Sciences biologiques,
Écologie et Environnement
CONFÉRENCES
JACQUES-MONOD

Roscoff (France), 14-18 Septembre 2013

Méthylation et déméthylation de l'ADN

DNA methylation and demethylation

PRESIDENT : **Pierre-Antoine DEFOSSEZ**
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Rapport sur la conférence

Conference report

CONFERENCE Jacques Monod
Centre national de la recherche scientifique

DNA methylation & demethylation

September 14-18 2013

Registration, food, and housing:
-460€ for students
-600 € for others

We can only accept 85 attendees
To express interest in the conference, please email an abstract and a publication list to:

pierre-antoine.defossez@univ-paris-diderot.fr

Speakers

Stephen B. BAYLIN JHMI, Baltimore	*Kristian HELIN* BRIC, Copenhagen	*Anjana RAO* LIAI, San Diego
Stephen BECK UCL, London	*Edith HEARD* Institut Curie, Paris	*Gilles SALBERT* CNRS, Rennes
Deborah BOURC'HIS Institut Curie, Paris	*Jean-Pierre ISSA* Temple U., Philadelphia	*Irina STANCHEVA* Wellcome Ctr, Edinburgh
Thomas CARELL LMU, Munich	*Albert JELTSCH* University of Stuttgart	*Dirk SCHÜBELER* FMI, Basel
Susan CLARK Garvan Inst., Sidney	*Peter JONES* USC, Los Angeles	*Toshikazu USHIJIMA* NCCRI, Tokyo
Luisa DANDOLO Institut Cochin, Paris	*Skirmantas KRIAUCIONIS* LICR, Oxford	*Michiel VERMEULEN* UMC, Utrecht
Claire FRANCASTEL CNRS, Paris	*Antonello MAI* University of Rome	*Michael WEBER* CNRS, Strasbourg
Ingrid GRUMMT DKFZ, Heidelberg	*Dinshaw PATEL* MSKCC, New York	*+8 short talks selected from abstracts*
Petra HAJKOVA MRC, London	*RADVANYI François* Institut Curie, Paris	

http://www.cnrs.fr/insb/cjm/cjmprog_e.html

Deadline

May 15, 2013

Organizing Committee

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Résume du Rapport

La conférence « Méthylation et Déméthylation de l'ADN » s'est déroulée du 14 au 18 septembre 2013 à Roscoff. Cette conférence était la première d'une série potentielle.

Parmi les 115 participants venant de différents pays (Allemagne, Australie, Belgique, Danemark, Etats-Unis, Espagne, France, Italie, Japon, Luxembourg, Lithuanie, Pays-Bas, Pologne, Royaume-Uni), 29 conférenciers de renommée internationale étaient invités auxquels se sont ajoutés 9 orateurs sélectionnés parmi les résumés soumis et 2 présentations attribuées aux 2 meilleurs présentateurs de poster, désignés par un jury ad hoc. De l'avis général, les posters dans leur ensemble étaient excellents.

Nous avons fait le choix stratégique de focaliser la conférence sur la méthylation et la déméthylation de l'ADN chez les mammifères, mais d'y représenter différentes disciplines complémentaires: biochimie et biologie structurale, biologie cellulaire et développementale, chimie, pathologies (avec notamment une emphase sur cancers et processus inflammatoires).

La conférence a reçu une réponse très enthousiaste. Les points soulignés sont l'excellente qualité des conférences proposée, la facilité des interactions avec les conférenciers et surtout la couverture d'une thématique au plein cœur de l'actualité, et qui n'est pas représentée correctement dans les conférences existantes.

Nous avons atteint le nombre maximal d'inscrits (115 personnes) bien avant la date limite et nous avons du refuser des inscriptions. Les questionnaires de satisfaction sont très positifs et souhaitent voir une pérennisation de ce cycle de conférence.
Les organisateurs et les participants soulignent la qualité de l'infrastructure locale fournie par le CNRS, et notamment l'excellent travail de Nathalie Babic, Alain Paoli, et du personnel de restauration.

Programm Overview

The conference took place in Roscoff from September 14th to September 18th, 2013. 115 participants (or attendees), including 29 invited speakers (of whom 3 gave plenary lectures), 9 short-talks selected from the abstracts, 2 talks selected as best posters by an ad hoc committee, and 75 other attendees.

The audience was international: from Australia (3), Belgium (4), Denmark (4), Germany (6), Italy (2), Japan (1), Lithuania (1), Luxemburg (1), Poland (2), Spain (1), the UK (8), and the US (2). There were 32 PhD students in attendance.

The conference was the first in a potential new cycle, dealing with "DNA methylation and demethylation". We focused on mammals, but chose to cover complementary approaches: biochemistry, structural biology, molecular biology, developmental biology, chemistry, human diseases (mainly cancer and inflammation).

Summary

The DNA methylation and demethylation conference was organized in 6 sessions, each consisting of 30-minutes talks given by the invited speakers and 15-minutes talks selected from the abstracts.

The conference also featured 3 keynote lectures and the first evening opening keynote lecture was given by **Pr Jean-Pierre Issa, (Philadelphia, PA, USA):** ***Epigenetic therapy using DNA methylation inhibitors.***
He addressed the paradox of how DNA methylation inhibitors, which should be completely non-specific, reprogram cancer cells but not normal cells. He then presented a clinical update of the use of the new DNA demethylation inhibitor, SGI110, a prodrug of 5-aza-deoxycytidine. Lastly, he presented recent biological screens designed at identifying new molecules that could reactivate methylated tumor suppressor genes in cancer.

Session 1. DNA methylation, pluripotency and gene expression

Dinshaw Patel (New York City, USA): ***Structural Biology of DNA Methyltransferases***
By using structural biology he investigated the control of DNMT1 activity. What prevents the enzyme from methylating a CpG across a non-methylated CpG? What molecular mechanisms are used to keep the enzyme inactive on the sites that are not appropriate targets ?

Déborah Bourchis (Paris, France): ***Small RNAs and DNA methylation in the oocyte***
Déborah discussed new paradigms in genomic DNA imprinting, showing that some loci are imprinted in a lifelong and ubiquitous manner, others in a lifelong but tissue-specific manner, others still in a transient manner. The molecular mechanisms were discussed.

Sebastien Smallwood (Cambridge UK): ***Transcription drives DNA methylome shaping in mouse oocytes.***

In his short-talk, Sebastien presented innovative techniques for mapping DNA methylation from a very small number of cells, applied these techniques to mouse oocytes and investigated the links between transcription and DNA methylation.

Michael Weber (Strasbourg France): ***Dynamics of DNA methylation in the mouse***
Michael Weber identified the genes that present dynamic DNA methylation during mouse embryonic development and established that DNA methylation is required for stable silencing of key genes *in vivo* (*e.g.* pluripotency, germ line genes), but it is not a general mechanism of gene repression.

Petra Hajkova (London UK): ***DNA demethylation in primordial germ cells***
Petra Hajkova investigates the mechanisms and kinetics of DNA demethylation that occur in the zygote and primordial germ cells. In particular, she studied the role of TET proteins, as well as the role of Base Excision Repair.

Irina Stancheva (Edinburgh UK): ***Establishing DNA methylation in the embryo***
To address the mechanisms that target DNA methylation to the appropriate regions of the genome, Irina focused on the protein LSH, a chromatin remodeler that is crucial for normal DNA methylation levels in mammals and plants.

Juliette Salvaing: ***DNA methylation and hydroxymethylation in rabbit embryos : effect of in vitro culture***
Juliette presented in her short-talk the kinetics of DNA methylation and hydroxymethylation in the rabbit zygote.

Session 2 Methylation dynamics in development

Skirmantas Kriaucionis (Oxford UK): ***roles of 5-hmC in the brain***
S. Kriaucionis described methods that permit the very precise isolation of nuclei from specific cell types in the mouse brain. Using these techniques, he discovered the presence of 5-hmC in Purkinje cells. He presented quantitative, genome-wide analysis of 5hmC, 5-methylcytosine (5mC) and gene expression in differentiated CNS cell types in vivo.

Ingrid Grummt (Heidelberg Germany): ***DNA methylation in the control of rRNA genes***
Ingrid Grummt and her coworkers analyzed the role of pRNA (promoter-associated RNA), a noncoding RNA that is complementary to the rDNA promoter, in mediating de novo CpG methylation of rRNA genes (rDNA). pRNA interacts with the target site of the transcription factor TTF-I, forming a DNA:RNA triplex that is specifically recognized by the DNA methyltransferase DNMT3b.

Hélène Bierne (Paris France): ***A role for the DNA demethylation pathway in persistent infection of hepatocytes by the bacterial pathogen Listeria***
H. Bierne has established a system in which human liver cells are persistently infected with the bacteria Listeria. She then studied the effect of this infection on the epigenome. Her hypothesis is that DNA hypomethylation might induce expression of genes involved in cell proliferation and survival. Thus DNA hypomethylation could promote bacterial infection.

Arnaud Krebs (Basel, Switzerland): ***Lessons from the methylome of mouse ES cells***
How are CpG islands protected against methylation? A. Krebs reported a new approach to determine the outcome of integrating CpG island fragments in a specific location of the mouse genome. He showed a contribution of both CpG richness and of transcription factor binding sites.

Luisa Dandolo (Paris France): ***DNA methylation, Methyl-binding proteins, and imprinting***
Luisa Dandolo and her group investigate the role of the non-coding RNA H19. She reported that H19 is a developmental reservoir of miR-675 that suppresses growth and Igf1r. She then showed that H19 interacts with a methyl-binding protein, which is required for H19 to exercise its role in gene regulation.

Keynote lecture 2 Anjana Rao (San Diego USA): ***Function of the TET Enzymes in DNA demethylation***
Anjana Rao gave an overview of the topics on DNA demethylation that are studied in her laboratory:
1) the mapping of 5hmC during thymus differentiation
2) the association of TET2 loss-of-function and low 5hmC levels with cancer
3) the role of TET proteins in reprogramming
4) new methods for the mapping of modified cytosines

Session 3 Technology and Genome wide mapping

Thomas Carell (Munich Germany): ***DNA Bases Beyond Watson and Crick***
Thomas Carell reported the results of his work on the demethylation pathway of mC and in particular on the oxidized bases, including hydroxymethyluracil. He has investigated the nature of proteins that recognize 5hmU.

Stephen Beck (London UK): ***Insights into cancer epigenome dynamics through methylome analysis***
This talk presented new technologies for the mapping and analysis of DNA methylation. These methods were applied to the epigenome of different cancers, in order to identify the underlying mechanisms driving aberrant hypermethylation and hypomethylation.

Saulius Klimasauskas (Vilnius Lithuania): ***New chemo-enzymatic approaches for epigenome profiling***
Dr Klimasauskas presented a method to identify the unmodified cytosines in the genome, the "unmethylome". This technique is complementary to the existing protocols for mapping methylated, hydroxymethylated, and other modifications of cytosine.

Elmar Weinhold (Aachen Germany): ***Expanding the chemical repertoire of methyltransferases with synthetic cofactor analogues***
He showed the use of methyltransferases to label DNA. By this technique he was also able to do nanostructuring of DNA and for example he mimicked a 3-way DNA junction.

Richard Meehan (Edinburgh UK): ***DNA Methylation Shapes the Polycomb Landscape***
The relationship between DNA methylation and Polycomb silencing is a longstanding question in the field. Pr Meehan mapped H3K27me3 in mouse cells almost devoid of DNA methylation

(Dnmt1n/n MEFs) His results establish that an intact DNA methylome is required for appropriate Polycomb-mediated gene repression by constraining Polycomb Repressive Complex 2 targeting.

Michiel Vermeulen (Utrecht, Netherlands): ***Exploring DNA methylation and demethylation by proteomics***
The Vermeulen team has used quantitative Mass spectrometry to identify the proteins that bind methylcytosine and its oxidized derivatives. This was done in three mouse cell types: ES, neural progenitors, and adult brain. The results reveal that these proteins are numerous, varied, and highly dynamic from one tissue to the other.

Gilles Salbert (Rennes France): ***5-hmC and enhancers***
Gilles Salbert and his lab investigates the regulation of enhancer activity and its link with cytosine hydroxymethylation. They have found that 5-hydroxymethylcytosine (5hmC) is dynamically associated with transcription factors binding to distal regulatory sites during cellular differentiation.

Nehme Saksouk (Montpellier France): ***Non-overlapping functions for histone and DNA methylation enzymes at pericentromeres uncover Polycomb dependent compensation mechanisms***
This short talk discussed the use of PiCH to discover the proteins associated with pericentromeres in mouse cells.

Sidney Hecht (Tempe USA): ***Novel Multifunctional Radical Quenchers Directed at Mitochondrial Energetics and Epigenetics***
A human being produces and burns 50-75 kg (100-150 moles) of ATP/day. During the process in the mitochondria there is a 2% linkage, we need something to trap these electrons. Multifunctional Radical Quenchers (MRQ) were discussed in this short-talk.

Keynote lecture 3 Edith Heard (Paris France): ***The paradigm of X inactivation***

In her plenary lecture, Edith Heard discussed the work of her team, aimed at understanding the molecular mechanisms of X chromosome inactivation in mammals. After illustrating the dynamics of X inactivation during development, she presented some recent work investigating the consequences of having 2 X chromosomes expressed in female ES cells.

Session 4 - Epidrugs

Stephen Baylin (Baltimore USA): ***DNA methylation in cancer.***
Dr Baylin showed results in the use of DNMTi in combination with inhibitors targeting other actors of the epigenetic machinery as NURD, a chromatin remodeler, in colon cancer.

Antonello Mai (Rome Italy): ***Targeting DNA methylation.***
Antonello has described a medicinal chemistry approach to develop inhibitors of the various epigenetic actors. In particular he has illustrated 2 strategies to find new inhibitors of DNMTs: the pharmacomodulation of an existing drug by changing the attachment orientation and the modulation of a histone methyltransferase inhibitor to transform it into a DNMT inhibitor.

Eric Letouzé (Paris France): ***SDH mutations establish a hypermethylator phenotype in paraganglioma.***
Eric presented in his short-talk a genome-wide study of DNA methylation in this cancer, and discussed the role of mutations in the gene SDH altering metabolism and the epigenome.

Peter Jones (Los Angeles USA): ***Resetting the Epigenome after 5-azadeoxycytidine Treatment.***
Dr Jones discussed the roles of DNMT3a and DNMT3b in resetting the epigenome after it has been demethylated by a 5-aza treatment.

Paola Arimondo (Toulouse France): ***Lessons from non-nucleoside inhibitors of DNMTs***
After presenting the development of new DNMT enzymatic tests for screening, Paola discussed the biological activities of catalytic inhibitors in comparison to the aza-cytidine analogs.

Albert Jeltsch (Stuttgart Germany): ***Structure, mechanism and regulation of the DNA methyltransferases Dnmt1 and Dnmt3a***
Albert reported his work, investigating how certain genomic sites are selected for methylation since the patterns are not random and that site-specificity of the DNA methyltransferases cannot explain all.

Session 5 Methylation and demethylation in disease

Santiago Uribe-Lewis (Cambridge UK): ***5-hydroxymethylcytosine marks promoters in colon that resist DNA hypermethylation in cancer***
Santiago analyzed genome-wide the cytidine modifications and the transcriptome in the gut.

François Radvanyi (Paris France): ***Regional epigenetic silencing in cancer***
François analyzed different epigenetic actors and their mutations in bladder cancer. In particular he pointed out the role of UTX/KDM6A mutations and EZH2 responsible of the H3K27me3 mark.

Toshikazu Ushijima (Tokyo Japan): **E*pigenomic alterations induced by chronic inflammation***
Dr Ushijima presented that the inflammation by *H. pylori i*nduces DNA methylation in colon and that aberrant DNA methylation is decreased after infection eradication. He also discussed the use of DNA methylation as cancer risk marker.

Claire Francastel (Paris France): ***Dnmt3b prefers germline genes and centromeric regions: identification of novel biomarkers for the ICF syndrome***
Claire presented her research to find biomarkers for diagnosis of the ICF syndrome and her efforts to validate them in patients.

Susan Clark (Sidney Australia): ***Epigenetic remodeling and replication timing in cancer***
Dr Clark presented the importance of the evolution of the techniques to measure DNA methylation and chromatin modifications in the understanding of the biological processes in normal and cancer cells. She then described the long-range epigenetic silencing (LRES) and the long-range epigenetic activation LREA) in prostate cancer.

Pierre-Antoine Defossez (Paris France): ***The genome-wide distribution and biological role of Zinc-finger-containing methyl-binding proteins***
Pierre-Antoine is interested in the proteins that interpret the DNA methylation signal in particular the methyl-binding proteins and their mode of binding to DNA. In particular he focused on ZBTB4 that he studied both at a molecular level (for example the DNA sequence recognition) and more recently in a mouse model.

Discussion: ”Where is the field going?” (Discussion leaders: Stephen Baylin, Susan Clark and Anjana Rao)
The major points that were discussed were:

- how the techniques have rapidly improved and ideally we would like to see all chromatin marks and actors in one experiment
- if the borders of the chromatin regions are also important and how the proteins interact with each other
- concerning 5hmC, what is its role? Is it just a transient mark or does it have a regulatory function.

Session 6 Mechanisms of DNA methylation and demethylation

Udo Müller (Munich Germany): R*ole and regulation of DNA modifications.*
In his presentation, Udo Müller recapitulated findings made by the Leonhardt lab in recent years concerning the methyl- and hydroxy-methyl binding proteins UHRF1 and UHRF2. By using a combination of cell biology, biochemistry and proteomics, the team has deciphered the role of these proteins in chromatin maintenance and in the regulation of DNMT1 activity.

Alexander Ishchenko (Villejuif France): I*nterplay of the base excision repair and mismatch repair pathways in active DNA demethylation.*
This winner of the best poster prize presented biochemical work aimed at deciphering the contribution of mismatch repair in DNA demethylation.

Martin Bachman (Cambridge UK): ***The dynamics of 5 hydroxymethylcytosine***
The second winner of the best poster prize presented a new technology aimed at investigating the half-life of 5-hydroxymethylcytosine in cells and in vivo.

Jörg Tost (Evry France): ***Large-scale DNA methylation analysis in complex diseases – deciphering the marks of environmental exposure***
Jörg Tost showed data how the farm environment that children were exposed to *in utero* can change the DNA methylation profile in cord blood at specific immune related genes. Potential effectors include microbial exposure and exposure of pregnant mice to bacteria from cowsheds leads to protection of the offspring to allergenic challenge through modification of the epigenetic profile at immune related genes. Further, the DNA methylation profile of sorted blood cell populations in the autoimmune disease Sjogren’s syndrome showed widespread alterations and interestingly some features were shared between different autoimmune diseases.

Conclusions and recommendations

This was the first meeting on DNA methylation and demethylation, not only for the Conferences Jacques Monod series but also worldwide. The participants highly appreciated the focus of the conference, covering a topic that is not usually covered adequately in the "Epigenetics and Chromatin" conferences that are typically offered.

Many of the world leaders in the field were present, and they mingled with the more junior attendees. The interactions among the participants were favored by the location, by the small size of the conference, and also by the walking moments due to the localization of the events. Many speakers presented unpublished results and stimulated the discussions. We also organized a discussion on the topic “Where the field is going” animated by three speakers that allowed an open exchange among all participants. The meeting has been extremely successful, the conference room was always full and we have received several emails thanking us for organizing such a specialized and exciting conference (some are cited here below). It was very appreciated the fact that all aspects of the fields were covered: from the chemistry to the clinics through biochemical and molecular studies of the fundamental biological process. All talks and poster presentation dealt with DNA methylation and demethylation, addressing fundamental aspects, as in development, to the development of technologies to detect these DNA modifications, drugs to target them and their role in diseases. It was generally considered that this was a highly successful, enjoyable and fruitful meeting resulting in excellent discussions regarding key issues yielding potential new collaborations. The participants were unanimous in their support to continue the series of meeting and have selected two candidates as Chair and co-Chair:
Deborah Bourch’is, CNRS Institut Curie, France,
Michiel Vermeulen, Utrecht Medical Center, The Netherlands.

As organizers we are very grateful for the practical organization and help by Mrs Nathalie Babic, who also was of great help to the participants and was able to solve several problems, such as baggage loss. The technical aspects were also extremely smooth thanks to Alain Paoli.
The location was also appreciated, as the food.

There are some slight changes that we would suggest for the next meeting.
The poster sessions were too shorts, it would be best to be able to leave all the poster during the entire meeting so that discussions can arise in front of the poster even outside the poster sessions.
A great success was the snack dinner (sandwiches and drinks) during the evening poster session.
More free time should be arranged in order to allow the participants to exchange together freely.

Email extracts:

« Many thanks for organizing and hosting an excellent meeting at Roscoff. The venue was excellent, talks highly informative and the participants were of a high quality.
Can't wait for the next one. »

« Juste un petit mot pour vous remercier pour ce beau congrès ! Je crois que cela a été un grand succès ! En tout cas, j'y ai pris beaucoup de plaisir ! »

« Congratulations again for putting together this outstanding conference in Roscoff. It was one of the best meetings I have been to; the talks were superb as was the discussion and the participation in poster sessions. Your hard work paid off. Thanks again for inviting me to this »

« The talks at the Roscoff meeting were all of very high quality. Thanks also for your help in recovering my bag. »

« Congratulations on a terrific meeting.
I was really honored to participate. »

« Just a brief note to thank and congratulate you all on a fantastic conference. I learnt a lot, enjoyment myself and already initiated new collaborations. I hope you will receive the support you need to continue this conference as a new series. »

« Thank you very much for your and your friends' dedication to the Jacques Monod conference.
I enjoyed the science there, meeting friends, and nice French food and wine. »