must respond to environmental changes with changes in gene expression to survive. Environmental responses include growth, movement, learning, homeostasis, immunity. All of these involve changes in gene expression in the relevant nuclei of the organism, and some of them may involve changes to the chromatin landscape that provide access to genes that are packaged in nucleosomes. The epigenomes of an organism can be challenged by many intrinsic and extrinsic stresses. Stress can be at the organismal level but may affect specific tissues (and epigenomes) to different extents. In particular specific protection of the germ cell and stem cells appear to exist although the nature of such protection is still open. What is clear is that chromatin is a critically important component of the cellular response to stress particularly in mediating the speed and amplitude of stress responses in cells. The lecture first dealt with heterochromatin responses to heat shock and other stress (replicative stress). Heterochromatin domains pose a particular challenge to genome stability. Failure to restore constitutive heterochromatin domains after replication owing to lack of histone deacetylation or chromatin remodeling can lead to chromosome breakages and aberrant chromosome segregation in mitosis. Heterochromatin instability can also lead to aberrant transposon repeat expression and mobility. Indeed there is now substantial evidence for stress-induced reactivation of LINE-1 expression and, potentially, activity with important implications for pathology (as in cancer, where newly mobilised repeat elements can be potentially mutagenic) but also in normal physiology. For example in the brain, several recent reports have suggested that LINE expression and potential mobility can participate in cellular diversity and may even have advantageous effects. The impact that genotoxic stress has on chromatin and the collaborations between the DNA repair and chromatin machineries to enable partial or complete recovery of chromatin landscapes after different types of DNA damage was also discussed. DNA damage can disrupt chromatin states locally – this damage has to be made accessible to DNA repair factors; this impacts on local chromatin states. Loss of pre-existing histones during stalled DNA replication or DNA repair represents a potential threat to maintenance of chromatin information the fate of parental histones and chromatin proteins is still unclear. The restoration of chromatin states requires new histone incorporation and recruitment of epigenetic machinery that can replace and remodify histones appropriately. There are thus multiple roles for histone variants, histone chaperones and chromatin remodeling complexes that help to “heal a wound” or trigger destruction in case the damage is too severe. Nutritional stress can also induce chromatin changes. Indeed, Cellular concentrations of metabolites can fluctuate as a function of a cell’s metabolic tate; Thus the activity of chromatin regulators may change as a function of metabolic status and so transduce a homeostatic transcriptional response. The potential impact that nutrition has on the availability of methyl-donors to a cell was specifically discussed and whether these effects are global or certain regions are more sensitive to them. Finally – the topic of whether such changes in chromatin state due to nutritional deficiency (after fasting and calorie restriction) can have lifelong beneficial or detrimental effects, and whether

they can influence inter-generational or transgenerational inheritance of chromatin states was also touched on. The influence of environmental fluctuations during early mammalian development is still not clear although clearly nutritionel deprivation or overexposure can impact on fetal growth and have longer term effects. Furthermore, nutritional stress can have very different consequences at different time in the life cycle. However, the extent to which the changes are simply at the gene regulation and cellular signaling levels that can affect growth and development of the fetus, with an impact on later physiology; or whether they are at the chromatin level, and whether they are truly epigenetic (in the sense that they can be inherited through cell divisions to the adult, or even across generations) – remains still very much an open question. Using model organisms such as rats and mice, as well as flies and worms, researchers are actively looking to see the extent and nature of maternal and paternal induction of intergenerational responses after short and long term fasting, calorie restriction, as well as modulation of dietary protein, fat and methyl-donor content. So far the conclusion for cross-generational effects in mammals seems to be that although changes induced by extremes in nutritional intake in utero can be inherited to the next generation (ie F1 for paternal transmission, or F2 for maternal transmission) – their mechanisms may not necessarily be chromatin based (RNA based mechanisms are very much favored currently), and furthermore the changes are not stably heritable (ie beyond F2 to F3) – in other words, in the absence of the initial trigger – the change induced rapidly wears off. Thus, it is premature to conclude that heritable chromatin changes can be induced and transmitted across generations in humans as there are too many confounding effects, particularly DNA sequence polymorphisms that can clearly underlie many so-called transgenerational heritable changes. It is even unethical to "advise" or even "treat" future parents from a chromatin-based epigenetic perspective.

The conclusions of this lecture and the preceding ones can be summarised as follows: (1) Chromatin memory is essential to buffer against changes in cell identity / fate, and ensure heterochromatin stability (prevent aberrant gene expression, repeat activity, centromeric instability.). (2) Chromatin plasticity is also essential during development and in some tissues to respond to hormonal and other signals, thus equilibrium versus epigenetic stability ("domains" rather than single nucleosomes are the functional units of chromatin). (3) Stress-induced changes can impact chromatin states – that are usually reversed but may sometimes lead to heritable changes in the soma or even the germ line. (4) Chromatin states are globally erased in the germ line of all organisms. Evolution appears to have gone to great lengths to prevent the carry-over of irrelevant (or deleterious) epigenetic information that would destabilise organisation of the next generation. (5) Stress induced changes can impact on the next generation (maternal and intergenerational effects) in rodents, flies, worms although the chromatin basis is still far from clear. (6) There is no evidence that stress-induced chromatin changes can be inherited transgenerationally (ie in absence of initial stress) in mammals and even in plants where transgenerational epigenetic changes clearly do exist. However, recent work in worms suggest that such stress induced transgenerational effect can occur in this organism. The reasons for this diversity remain unclear but open up exciting new avenues of research.

SÉMINAIRES

Conférences

Trois conférences d'actualité en lien avec les cours :

Dr Robin Allshire (université d'Édimbourg, Royaume-Uni), le lundi 9 fevrier : « Epigenetic inheritance of specialised states ».

Dr Deborah Bourc'his (Institut Curie, Paris), le lundi 23 février : « Rôle de la méthylation de l'ADN dans la préservation du paysage chromatinien méiotique ».

Dr. John Greally (Albert Einstein Institute, États-Unis), lundi 2 mars : « Stress, Genomic Regulation and Heritability ».

Colloque : *Epigenetics and Cellular Memory: a role for chromatin inheritance?*

Le colloque s'est tenu les 18 et 19 mai 2015, avec les interventions suivantes :

Dr Mark Ptashne, Memorial Sloan–Kettering Cancer Center, New York, États-Unis : « The mechanism and use of transcriptional silencing ».

Dr Genevieve Almouzni, Institut Curie, Paris : « Shaping chromatin in the nucleus, the bricks and the architects ».

Dr Sara Buonomo, University of Edinburgh, Royaume-Uni : « The late identity ».

Dr Caroline Dean, Sainsbury Institute, Norwich, Royaume-Uni : « Polycomb-based epigenetic switching ».

Dr Susan Strome, UCSC, Californie, États-Unis : « Transmitting an epigenetic memory of germline across generations and through development ».

Dr Jürg Müller, Max Planck Institute, Munich, Allemagne : « Heritability of Polycomb-repressed chromatin ».

Dr Michel Wassef (Margueron Team), Institut Curie, Paris : « Epigenetic memory and cancer: The example of PRC2 ».

Dr Anja Groth, BRIC, Copenhague, Danemark : « Chromatin Replication and Epigenome Maintenance ».

Dr Jérôme Dejardin, IGM, Montpellier : « Setdb1 stimulates telomere transcription ».

Dr Francis Stewart, BIOTEC, Dresde, Allemagne : « H3K4 methylation in early mouse development ».

Dr Valeria Cavalli, Washington University, St Louis, États-Unis : « Epigenetic and Transcriptional Control of Axon Regeneration ».

Dr Olivier Cuvier, université de Toulouse, France : « Chromatin-based memory in cellular clones: novel views from single cell transcriptomics ».

Dr Danesh Moazed, Harvard University, Boston, États-Unis : « Epigenetic Inheritance of Histone H3 Lysine 9 Methylation ».

Dr Jonathan Weitzman, Epigenetics and Cell Fate, Paris : « Host-parasite interaction, an epigenetic relationship ».

Dr Rick Young, Whitehead Institute, Boston, États-Unis : « Regulatory landscape of embryonic stem cells ».

Dr Pauline Audergon, University of Edinburgh, Édimbourg, Royaume-Uni : « Restricted epigenetic inheritance of H3K9 methylation ».

Dr Geno Shi, Boston Children's Hospital, Boston, États-Unis : « Histone and DNA demethylases ».

Enseignement à l'étranger

Université d'Uppsala, 13 mars 2015, 1 cours sur : « Exploring Epigenetics in Development and Disease in the Context of X--Chromosome Inactivation ».

Université d'Oxford et Maison française d'Oxford, juin 2015, 1 cours sur : « Chromatine et mémoire cellulaire ».

Publications

Articles originaux

Chaligné R, Popova T, Mendoza-Parra M.A., Saleem M.A., Gentien D., Ban K., Piolot T., Leroy O., Mariani O., Gronemeyer H., Vincent-Salomon A., Stern M.H., Heard E., « The inactive X chromosome is epigenetically unstable and transcriptionally labile in breast cancer », *Genome Res*., 25, 2015, 488-503.

Chu C., Zhang Q.C., da Rocha S.T., Flynn R.A., Bharadwaj M., Calabrese J.M., Magnuson T., Heard E., Chang H.Y., « Systematic discovery of Xist RNA binding proteins », *Cell*,161(2), 2015, 404-16.

Sanulli S., Justin N., Teissandier A., Ancelin K., Portoso M., Caron M., Michaud A., Lombard B., da Rocha S.T., Offer J., Loew D., Servant N., Wassef M., Burlina F., Gamblin S.J., Heard E., Margueron R., *Mol. Cell*, 57, 2015, 769-83.

Chapitres de livres

Giorgetti L, Piolot T, Heard E., « High-Resolution 3D DNA FISH Using Plasmid Probes and Computational Correction of Optical Aberrations to Study Chromatin Structure at the Sub-megabase Scale », *Methods Mol. Biol.,* 1262, 2015, 37-53, DOI: 10.1007/978-1-4939-2253-6_3.

Revues, commentaires

Dekker J, Heard E., « Structural and functional diversity of Topologically Associating Domains », *FEBS Lett*., 589, 2015, 2877-84.

Galupa R. et Heard E., « X-chromosome inactivation: new insights into cis and trans regulation », *Curr Opin Genet Dev*., 31, 2015, 57-66.

Guénet JL, Panthier JJ, Avner P, Heard E, Montagutelli X., « The legacy of Mary F. Lyon (1925-2014) », *Med Sci* (Paris), 31, 2015, 687-9.

Autres activités

Principales conférences invitées 2014-2015

Séminaires

Gurdon Institute Seminar (Cambridge, Royaume-Uni) : novembre 2015 – invitée par Sir John Gurdon et Prof. Azim Surani.

EFPL (Lausanne, Suisse) : décembre 2015 – invitée par Prof. Didier Trono et Prof. Denis Duboule.

Colloques / symposia

Colloque *Hommage à Francois Jacob*, novembre 2015, Collège de France, Paris (organisatrice, oratrice).

Transgenerational Epigenetic Inheritance – Company of Developmental Biologists meeting, octobre 2015, Wiston House, Royaume-Uni (organisatrice, oratrice).

EMBO – Institut Pasteur Conference: Genetic Control of Development and Evolution - A tribute to Francois Jacob, septembre 2015, Institut Pasteur, Paris (oratrice).

Cold Spring Harbor Transcription Meeting, août 2015, CSH, NY, États-Unis (oratrice).

Cold Spring Harbor 80th Symposium, mai 2015, CSH, NY, États-Unis (oratrice).

The Wellcome Trust Waddington Symposium « Epigenetics: in dialogue with the genome », juin 2015, Édimbourg, Royaume-Uni (oratrice).

EMBO Chromatin Symposium, mai 2015, Heidelberg, Allemagne (oratrice).

British Society of Developmental Biology, avril 2015, Warwick, Royaume-Uni (oratrice).

Keystone DNA Methylation and Epigenomics meeting, mars 2015, Keystone, États-Unis (oratrice).

21st Century Genetics, *Cologne Meeting*, février 2015, Allemagne (oratrice).

Participation aux programmes nationaux et internationaux

Coordination d'un Laboratoire d'excellence « DEEP » (Développement, épigenèse, épigénétique et potentiel), conçu dans le cadre des « Investissements d'avenir » au sein de PSL (depuis 2012).

Membre du réseau européen « Epigenesys » (2010-2015).

Partenaire du projet européen intégré FP7 « Syboss » (2010-2015).

Partenaire du projet européen intégré FP7 « MODHEP » (2010-2015).

Partenaire du projet international « Epigenetics » BIOGEN Idec (2014-2017).

Membre du Conseil stratégique de la recherche (CSR) 'depuis décembre 2013).

Membre de la Comité de la recherche de la FRM (Fondation pour la recherche médicale) (depuis 2012).

Conseil scientifique de l'Institut de génétique humaine (Montpellier, France) (depuis 2011).

Membre du « EMBO Membership Committee » (depuis 2013).

Membre du Conseil scientifique de la Fondation Bettencourt Schueller (depuis 2014).

Membre du « Section Committee 7 of the Royal Society » (depuis 2013).

ACTIVITÉS DE RECHERCHE

1. Direction de l'unité de Génétique et biologie du développement à l'Institut Curie (INSERM U 934, CNRS UMR 3215)

Depuis 2010, je dirige l'unité de Génétique et biologie du développement à l'Institut Curie. L'ambition de cette unité repose sur un concept simple, mais fondamental : mieux connaître les processus qui régissent le développement normal

pour identifier l'origine des désordres pathologiques. Au cours du développement, les cellules doivent en permanence tenir compte de repères moléculaires et physiques, qui leur permettent de percevoir leur environnement, d'interagir ou de se synchroniser avec d'autres cellules, de proliférer, de prendre la décision de maintenir un état de pluripotence ou de s'engager vers la différenciation et d'acquérir une spécialisation à l'origine de fonctions tissulaires complexes. La transformation cancéreuse peut résulter de perturbation à chacun de ces niveaux, et induire un programme spatio-temporel aberrant de différenciation, de prolifération, et de maintenance de l'identité cellulaire. Les interactions au sein de l'Institut Curie assurent la continuité entre une recherche fondamentale et une recherche appliquée visant à l'amélioration du diagnostic des pathologies tumorales et au développement de traitements anti-cancéreux innovants.

L'unité de Génétique et biologie du développement fournit une trame multithématique et multidisciplinaire unique pour l'étude des événements qui affectent l'identité cellulaire dans un contexte développemental. À partir d'organismes modèles tels que la drosophile, la souris et le poisson zèbre, les chercheurs de notre unité étudient les mécanismes fondamentaux du développement, depuis la formation des cellules souches germinales, la différenciation et la morphogenèse de l'embryon, jusqu'à l'acquisition de fonctions complexes. L'année 2013-2014 a été marquée par plusieurs découvertes et publications dans les domaines de l'épigénétique et de la biologie du développement, ainsi que l'attribution d'un ERC (équipe de D. Bourchis).

Depuis 2012, notre unité, ainsi que l'UMR 3664 de l'Institut Curie, bénéficient d'une labélisation « LABEX ». Financé pour 8 ans dans le cadre des « Investissements d'avenir », ce Labex relève de nouveaux défis scientifiques dont l'impact est important tant sur le plan cognitif que pour les applications potentielles en santé humaine. Le projet DEEP (Développement, épigenèse, épigénétique et potentiel) est réalisé dans le contexte de l'Idex PSL (Initiative d'excellence, Paris Sciences et Lettres), permettant des liens forts avec d'autres instituts, en particulier le Collège de France. Les activités fédératrices, scientifiques et d'enseignement de ce LABEX en 2013-2014 sont décrites sur le site web : (http://www.labex-deep.fr/).

2. Direction de l'équipe Épigénèse et développement chez les mammifères

J'anime une équipe de recherche au sein de l'unité de Génétique et biologie du développement à l'Institut Curie (Paris). Notre but est de comprendre comment au cours du développement et de la différenciation cellulaire l'acquisition de caractéristiques cellulaires spécialisées est assurée non pas par un changement de la nature et de la séquence des gènes, mais de la manière dont ces gènes sont exprimés. Le développement embryonnaire précoce des mammifères femelles s'accompagne de l'inactivation transcriptionnelle de l'un de leurs deux chromosomes X, achevant ainsi la compensation de dose vis-à-vis des mâles XY. Ce processus, connu sous le nom d'inactivation du chromosome X, représente un paradigme de l'épigenèse développementale. En étudiant le contrôle de l'inactivation du chromosome X, nous développons des méthodes et des techniques permettant la compréhension de mécanismes fondamentaux qui sous-tendent la régulation de

l'expression des gènes, à la fois au cours du développement et de la différenciation cellulaire, mais aussi lors de la tumorigenèse.

L'inactivation du chromosome X est un modèle de choix pour décrypter les mécanismes moléculaires mis en jeu lors de la prise de décisions développementales, ainsi que pour assurer leur maintien. Notre recherche est organisée autour de quatre axes principaux de recherche :

1. Quels sont les mécanismes contrôlant l'initiation de l'inactivation du chromosome X ?
2. Comment la répression transcriptionnelle du chromosome X est-elle établie ?
3. Comment l'état inactif est-il fidèlement transmis au cours des générations cellulaires ?
4. Comment le développement tumoral affecte-t-il le maintien de l'état inactif du chromosome X ?

Résumé des découvertes récentes de l'équipe (pour plus d'information, consulter le site web de l'équipe http://ugbdd.curie.fr/fr/equipe_heard) :

1. Développement d'un modèle physique permettant une meilleure compréhension des fluctuations structurelles de la chromatine, qui pourraient expliquer la dynamique transcriptionelle des gènes impliqués dans la mise en place de l'inactivation du chromosome X. *Giorgetti et al, Cell,* 2014.
2. Découverte de plusieurs gènes exprimés de manière monoallélique au cours du développement chez la souris, et impliqués dans des maladies autosomiques dominantes chez l'homme. Gendrel *et al*., *Developmental Cell,* 2014.
3. Identification de la protéine Jarid2 comme facteur principal dans le recrutement du complexe polycomb PRC2 à la chromatine via l'ARN Xist. Rocha *et al., Mol. Cell,* 2014.
4. Découverte que la présence de deux chromosomes X actifs empêche la différenciation des cellules souches embryonnaire, via la modulation des voies de la signalisation. Schulz *et al., Cell Stem Cell,* 14.

Faits illustrant le rayonnement ou l'attractivité académiques de l'équipe :

1. Deux ERC « Advanced Investigator Award » : de 2010 à 2015 (EpigenetiX) et de 2015 à 2020 (XPRESS).
2. Labellisation « La Ligue contre le cancer » (2012).
3. Participation à trois projets européens (SYBOSS, MODHEP, Epigenesys) et un projet NIH actuellement (2010-2015).
4. Fellow of the Royal Society (2013) et Membre de l'EMBO (depuis 2005).
5. Prix de la Fondation Allianz Institut de France (2013), médaille d'argent du CNRS, Grand Prix de la FRM.

Principales contributions de l'équipe à des actions de formation :

1. Comité scientifique de plusieurs cours internationaux (Masters/PhD) à l'Institut Curie (cours Épigénétique, génome non-codant, biologie du développement et d'autres).
2. Enseignement à différents cours M2/PhD à l'Institut Curie, l'Institut Pasteur, ENS et autres (environs 9 cours/an).
3. Accueil de stagiaires (collégiens, lycéens, etc.), par exemple « Opération apprentis chercheurs » au sein de l'équipe.