CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

Sciences du Vivant - Environnement et Développement durable

CONFÉRENCES JACQUES-MONOD

CONFERENCE REPORT

EPIGENETICS IN DEVELOPMENT AND DISEASE: PERSPECTIVES FROM MULTIPLE ORGANISMS

EPIGENETIQUE, DEVELOPPEMENT ET PATHOLOGIE : DE LA LEVURE A L'HOMME

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1. Report on administrative aspects

The first Epigenetics Jacques Monod Conference took place in Aussois, in the French Alps, from January 4th to January 8th 2006.
The date of the conference was carefully chosen so as not to overlap with other Epigenetic conferences such as the Keystone or the EMBL meetings, thus ensuring the presence of some of the foreign invited speakers, who frequently attend both meetings.

The total number of participants was 107. Among the 27 invited speakers, 5 came from overseas (USA, Japan, Australia) and 9 from European countries. In addition, 14 speakers were selected from submitted abstracts, providing a forum for the newest data to be discussed and an opportunity for younger investigators to present their work.
Three speakers could not participate in the meeting, for family or illness reasons, and two of them were replaced by members of the corresponding laboratories.

The nationality composition of the participants was as follows :

Country	Invited Speakers	Other participants	Students/ Post-Docs
Australia	1		
Austria	1		
Belgium		4	
France	12	57	27
Germany	2	2	
Italy	1		
Japan	1	1	
New Zealand		1	
Sweden		1	1
Switzerland		2	1
The Netherlands	1	2	1
UK	3	8	3
USA	3	1	1
Total	**25**	**79**	**including 34**

Among the participants, the proportion of students and post-docs was 32% and it was noted that a very high proportion of the speakers and participants were women (53 %).

We chose to ask for a reduced price for student and post-doc registration (350€ versus 550€). The meeting expenses were totally covered by the CNRS contribution. From the final calculations obtained by Dominique Lidoreau, the budget was fully equilibrated, with a slight profit for the CNRS. This was achieved with no compromise to the quality of the hospitality or scientific participation.

The meeting was advertised by different strategies.
The CNRS Jacques Monod committee (Dominique Lidoreau, Paul Hossenlopp and Didier Hatat) sent out advertising posters to a series of laboratories in France and set up a very functional Web page.

We used the mailing lists from different epigenetic networks to send the information out to foreign and french laboratories, as well as advertising the conference on the European Network Web page and at the Epigenetics Gordon conference in August 2005. One thing that future organisers should be aware of is that scientists will register up to the last minute…

The presence of the Jacques Monod committee and especially of Dominique Lidoreau offered an extraordinary comfort for the meeting organisers. We could constantly rely on her competence and experience to foresee and solve any potential problem …

The meeting was composed of three plenary lectures of 60 minutes and of seven sessions with 30 minute talks interspersed with short talks from 14 selected participants. Each plenary speaker was formally introduced by different senior scientists.
We invited 25 speakers, all working on epigenetics, of course, but we took great care in selecting scientists whose focus was on different organisms. As a consequence, all speakers made a special effort to give very comprehensible presentations.

The plenary lectures, given by pioneers in the field, gave an excellent overview on very different epigenetic processes in three different models (RNA silencing in Plants and Yeast, DNA methylation in Neurospora and Genomic Imprinting in mouse and human).
In most cases, speakers presented an introduction to their subject, with published results and then added unpublished material as well as future prospects. A large number of questions and dicussions occurred after each talk, with enthusiastic discussions carried into the dining room. We were very grateful to the catering staff for their patience when we were sometimes slightly late for a meal.

Poster sessions were set in the evening, after dinner in the spacious mezzanine area. These sessions were very successful with extensive interactions that actively involved the young and more senior investigators alike. We had originally planned for one hour and a half sessions, but they clearly lasted for over two hours. We had 40 posters for 40 boards, which allowed us to keep the posters up for the two sessions and throughout the meeting. This was greatly appreciated by all, since it gave more time to participants to see all the posters in the evenings, and also to refer to the posters during the day, if needed for additional discussion.

The meeting was organised such that the afternoons were free, with the possibility for participants to go skiing, or walking around Aussois. The snow and the nice weather were a very positive asset of this meeting.
The structure of the Paul Langevin Centre was highly appreciated, since the conference room, the meals, the posters and the bedrooms were all in the same location, greatly favoring informal interactions between all the participants.
A very convenient WiFi set up has recently been installed in the Centre and was especially appreciated by senior scientists.

This meeting was a great success, which we sensed during the conference but also afterwards from the very positive feedback we have since obtained from participants. For example, several students and post docs mentionned to us how much they had appreciated the incredible opportunity to be with senior scientists in the field and to listen to their discussions at the dining room table. .

During the last evening session, we raised the question of whether we should have another Epigenetics meeting in two years time. A strong majority of the participants were in favor of this. Anne Ferguson-Smith accepted to become the President and Edith Heard the Vice-President (Institut Curie, France). It was requested that if a future meeting was approved, that the meeting be scheduled in Aussois for the beginning of February (off-peak) to keep travel costs lower for international participants.

2. Report on scientific aspects

Plenary lectures

The first Plenary Lecture given by Rob Martienssen (Cold Spring Harbor, USA) had a stimulating title : « Making sense of junk RNA ». His working models are the plant, Arabidopsis and fission yeast, in which transposons and heterochromatic repeats can regulate neighbouring genes. Inspired by the nobel prize winning work of Barbara McClintock, he explained that heterochromatic (junk) RNA is widespread and processed by RNAi, notably from tandem repeats. In Arabidopsis, the SWI/SNF remodeler DDM1 targets DNA methylation and histone H3 K9 methylation to transposons, probably via siRNA. It was speculated about the application of these findings to euchromatic regions and to higher organisms.

The second Plenary Lecture was given by Eric Selker (Eugene, Oregon, USA), who presented findings from his lab on DNA Methylation and Genome Defense in the model eukaryote *Neurospora crassa*. Nearly all methylated regions of Neurospora are products of RIP (repeat-induced point mutation), a premeiotic homology-based genome defense system that Eric Selker discovered in the 80's. Their recent work has revealed clear ties between modifications of DNA and chromatin, most notably that the DIM-2 DNA methyltransferase is directed by heterochromatin protein 1 (HP1), which in turn recognizes trimethyl-lysine 9 on histone H3, placed by the DIM-5 histone H3 methyltransferase. Using this model system, Eric Selker presented the evidence that histones can serve to integrate diverse signals to control DNA methylation. This was useful because it showed that in biology, histone modifications have the potential to confer DNA methylation *de novo*.

The third Plenary Lecture focused on Genomic Imprinting and was given by Denise Barlow (Vienna, Austria). Genomic imprinting in mammals is regulated by the cells normal epigenetic machinery. Imprinted expression of the mouse *Igf2r* gene is directly regulated by the *Air* non-coding RNA. At this time it is not known if the *Air* ncRNA itself is used for gene silencing, or, if its expression is sufficient. She examined the behaviour of the *Air* ncRNA and also looked at chromatin modifications around the imprinted *Igf2r* gene to investigate the relationship between non-coding RNA expression, DNA methylation and regional histone modification. This brought together themes from the previous plenaries in a mammalian model system. Surprisingly *Air* was discovered to be a highly unstable RNA and to affect chromatin only at the promoters of the genes it silences. Together, this data does not support a spreading model whereby the *Air* ncRNA is itself used for silencing such as has been suggested in mammalian X inactivation. Instead, it indicates that expression itself may be sufficient.

Sessions

The first session addressed **Epigenetic phenotypes, mechanisms and disease**.
Anne Ferguson-Smith (Cambridge, UK) considered the mechanism of imprinting from an evolutionary perspective. She proposed that domains with methylation marks on the paternal chromosomes acquired a protection from methylation in the maternal germline in order to evolve imprinting control. In addition, methylation marks on the maternal chromosome were actively acquired specifically to regulate imprinting. Both scenarios suggest a mechanism in which imprints are regulated by the maternal germline; termed the Matriarch Model. Mother's influence on imprints continues in the newly fertilised egg where major genome-wide reprogramming events take place regulated by the egg cytoplasm which protects germline methylation imprints from this reprogramming.
We then switched to plants in which epigenetic alterations in gene expression are often transmitted meiotically, and could therefore contribute significantly to natural genetic variation. Vincent Colot (Evry, France) presented data which indicate that approximately 1% of Arabidopsis genes have the capacity to adopt distinct epigenetic states that are stably inherited across mutiple generations. Remarkably, most "epimutable" genes are characterized by ancestral adaptations of transposable element sequences. These events are therefore likely to play a major role in conferring epimutability to genes and has wider implications for our understanding of the heritability of acquired epigenetic states and the role of epigenetic changes in disease.

Coming back to genomic imprinting, Marisa Bartolomei (Philadelphia, PA, USA) discussed the dynamic programming event affecting early imprinting marks on the *H19-Igf2* locus. Imprinted genes rely on epigenetic modifications that are set in the germline or early embryo for parental identity to be assumed in the developing organism. These marks must be properly conferred and subsequently maintained for normal development--absence of appropriate epigenetic modifications leads to disease. She described recent data from her group showing that multiple trans-acting factors, including CTCF and MBD3, are required to set and maintain epigenetic marks and protect the normally unmarked chromosome from acquiring the wrong imprint.
Continuing with the *H19-Igf2* locus, Luisa Dandolo (Paris, France) described a potential role for the *H19* non-coding RNA. By producing gain of function transgenic *H19* mice, she showed that the reduced size phenotype of these mice was linked to a decrease in the level of *Igf2* mRNA, thus suggesting that the *H19* RNA could have a transcriptional or post-transcriptional silencing *trans* effect on other genes. The mechanism by which this RNA can act as a repressor still remains to be identified.

The second session was entitled **Nuclear organisation and chromatin interactions.**
Geneviève Almouzni (Paris, France) presented recent work on histone H3 variants and their post-translational modifications in mammalian cells. She compared modifications before and after incorporation into chromatin for each individual variant. Based on these findings she discussed how the history of histone modifications for each variant in combination with local activities can impact on the final state of equilibrium that is reached in specific domains of the nucleus.

The third session adressed **Dosage compensation.**
Asifa Akhtar (Heidelberg, Germany) reported on the purification of the dosage compensation complex, which is required for hyper-transcription of the single male X chromosome in

Drosophila. She showed that several nucleoporins biochemically co-purified with male-specific lethal (MSL) proteins, and that these nucleoporins are required for the proper localization of the MSL complex and dosage compensation of X-linked genes. These results highlight the role of nucleoporins in gene regulation. The application of this model system for disease processes in mammals was also discussed.
Edith Heard (Paris, France) addressed the importance of nuclear localisation of the *Xist* RNA, responsable for X inactivation in mammals. Using immunofluorescence and RNA FISH in female ES cells, she showed that the *Xist* RNA domain was a repressive compartment in the nucleus, with exclusion of RNA PolII. She also showed that X-linked genes become relocated within the *Xist* RNA domain during X inactivation.
Takashi Sado (Mishima, Japan) spoke about the *Tsix* gene which silences *Xist* through modification of the chromatin structure. It is still unknown, however, whether its RNA product is required for the function of *Tsix* or whether just the act of antisense transcription through *Xist* is sufficient. To get more insight into this, he eliminated spliced *Tsix* RNA in mouse while keeping antisense transcription unaffected. He also generated mice in which *Tsix* is truncated before it runs across the *Xist* promoter. Analyses of these mice suggest that antisense transcription across the *Xist* promoter is more critical than the RNA itself for *Tsix*-mediated *Xist* silencing.
Claire Rougeulle (Paris, France) also presented data on the function of *Tsix* and showed that this gene is a chromatin remodeler, capable of acting at two levels. It triggers a long range H3K4 dimethylation, suggesting it is involved in the transition between imprinted to random X inactivation. It also induces a repressive chromatin structure at the *Xist* promoter, suggesting a role in the choice of which X is silenced during random X inactivation.

The fourth session described **Regulatory silencing proteins.**
Pierre-Antoine Defossez (Paris, France) spoke about five known proteins binding to methylated DNA in mammals: MBD1, MBD2, MBD4, MeCP2, and Kaiso. Genetic experiments suggest that other actors involved in methylation sensitive DNA binding are yet to be discovered. With this in mind, he searched the human genome for Kaiso-related proteins and found two proteins: ZBTB4 and ZBTB38. These proteins, like Kaiso, bind methylated DNA and repress transcription and their role is now being investigated.
Robert Feil (Montpellier, France) talked about imprinting control regions in the mouse, and how their differential epigenetic organisation is maintained during development with particular emphasis on histone modification and the roles of Polycomb group proteins in that process He spoke about the Kcnq1 imprinted domain on distal chromosome 7 where many genes are imprinted in the placenta only. This imprinting seems to be independent of maintenance DNA methylation, and correlates with H3 K9 and K27 methylation on the repressed chromosome. He found the K9 specific HMT G9a to be important for the somatic maintenance of this placenta-specific imprinting.
Continuing on the important function of Polycomb group proteins, there were also two talks which dealt with the Polycomb mediated silencing in Drosophila (Valerio Orlando, Naples, Italy) and in X chromosome inactivation (Neil Brockdorff, London, UK). Together these talks provided a useful comparative framework to consider the role of these proteins in conferring silent states in different epigenetically regulated contexts.

The fifth session dealt with **Epigenetic reprogramming in the early embryo and germline.**
The fact that the nucleus of differentiated somatic cells can be reprogrammed by nuclear transfer (cloning) in order to sustain embryonic development is now well established. Nathalie Beaujean (Jouy-en-Josas, France) described results in the mouse which demonstrate that the mechanisms of this reprogramming include reorganization of the pericentric heterochromatin

as a step-wise process occuring immediately after the nuclear transfer, spreading over the whole first cycles and leading in half of the embryos to a normal nuclear structure able to initiate embryonic genome activation.
Wolf Reik (Cambridge, UK), focused on higher order chromatin architecture and the role of DNA methylation, Matrix attachment regions and the CTCF insulator protein in organisation of long range regulatory interactions. He also described challenging experiments assessing changes in histone modifications in primordial germ cells, in an attempt to define the imprinting mark and the dynamics of its erasure.
Jörn Walter (Saarbrucken, Germany) spoke about mechanisms of epigenetic reprogramming in the zygote. He described experiments preformed in his group to a) analyse the sequence of chromatin modifications in parallel with DNA demethylation and b) to investigate if the active demethylation processes in male chromosomes of the zygote are coupled to DNA repair processes. He discussed the possible link between histone modification at histone H3K9 and DNA-demethylation processes. He furthermore showed that he does not observe are clear sign of DNA strand breaks in the zygotic DNA during early stages but found a striking association of the double strand repair marker gamma H2A.X with zygotic chromosomes.
Deborah Bourc'his (Paris, France), a new young principal investigator recently returned to France from the US, suggested that methylation imprints were strikingly biased towards the maternal genome. She presented a mutability model explaining the erosion of paternal methylation imprints as a result of the earlier timing of paternal imprint establishment through the action of Dnmt3L during spermatogenesis. Using a mouse model devoid of imprints of both parental origins, she provided functional data illustrating the more important role of maternal imprints compared to paternal imprints in early development.
Saadi Khochbin (Grenoble, France) showed that spermiogenesis in the mouse is associated with a spectacular re-programming of the pericentric heterochromatin, which evolves towards a structure accumulating hyperacetylated histones just before their replacement by new histone variants forming a new type of DNA-packaging structure. These histones survive in mature spermatozoa and have the potential to convey a male-specific epigenetic information.

The sixth session was entitled **Genome rearrangements and small non coding RNAs** and emphasised the value of model organisms for the study of small non-coding RNAs in vivo.
Three different examples of epigenetic effects were described by Eric Meyer (Paris, France) in ciliates, by Benoit Arcangioli (Paris, France) in S. pombe and by Stéphane Ronsseray (Paris, France) in Drosophila.

The last session adressed **Epigenomics and evolution.**
Bas van Steensel (Amsterdam, The Netherlands) reported the genome-wide mapping of genes that interact with the nuclear lamina in Drosophila. Using the DamID technology, nearly 500 genes were identified that interact with lamin. Detailed characterization of these genes provided new insights into the links between various chromatin modifications and interactions of the genome with the nuclear lamina.
Robert Rapkins (Canberra, Australia) discussed evolution of genomic imprinting by comparing the extent to which imprinting in eutherian mammals is conserved in the meta and prototherian mammals, the marsupials and monotremes.

Hence the scientific programme encompassed a large number of epigenetic processes in a wide-range of model systems and organisms. The discussions allowed useful comparisons to be made between the results presented, and stimulated considerable debate and speculation around new ideas and the development of new theories. This was

an active and energetic symposium reflecting the quality and importance of Epigenetics research.

3. Concluding remarks

Epigenetic effects on gene regulation have been known for only a little more than twenty years and are thus a relatively new field in biology. DNA methylation was the first epigenetic mechanism to be described and studied for a long time. More recently, several other mechanisms have been suspected to be involved in epigenetic control, such as histone modifications, silencing proteins, RNAi based controls and sub-nuclear localisation. With the rapid evolution of these new aspects, epigenetics is becoming more and more complex but at the same time, there is an increasing excitement about understanding the basic mechanisms underlying the epigenetic processes.

The strength of this meeting resided in the fact that all participants were familiar with epigenetic terms but maybe not with the different organisms used to study this gene regulation. Several models, each with specific characteristics and tools, were described in front of a very receptive audience who were not inhibited to ask lots of questions. This will most certainly lead in the near future to collaborative interactions between groups as well as investigations of different mechanisms in our own favorite biological model.

This conference successfully brought together scientists all working on epigenetics but on many different aspects of the question. It gave an excellent overview of the current state of this very rapidly evolving field, which we think was of great benefit to the younger students and post docs, as well as to the other participants.

A suggestion for a future meeting would be to consider the possibility of inviting an epistemologist interested in the development of epigenetics, such as Michel Morange. In addition, representatives from the field of cancer epigenetics could also be encouraged to attend.

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