Epigenetics and Periconception Environment

Proceedings of the EPICONCEPT Workshop 2013
Epigenetics for Improved Food Production: from Model to Practice

Sant Feliu de Guíxols, Spain
13 - 16 October 2013

Editors
Ann Van Soom, Alireza Fazeli, Amos Tandler, Francesc Piferrer

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www.cost.esf.org
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Welcome from the Chairman

We have had a great summer in most of Europe, and I hope you all had some time to relax and enjoy the European culture and food. While some of us were on holidays other COST members have been very active in preparing an excellent Workshop which will take place near the blue Mediterranean Sea in Sant Feliu de Guíxols in Spain on 13-16 October 2013. The title of the Workshop which is organized by Working group 3 is 'Epigenetics for Improved Food Production: from Model to Practice'

When you look up the word 'Workshop' in the dictionary there are two meanings for this noun: 1. a room or building in which goods are manufactured or repaired, 2. a meeting at which a group of people engage in intensive discussion and activity on a particular subject or project.

This second meaning is what this workshop is meant for: to engage in intensive discussion and activity. In order to get you all activated, we have invited internationally renowned keynote speakers and top researchers which will stimulate your neurons and brains with the latest findings in the area of epigenetics. Interspecific differences in their epigenetic response to environmental and nutritional cues will be presented and examples will be provided about how environment and nutrition can affect animal physiology in the long term, focusing on items such as sex control, growth, malformations and metabolism.

I am really looking forward to this workshop. I expect a great deal from the Science on Epigenetics, but also from the Environment. I would therefore like to take this opportunity to thank Francesc Piferrer and all the other members of the local organizing committee for volunteering to organize this workshop on a very short notice at the beautiful Costa Brava. Amos Tandler and Pascale Chavatte-Palmer together with the members of the Scientific Committee have managed to compile a mesmeric scientific programme. I will probably not find the time to enjoy the pool or the beach since all sessions look equally interesting.

Many thanks to Laszlo Tecsi for his invaluable assistance in administering and putting together the website and this abstract book. Thank you Alireza for keeping everyone's attention to the deadlines during the holidays.

Welcome to Sant Feliu de Guíxols!

Ann Van Soom

Chairman of Epiconcept

Welcome from the Scientific Committee

The study of epigenetics has been gaining popularity lately as it became clear that the expression of functional genes, important for health and for food production, is controlled via gene silencing and expression. This repertoire of effects is based on the epigenomic mechanisms such as DNA methylation, histone modifications, the influence of non-coding RNAs and post-translational alterations. While much of these mechanisms are gradually being revealed the targeted control of functional gene expression is still in its infancy. It is interesting to note that present epigenetic research focuses on health issues while the use of epigenetic tools to increase food production is negligible. For instance an Internet search of the string that associates epigenetics with health produces about 1 million hits while the association of epigenetics with food production results in only 3800 hits!

A dramatic augmentation of food production is an imminent need for the world's growing population which according to UN forecasts will reach 9 billion by 2043. The present workshop and our COST Action focus on the issue of improved food production in farmed animals, including mammalian, avian and piscine species by using epigenetic tools. During the two days our workshop we will deal with a variety of aspects of epigenetics ranging from novel tools to the environmental and nutritional effects on the epigenome in various organisms. On purpose we decided to limit the number of participants to create an intimate atmosphere that will give ample opportunities for participants to mingle and interact. We hope that this intimacy will create opportunities for future collaborations in the form of joint research via short term scientific missions of our COST Action and grant proposals.

We wish you an interesting and fulfilling meeting.

Members of the Scientific Committee:
Nathalie Beaujean, France
Amir Bitan, Israel
Pascale Chavatte-Palmer, France
Sofia Engrola, Portugal
Jorge Fernandes, Norway
Yael Heifetz, Israel
Elin Kjorsvik, Norway
Francesc Piferrer, Spain
Marco Saroglia, Italy
Amos Tandler, Israel (Chairman)

Welcome from the Organising Committee

Welcome to the Epiconcept Workshop 2013 on the Costa Brava which is one of the most emblematic and beautiful areas of Catalonia. This is a region with a strong personality known for many things such as its largest city and capital Barcelona, its architecture, its gastronomy and lately as a science hub in southern Europe.

This workshop is expected to be a forum for both established and young scientists to exchange ideas about basic epigenetic mechanisms, and how the new knowledge, brought about by epigenetics, can be used to improve animal production. This is a truly international event. We are convinced that the lectures given by the seventeen invited speakers from eleven different countries and the oral and poster presentations of the younger researchers will be of high interest for all of us.

The number of participants and the two-day format will maximise the chances of informal discussions and idea exchanges. The venue with its extraordinary natural beauty provides an excellent framework and atmosphere for such a workshop.

The social event of the workshop includes two excursions. One will be to the Dalí Museum in Figueres and the other to the old city of Girona. Salvador Dalí, the world famous surrealistic painter, was born and lived nearby, and the museum holds an impressive collection of his works. The exhibits are mainly paintings but sculptures and jewelry can also be seen here. The city of Girona boasts with its well-preserved Jewish quarters, the Cathedral, the Arabian baths and the archeological promenade. We hope that participants will take the opportunity to spend some extra days here, and explore many beautiful places on the Costa Brava. The ‘cales’, which are small beaches surrounded by cliffs and the crystal clear water of the Mediterranean, the fishing towns such as Calella de Palafrugell, Cadaqués or Tossa are ‘must-see’ attractions. You can see the Baroness Carmen Thyssen collection of paintings and the Benedictine monastery in Sant Feliu de Guíxols. Please make sure that you try the dishes of the local gastronomy based on seafood delicacies including the renowned anchovies from L'Escala or shrimp from Palamós.

The organisation of our workshop would not have been possible without the generous support of the Catalan Society of Biology (CSB), the University of Sheffield (UOS), the Society of Israeli Aquaculture and Marine Biotechnology (SIAMB) and the personal efforts of many people. I would like to thank the members of the scientific committee: Nathalie Beaujean, Amir Bitan, Sofia Engrola, Jorge Fernandes, Yael Heifetz, Elin Kjorsvik, Marco Saroglia, Pascale Chavatte-Palmer and Amos Tandler (chairmain). The latter two members along with Ann Van Soom and Alireza Fazeli (chairman and vice chairmain of the EPICONCEPT Executive Committee, respectively) formed the organising committee. Special thanks to Laszlo Tecsi (UOS), Amir Bitan (SIAMB) and Mariàngels Gallego and Maite Sánchez (SCB) for their help with setting up the webpage, payments and logistics.

Welcome to Sant Feliu de Guíxols. Enjoy the workshop and the location!

Francesc Piferrer

Chairman of the Organising Committee

Programme

Day 0
Sunday 13 October 2013

18:00-19:30 ***Arrival and Registration of Participants***
19:30-20:00 ***Welcome Cocktail***
20:00-21:00 ***Dinner***

Day 1
Monday 14 October 2013

08:30-08:45 ***Meeting Opening***

Session 1: Overview of the advances in the area of epigenetics

08:45-09:35 **Keynote: Moshe Szyf (McGill University, Canada)** Epigenetic processes mediating genome adaptation to experience and exposure
09:35-10:00 **Antoine Peters (Friedrich Miescher Institute, Switzerland)** Parental epigenetic control of embryogenesis: a balance between inheritance and reprogramming
10:00-10:25 **Wendy Dean (Babraham Institute, United Kingdom)** High resolution analysis of reprogramming in the mouse germline
10:25-10:50 **Helene Jammes (French National Institute for Agricultural Research, INRA, France)** Epigenetic of gametes and fertility in male: Potential consequences on progeny

10:50-11:20 ***Coffee Break***

Session 2: Tools and technology

11:20-12:10 **Keynote: Andreas Gnirke (Broad Institute, United States)** Genome-scale DNA-methylation analysis
12:10-12:35 **Juanma Vaquerizas (Max Planck Institute, Germany)** Computational approaches to study epigenetics regulation
12:35-13:00 **Amir Sagi (The Ben Gurion University of the Negev, Israel)** Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture

13:00-14:00 ***Lunch***

14:30-15:00 **Mary Goll (Memorial Sloan-Kettering Cancer Center, United States)** Fluorescent reporters of heterochromatin mediated silencing reveal parent of origin effects on transcription in the early zebrafish embryo

15:00-15:30 **Sari Pennings (Centre for Cardiovascular Science, United Kingdom)** Epigenetic control in model systems and development

Session 3: Presentations by Early Stage Researchers

15:30-15:50 **Herve Acloque (French National Institute for Agricultural Research, INRA, France)** DNA methylation analysis in sperm from infertile/subfertile boars

15:50-16:10 **Alfonso Gutierrez-Adan (Spanish National Institute for Agricultural and Food Research and Technology, INIA, Spain)** Zrsr1 splicing factor controls spermatogenesis

16:10-16:20 **Florence Naillat (Babraham Institute, United Kingdom)** Wnt-4 signalling in the regulation of DNA methylation during early female germ cell development

16:20-16-50 ***Tea Break***

16:50-17:10 **Josep Rotlland (Spanish National Research Council, CSIC, Spain)** Transcriptional regulation of Sparc (Osteonectin) gene by DNA methylation in teleosts

17:10-17:30 **Bartosz Wojciechowicz (University of Warmia and Mazury in Olsztyn, Poland)** Endometrial steroidogenesis during peri-implantation period in pigs - state of the art

17:30-17:40 **Jerome Jullien (University of Cambridge, United Kingdom)** Sperm is programmed to support transcription of developmentally-important embryonic genes

17:40-17:45 **David Kradolfer (Swiss Federal Institute of Technology, ETH, Switzerland)** An imprinted gene underlies post-zygotic reproductive isolation in Arabidopsis thaliana

17:45-17:50 **Francisco Otero-Ferrer (Canary Islands Institute of Marine Sciences, Spain)** Seahorses: A new and unique epigenetic model

17:50-19:15 **Session 4: Roundtable discussion on the issue of suitability of various model organisms for specific epigenetic studies**

19:20-20:20 ***Dinner***

20:30-22:00 ***Poster Session and Open Bar***

Day 2
Tuesday 15 October 2013

Session 5: Epigenetics based on nutritional programming

09:00-09:50 **Keynote: Marisol Izquierdo (University of Las Palmas, Spain)** Nutritional programming and progeny performance in marine fish

09:50-10:15 **Chris Ashwell (North Carolina State University, United States)** Nutritional conditioning in poultry

10:15-10:40 **Luisa Valente (Interdisciplinary Centre of Marine and Environmental Research, CIIMAR, Portugal)** Programming of muscle growth in fish: Epigenetic regulation of development and growth in Senegalese sole (Solea senegalensis) - EPISOLE

10:40-11:05 **Ann Gabory (French National Institute for Agricultural Research INRA, France)** Epigenetics of sexual dimorphism under different maternal diets in mouse placenta

11:05-11:35 ***Coffee Break***

11:35-12:00 **Alex Evans (University College Dublin, Ireland)**; Developmental programming in cattle and sheep: offspring phenotypes

Session 6: External environmental programming of epigenetics

12:00-12:50 **Keynote: Bryan Turner (University of Birmingham, United Kingdom)** The nucleosome as a transducer of environmental signals

12:50-13:15 **Yoav Soen (Weizmann Institute of Science, Israel)** Epigenetic and symbiotic mechanisms of inheritance of parental response to toxic stress

13:15-13:40 **Jorge Fernandez (University of Nordland, Norway)** External environment and muscle development in fish

13:45-14:45 ***Lunch***

Session 7: Presentations by Early Stage Researchers

15:15-15:35 **Noelia Diaz (Institute of Marine Sciences (ICM-CSIC), Spain)** Effects of early temperature on European sea bass (Dicentrarchus labrax) sex differentiation: Gonadal whole transcriptome analysis

15:35-15:55 **Elisa Haucke (Martin Luther University, Germany)** Accumulation of advanced glycation end products and the

associated increase in oxidative stress in the rabbit blastocys of diabetic mothers

15:55-16:15 **Anna Mallol (Autonomous University of Barcelona, Spain)** Effect of the epigenetic modifiers Psammaplin A and Vitamin C on the nuclear reprogramming of mouse somatic cell nuclear transfer embryos

16:15-16:45 ***Tea Break***

16:45-17:05 **Anna Slawinska (University of Technology and Life Sciences, Poland)** Pre- and synbiotics injected in ovo stimulate performance traits and immune responses in adult chickens

17:35-17:45 **Anita Franczak (University of Warmia and Mazury in Olsztyn, Poland);** Transcriptomic profile of the porcine myometrium - novel players during peri-implantation period

17:45-17:55 **Parisa Norouzitallab (Ghent University, Belgium)** Epigenetic control of phenotypes relevant for aquatic species

17:55-18:05 **Hanne Johnsen (Norwegian Institute of Food, Fishery and Aquaculture, NOFIMA, Norway)** Influence of embryonic stress on HPI axis development and functionality in Atlantic salmon (Salmo salar L.)

18:05-18:15 **Kaja Helvik Skjaerven (National Institute of Nutrition and Seafood Research, NIFES, Norway)** Transgenerational epigenetic effect of parental methyl supplements in Zebrafish diets, project description and preliminary results

18:15-19:15 **Session 8: Roundtable discussion on external environmental programming of epigenetics**

19:20-20:20 ***Dinner***

20:30-24:00 ***Farewell Event***

Day 3
Wednesday 16 October 2013

07:30-08:30 ***Breakfast***

08:30-12:30 ***Transfer to Barcelona Airport Terminal 1 or excursions to either to the Old City of Girona or to the Dali Museum in Figueres***

Option 1: Departure to Barcelona Airport

08:30-10:00 *Transfer to Barcelona Airport Terminal 1*
Free shuttle buses are available between Terminal 1 and Terminal 2. The airport transfer bus can also drop participants off to Barcelona in case they wish to stay in the city for a few extra days.

Option 2: Dali Museum in Figueres

08:30-10:00 *Journey from hotel to Figueres by coach*
10:00-11:30 *Guided tour of the museum*
11:30-12:00 *Free time for shopping*
12:00-13:30 *Journey from Figueres to hotel by coach*

Option 3: Old City of Girona

09:00-10:00 *Journey from hotel to Girona by coach*
10:00-12:00 *Tour of the city ('El Call' Jewish quarter, cathedral, Arabian baths and archeological promenade)*
12:00-13:00 *Journey from Girona to hotel by coach*

13:00-14:30 ***Lunch at Hotel Eden Roc***

14:30-16:00 ***Transfer to Barcelona Airport Terminal 1***
Free shuttle buses are available between Terminal 1 and Terminal 2. The airport transfer bus can also drop participants off to Barcelona in case they wish to stay in the city for a few extra days.

Abstracts of Presentations

O-1

Oral Presentation

Acloque, Herve

Genetics and Cell Laboratory, French National Institute for Agricultural Research (INRA)

Congras A, Vignoles F, Foissac S, Lhuillier E, Bouchez O, Riquet J, Pinton A, Delcros C, Bouissou-Matet-Yerle M, Acloque H

DNA methylation analysis in sperm from infertile/subfertile boars

Male infertility is an increasing health challenge for our societies, either for human or livestock's populations. As diffusion of the genetic progress goes through sires, male infertility consequently slows the improvement of animal selection schemas and farms' productivity.
We are studying the epigenetic marks in sperm and somatic DNA from fertile and subfertile/infertile boars. As some imprinted loci reported to be altered in infertile humans, we focused our analysis on several imprinted loci in the pig. We first used MeDIP-qPCR techniques to globally compare the methylation level of almost a hundred imprinted loci both in fertile and subfertile/infertiles animals. We then selected specific loci showing a variation in their methylation level and we are currently going deeper in the analysis of those regions by bisulfite conversion and pyrosequencing. We noticed at least one imprinted gene with a high DNA hypermethylation in the spermatic DNA of oligo-astheno-teratospermic animals compared to fertile animals, two of them carrying chromosomal rearrangements. No change was observed in the somatic DNA.
In addition to these local analysis, we provide here the genome-wide distribution of methylation and hydroxymethylation in sperm DNA from fertile Large White boars using MeDIP-seq technology. We also compare these data with the human sperm methylome, integrating a specific focus on imprinted genes and genes involved during early development and pluripotency.
Our aim is to define specific or common epigenetic signatures of farm animals' infertility. These epigenetic signatures will be an additional parameter to evaluate the sperm quality and will finally help to lower the number of potentially infertile males introduced in the selection schemas. Altogether, this study will provide novel data on sperm methylome in addition to the mouse and human data and will help to understand the function of DNA methylation and hydroxymethylation in mammals gametes.

Oral Presentation

Ashwell, Christopher
Prestage Department of Poultry Science, North Carolina State University

Ashwell CM

Dietary conditioning in poultry by limiting specific nutrients early in life

Science is just beginning to learn how the diet may have long-term influence on performance and health. A form of epigenetic regulation has been recently described called fetal “programming”. Fueled by epidemiological data the “fetal origins” hypothesis suggests that a poor in utero environment resulting from maternal dietary or placental insufficiency may “program” susceptibility in the fetus to cardiovascular or metabolic disorders. We have observed similar apparent programming by dietary manipulation in the chicken. When birds are challenged with a diet low in specific nutrients they obtain the ability to better utilize these nutrients later in life. This increased nutrient retention from the diet can partially be explained by an enduring increase in the expression of the intestine-specific transporters during programming as well as later in life. These observations have been made in chickens, turkeys and quail. The ability to manipulate bird efficiency for a particular nutrient may be very useful in periods of changing environments or changing feed component availability.

Oral Presentation

Dean, Wendy
Epigenetics Program, Babraham Institute

Dean W

High resolution analysis of reprogramming in the mouse germline

Epigenetic reprogramming of primordial germ cells (PGCs) culminates with genome-wide erasure of DNA methylation. This erasure is essential in order to reset the parent of origin specific marks that must undergo reprogramming in each generation during development of primordial germ cells. Beyond the resetting of genomic imprints this erasure guards against transgenerational epigenetic inheritance that may harbour deleterious epimutations in the future oocyte. Initial studies of PGCs from 13.5da fetuses at base-pair resolution indicated that the maternal germline becomes virtually without DNA methylation and that AID, a cytosine deaminase, may play a role in this process. We have significantly expanded this window from da 9.5 until da 16.5 using Next Generation Sequencing and BS –Seq. Extension of this investigation to include the da6.5 epiblast (prior to PCG determination) and PGCs from da9.5 onward reveals a more complex two-stage demethylation which reduces DNA methylation (%CG) by more than 50% in the early phases leaving loss of imprinted methylation to be initiated from da 11.5 and completed at da 13.5. This occurs by a largely passive mechanism that includes the exclusion of Np95, an obligate chaperone of Dnmt1, thus reducing DNA methylation owing to the failure of maintenance methylation. At this pivotal point when PGCs become meiotically arrested the maternal germline is virtually DNA methylation free.
Thus reprogramming is initiated prior to germ cell migration and is not completed until imprinted methylation is erased at meiotic arrest. Despite profound genome-wide DNA methylation reprogramming, some loci retain a substantial methylation signature that is carried forward into mature oocytes and sperm.

Oral Presentation

Diaz, Noelia
Marine Renewable Resources, Institute of Marine Sciences (ICM-CSIC)

Diaz N, Piferrer F

Effects of early temperature on European sea bass (Dicentrarchus labrax) sex differentiation: Gonadal whole transcriptome analysis

European sea bass (Dicentrarchus labrax) is a gonochoristic species with a polygenic sex determination system with environmental influences. High temperatures, as used in sea bass hatcheries, masculinize many genetic females hence skewing sex ratios to 3:1 in favor of males. Previous studies in our group showed that temperature exerted an effect on the final sex ratio of the population by differences in the methylation levels of the gonadal aromatase promoter (aromatase is the enzyme that catalyzes the conversion of androgens into estrogens thus controlling the final sex of the individual) when fish are one year old. However, these epigenetic gender-related and temperature-related differences were not present at 170 days post hatch (dph). But the effects of this early temperature exposure at the time of sex differentiation are unknown. In the present study, sea bass larvae were exposed to either natural (15-17ºC; NT) or artificially high temperature (21ºC; HT) from 20 to 90 dph, comprising the period of early gonad formation. Gonads were analyzed shortly after the beginning of sex differentiation at 170 days post hatch at the whole transcriptome level with a custom-made microarray, and results were confirmed by RT-qPCR. HT masculinized genetic females, with twice as males as in the NT group, and 34 genes were found to be differentially expressed (21 up- and 13 down-regulated genes) in the HT group. An in-depth analysis of these genes by RT-qPCR showed two patterns: an increase in the expression of genes related to the male pathway and a decrease in the ones related to the female pathway. Furthermore, a search among the genes of our array, based on a previous bibliographical analysis showed the presence of epigenetic-related genes. The analysis of 5 of them showed a different behavior due to the early temperature treatment. Supported by Epigen-Aqua grant AGL2010-19539 to F.P.

Oral Presentation

Evans, Alexander
School of Agriculture and Food Science, University College Dublin

Evans ACO

Developmental programming in cattle and sheep: offspring phenotypes

It is now well established that the maternal environment has profound effects on embryo and fetal development and that this persists into adulthood. Few studies have investigated the effects of maternal environment on reproductive system, especially in single-ovulating species like humans and cattle. Since numbers of follicles in ovaries are determined during gestation, we conducted a study where maternal nutrition in cattle was restricted to 60% of maintenance shortly before conception to the end of the first trimester of pregnancy (coinciding with follicle proliferation and the peak numbers of follicles in the foetal ovaries). Although weight of offspring was unaltered at birth, which is unlike results of the severe nutrition restriction model in many other studies, the numbers of follicles in the ovaries in calves born to nutritionally restricted mothers were 60% lower compared with calves born to control mothers (Mossa et al., 2013 Biol Reprod, 88(4): 92, 1-9).
Most studies that investigate the effects of the maternal environment during gestation on embryo and fetal development examine under nutrition. However, over nutrition during gestation is an issue in humans and has implications for animal production in agriculture. To investigate this, we studied lambs born to pregnant ewes that received a twice daily high glycaemic oral dose of propylene glycol or water (control) in addition to their normal meals during the last trimester of pregnancy. We found that short duration, high glycaemic intake during late gestation, that causes transient elevations in glucose and insulin concentrations (analogous to snacking on high glycaemic foods) compared to the control ewes, had substantial effects to increase offspring birth weight and postnatal growth rates (Smith et al 2009, BJ Obstet Gynaecol 116, 975-983).
The challenge for human medicine and animal production systems is now to understand the mechanism by which maternal environment affects offspring development and, of greater urgency, is to recommend diets that have a positive impact on the health, development and welfare of offspring.

Oral Presentation

Fernandes, Jorge
Faculty of Biosciences and Aquaculture, University of Nordland

Fernandes J

Epigenetic regulation of fish muscle growth by abiotic factors

Several teleost species display a remarkable phenotypic plasticity of muscle growth that is influenced by abiotic factors. It is known that water temperature and light conditions during early ontogeny stages can significantly influence the number and size of muscle fibres by regulating cell rates of hypertrophy and hyperplasia but the molecular networks underlying this plasticity are poorly understood. DNA methylation of specific cytosine residues in the genome by DNA methyltransferases (dnmt) is one of the main epigenetic mechanisms regulating gene expression. It is not only associated with transcriptional silencing of protein-coding genes but also microRNAs (miRNAs). These small non-coding RNAs are involved in post-transcriptional regulation and they are critical regulators of myogenesis. In the present study we have used Atlantic cod (Gadus morhua) and Senegalese sole (Solea senegalensis) as model species to investigate the epigenetic regulation of muscle growth by photoperiod and temperature, respectively.

Six-month old Atlantic cod were kept in two groups of triplicate tanks under continuous light (LL) or natural photoperiod conditions (NL) for 6 months. The mean weight of Atlantic cod juveniles reared under LL was found to be 13% greater than those kept under NL conditions for 120 days. Dnmt1 and dnmt3a transcript levels showed a significant increase in fast muscle of juvenile cod from the LL group. SOLiD sequencing revealed a number of differences in muscle miRNA transcriptomes, which may be associated with repression of key genes involved in inhibition of muscle growth. Senegalese sole eggs were incubated at 20 °C until hatching and then larvae were transferred to triplicate tanks at three different temperatures (15, 18 or 21 °C). At stage 2 of metamorphosis, larvae reared at 21 °C had larger muscle fibres and a mean weight 2-fold greater than those from the 15 °C group. Next-generation pyrosequencing showed that transcript levels of several growth-related genes were affected by temperature, including myogenin (myog). Methylation of the myog promoter increased at 15°C relatively to higher rearing temperatures. Moreover, a higher incubation temperature promoted expression of some miRNAs positively related with growth.

Taken together, our data provide a global perspective of the different layers involved in epigenetic regulation of muscle growth in fish by environmental factors and indicate that miRNAs may play a crucial role in this process.

Oral Presentation

Gabory, Anne

Developmental Biology and Reproduction Unit, French National Institute for Agricultural Research (INRA)

Gabory A, Panchenko P, Ferry L, Junien C

Epigenetics of sexual dimorphism under different maternal diets in mouse placenta

The recent and rapid worldwide increase in noncommunicable diseases challenges the assumption that genetic factors are the primary contributors to such diseases. A new dimension, that of the “developmental origins of health and disease” (DOHaD), is at stake and therefore requires a paradigm shift. Maternal obesity and malnutrition predispose the offspring to develop metabolic syndrome, a vicious cycle leading to transmission to subsequent generation(s), with differences in response and susceptibility according to the sex of the individual. Placenta is a programming agent of adult health and diseases. Adaptation in placental phenotype in response to maternal diet and body composition alter fetal nutrient provision. This implies important epigenetic changes that are however still poorly documented in DOHaD studies, particularly concerning overnutrition. Our objective was to investigate the effects of a high fat diet (HFD) on mouse placental development. We used transcriptomic and epigenetic techniques and showed for the first time that not only the gene sets but also the biological functions affected by the HFD differed markedly between the two sexes. Moreover, the expression of the epigenetic machinery enzymes, as well as global DNA methylation, were highly dynamic during the fetal period (3 stages), clearly differed between the 2 layers of placenta (labyrinth and junctional zone) and showed conspicuous sexual dimorphism. Thus, these findings demonstrate a striking sexual dimorphism of programming trajectories in response to the same environmental challenge. Explaining the sex-specific causal variables and how males versus females respond and adapt to environmental perturbations should help physicians and patients anticipate disease susceptibility.

Oral Presentation

Gnirke, Andreas
Technology Laboratory, Broad Institute of MIT and Harvard

Gnirke A, Gu H, Ziller M, Boyle P, Clement K, Fostel JL, Hollinger A, Smith Z, Epstein C, Meissner A

Genome-scale DNA-methylation analysis

Two main methods for genome-scale DNA-methylation analysis are routinely used within our epigenomics group: Reduced Representation and Whole Genome Bisulfite Sequencing (RRBS and WGBS, respectively). This presentation will focus on the basic methodology and showcase selected highlights from past and current research projects.

RRBS is a genome-scale spot test whereby the ends of a size-selected subset of restriction fragments are bisulfite sequenced. Illumina RRBS of MspI fragments is efficient and cost-effective because it is biased towards genomic features such as CpG islands and promoters in vertebrate genomes, and because each (short) sequencing read covers at least one CpG. Since its inception in 2005, our RRBS protocol has evolved into two versions that differ in DNA input requirements and throughput. For low-input samples we run a manual protocol that produces meaningful (and interesting) data even for DNA samples with <1 ng. Our largely automated production implementation (batch size ≥24, typically 96 samples) works well for DNA samples with ≥200 ng. Both protocols typically generate ≥10x sequence coverage of ≥1M unique CpGs.

To assess the extent of differential methylation in the human genome we analyzed WGBS data sets representing 30 diverse human cell and tissue types. Interestingly, only ~22% of autosomal CpGs show dynamic (≥30%) methylation changes in a normal developmental context, whereas the majority of CpGs are essentially static. Dynamic CpGs tend to co-localize with gene-regulatory elements as well as with SNPs associated with cell-type specific disorders. From a technical perspective, WGBS is relatively inefficient and expensive in that 90% of WGBS sequencing reads cover static CpGs, or no CpG at all, and thus offer little biologically relevant methylation information. Targeted bisulfite sequencing of dynamic CpGs, or a subset thereof, may prove more cost-effective than WGBS, and more comprehensive than RRBS, for profiling the dynamic fraction of the human methylome.

Oral Presentation

Goll, Mary
Developmental Biology, Memorial Sloan-Kettering Cancer Center

Schneiderman JI, Gutierrez D, Rajshekar S, Goll MG

Understanding heterochromatin establishment and maintenance in the zebrafish embryo

The zebrafish offers unique opportunities to understand the mechanisms required for establishment and maintenance of repressive chromatin during vertebrate development. To this end, we have developed transgenic zebrafish lines carrying integrated fluorescent reporters that recapitulate molecular features found at sites of endogenous heterochromatin.These lines provide an in vivo platform to rapidly identify genetic, chemical and environmental conditions that alleviate epigenetic silencing of transcription in a developing vertebrate embryo. As part of an ongoing screen, we have used these lines to identify several vertebrate specific genes with roles in transcriptional silencing. In addition, these transgenic lines have revealed unexpected epigenetic phenomena, including the first clear evidence for parent-of-origin effects on transcription in zebrafish.

Oral Presentation

Gutierrez-Adan, Alfonso

Department of Animal Reproduction, Spanish National Institute for Agricultural and Food Research and Technology (INIA)

Lopez-Cardona AP, Ramos-Ibeas P, de Frutos C, Pericuesta E, Calle A, Pintado B, Fernandez-Gonzalez R, Ramirez de Paz MA, Chitwood JL, Ross PJ, Gutierrez-Adan A

Zrsr1 splicing factor controls spermatogenesis

The alternative splicing of precursor messenger RNA (pre-mRNA) is one of the processes by which a single gene produces distinct mRNAs through differential selection of potential splice sites. Recent studies suggest that epigenetic regulation determines not only what parts of the genome are expressed, but also how they are spliced. Alternative splicing plays critical roles in differentiation, development, and disease, and is a major source for protein diversity in higher eukaryotes; however, key factors that control tissue-specific alternative splicing remain largely undefined. Zrsr1 is part of the heterodimeric protein U2AF, composed of two evolutionary conserved subunits (U2AF65/U2AF35) that play a critical role in the exon definition process. The protein contains 2 CCCH zinc finger motifs and shares between them an RNA-recognition motif. It has been suggested that expression of the U2AF family members could be regulated in a tissue specific manner. To determine the function of Zrsr1 (a protein only expressed in male embryos) in early gamete generation and sex differentiation, we have created a dominant negative transgenic mouse model. Using zinc finger nucleases we introduced a nonsense mutation in the RNA-recognition motif that produces a truncated protein but maintains one of the zinc finger (CCCH type) motifs. Homozygoous males, but not female mice, exhibited hematocrit alterations, highlighting a high proportion of blister cells and indicating a role for Zrsr1 in erythrocyte maturation. Male mice also exhibited sterility with spermatocyte arrest, germ cell sloughing and apoptosis, which ultimately led to azoospermia. RNA-seq analysis of testis at day-15 (immediately after the highest expression of Zrsr1 in developing testis) indicated that altered splicing of Zrsr1 target pre-mRNAs affected expression of 1,444 genes representing several functional pathways, including those implicated in gamete generation, nucleotide binding, chromosome organization, and cell cycle process. Also, we identified 110 alternative splicing transcripts, where the mutation in Zrsr1 resulted in a shift in isoform ratios, representing putative Zrsr1 targets. These were enriched for cell cycle process, nucleotide binding, and regulation of transcription. In addition to providing significantly novel insights into Zrsr1 functions in this transgenic model, our results reveal a critical role for testis-specific functions for Zrsr1 as a pre-mRNA splicing regulator of male fertility.

Oral Presentation

Haucke, Elisa
Department of Anatomy and Cell Biology, Martin Luther University

Haucke E, Navarrete-Santos A, Simm A, Henning C, Glomb M, Grune T, Fischer B, Navarrete-Santos A

Accumulation of advanced glycation end products and the associated increase in oxidative stress in the rabbit blastocys of diabetic mothers

About 7% of pregnancies are complicated by diabetes mellitus (DM). Although our understanding and management of DM have improved over the last decades, diabetic pregnancies are still on risk for congenital malformations. The underlying mechanisms are unclear. Advanced Glycation End products (AGEs) are known to play a critical role in the development of diabetic complications. AGEs are formed non-enzymatically via reactions between reducing sugars and amine groups. They are known to alter biological properties of proteins and to cause oxidative stress. We have investigated whether a poorly controlled maternal DM induces metabolic stress in the preimplantation embryo, employing a rabbit model.
Blastocysts developed under diabetic conditions showed an increased protein bound CML and Arg-pyrimidine concentration in embryonic cells; whereas protein bound pentosidine was not affected by slot blot analysis. The blastocyst cavity fluid (BFC) showed enhanced AGE-fluorescence with excitation and emission 330/405 nm and 360/440 in blastocysts. Besides fluorescent AGEs soluble CML was detectable in the BCF by high-performance liquid chromatography. Various AGE inductors (glucose, methylglyoxal) were used to simulate the AGE accumulation in the blastocyst in vitro. As AGE formation is closely related to oxidative stress we determined first the oxidative status in embryos. Oxyblot analysis revealed a higher rate of carbonylated proteins in diabetic blastocysts. The 20s proteasome takes a major part in elimination of damaged proteins in vivo. Measuring their activity via peptide substrate suc-LLVY-MCA showed a noticeable activity in blastocysts.
Taken together poorly controlled maternal diabetes during the preimplantation period leads to metabolic stress in the embryo. Although these results do not provide strict causation between congenital malformation and diabetes, it is likely that AGEs play an important role as stimuli for activating intracellular stress pathways.

Oral Presentation

Izquierdo, Marisol
Aquaculture Research Group, University of Las Palmas

Izquierdo MS

Nutritional programming and progeny performance in marine fish

Seafood has a high nutritional value for human health and its demand is increasing, being expected to reach over 150 million MT/year in 2030. However, world fisheries production remained stagnant for the last 20 years with over 85% of the stocks being over-exploited or fully-exploited. Only aquaculture can meet this seafood demand, being the food production sector with the fastest growing rate. Fishmeal and fish oil were main ingredients in aquafeeds, but they are unsustainable and insufficient to maintain aquaculture growth. Thus, these ingredients have been reduced by 50% in aquafeeds during the last decade. Nevertheless, the complete removal of fishmeal and fish oil is required to promote the sustainable development of aquaculture and for that, fast growing high quality fish that are better adapted to utilize diets without fishmeal and fish oil are necessary. Two different strategies are investigated to achieve this goal: nutritional programming and inheritance.
Fish oil is rich in omega 3 highly-unsaturated fatty acids (n-3HUFA), essential for marine species, due to their low bio-conversion ability. Plant oils lack n-3HUFA but contain their precursors. The genes of enzymes bio-converting these precursors may be nutritionally regulated. Nutritional conditioning would allow fish genome adaptation for a better utilization of diets without fishmeal or fish oil. To define the dietary factors that influence the epigenetic profile, the most sensitive time-windows and the lenght of intervention we conducted three types of programs by early exposure of embryos, larvae or postlarvae to different diets. After several months of monitoring growth and metabolism, fish were challenged with either plant diets or fishmeal diets. The studies showed the very long-term effects resulting from parental epigenetic control of embryogenesis, perhaps along the whole life cycle of the fish, as well as the potential of nutritional conditioning during early larval stages.

O-13

Oral Presentation

Jammes, Helene

Developmental Biology and Reproduction Unit, French National Institute for Agricultural Research (INRA)

Jammes H, KIiefer H, Campion E

Epigenetic of gametes and fertility in male: Potential consequences on progeny

A global increase of the male infertility is described for different species. Human infertility affects 10-15% of couples and among infertility causes, one third has a male origin (Louis, 2013). The genetic causes of male infertility have been searched (karyotypic abnormalities, microdeletions, mutations ; Maduro, 2003). In cattle, the importance of male contribution to reproductive success is well documented, bull breeding soundness evaluation are a commercial practice and there are specific guidelines for semen evaluation. Hovewer, a surprisingly low number of quantitative trait loci (QTLs), genome-wide association studies (GWAS) and genomic selection (GS) approaches have reported candidate genes associated with fertility traits (Fortes 2313). In recent years, it become increasingly clear that epigenetic mechanisms are critical during spermatogenesis and that perturbations in these mechanisms can result in male sub/infertility (Zamudio, 2008; Carrell, 2012; Chalas-Boissonnas, 2013). The current knowledge on epigenetic that occur during male meiosis is discussed in this review. A special attention is focused on DNA methylation, histone tail modifications, targeted histone retention and protamine incorporation into the chromatin, and small and long non coding RNA production. All these epigenetic mechanims are involved in highly regulated processes such as silencing of gene and retrotransposon, chromatin condensation and packaging of DNA. We will discuss the importance of a better understanding of how epigenetic changes in the sperm may have a causative role in etiology of infertility. Perturbations in the establisment and /or maintenance of any of these epigenetic marks could be induced by environmental factors and could affect the fertility status. These epigenetic marks constitute also the paternal contribution to embryo development. Finally, the recent studies focused on transgenerational and paternal effects in mice and in human are discussed.

Oral Presentation

Mallol, Anna
Department of Cellular Biology, Autonomous University of Barcelona

Mallol A, Pique L, Santalo J, Ibanez E

Effect of the epigenetic modifiers Psammaplin A and Vitamin C on the nuclear reprogramming of mouse somatic cell nuclear transfer embryos

Nuclear reprogramming of differentiated cells towards an embryonic totipotent state through somatic cell nuclear transfer (SCNT) is an inefficient process and many epigenetic abnormalities have been found in cloned embryos. The aim of the present work was to investigate the effect of two epigenetic modifiers, psammaplin A (PsA) and vitamin C (VitC), on mouse SCNT efficiency in terms of both offspring production and embryonic stem cells (ESC) derivation. To this aim, enucleated oocytes were reconstructed with a cumulus cell nucleus, parthenogenetically activated and treated for 16 h with 100 μM VitC, 10 μM PsA or a combination of both (VitC-PsA). The resulting blastocysts were stained to analyze the number of inner cell mass (ICM) cells and the expression of Cdx2, Oct4 and Nanog, or were used to derive NT-ESC lines. Alternatively, 2-cell embryos were transferred to recipient females to assess full-term development. We found that, when compared with untreated SCNT embryos, VitC-PsA treatment significantly increased blastocyst rates (45.5% vs. 56.5%), ICM cell number (7.1 vs. 14.2) and Oct4 expression (2-fold), whereas PsA alone only increased ICM cell number (16.9) and Oct4 expression (2-fold), and VitC alone resulted in a significant increase in Cdx2 expression (1.5-fold). In spite of this, all three treatments significantly improved full-term development, and to a similar extent, when compared with untreated embryos (3.1-4.9% vs. 0%). PsA- and VitC-treated SCNT embryos also gave rise to more NT-ESC lines when compared with untreated embryos (28.6%, 33.3% and 9.5%, respectively), although in this case the differences were not statistically significant, possibly due to a still low number of samples. In conclusion, both epigenetic modifiers appear to improve nuclear reprogramming and the efficiency of mouse SCNT. Studies are currently being performed to determine the effect of VitC and PsA treatments on histone acetylation and methylation levels.

Oral Presentation

Naillat, Florence
Epigenetics Program, Babraham Institute

Naillat F, Tomizawa S, Ivanova E, Kelsey G, Vainio S

Wnt-4 signalling in the regulation of DNA methylation during early female germ cell development

The germ cells are the foundation of an individual and have several fundamental molecular processes which are critical for the next generation. For example, the methylation status of the germ cells is modified during their formation, their migration into the bipotential gonad and their maturation. Of the Wnt family of signalling molecules, Wnt-4 is a crucial factor in the control of female sexual development. The lack of Wnt-4 expression in mouse leads to partial female to male sex reversal during embryogenesis. The Wnt-4 knockout presents absence of the Müllerian ducts and instead maintenance of the Wolffian ducts. Besides these morphological defects, only 20% of the Wnt-4 deficient germ cells undergo meiosis. This suggests a critical role for Wnt-4 signalling in female germ line development. We have addressed the roles of Wnt-4 signalling in the control of female germ line development in greater detail by addressing potential changes in epigenetic regulation. We found that markers of histone methylation H3K27me3 and the de novo DNA methyltransferase Dnmt3b were still expressed at E14.5 in Wnt-4 deficient female germ cells, whereas they are lost by this stage in the wild-type female germ cells. Based on these findings, we used bisulfite sequencing to identify potential changes in the methylation status of promoters of genes involved in meiosis. We compared Wnt-4 deficient germ cells to wild type cells obtained from FACS analysis at E14.5. The Wnt-4 deficient germ cells were hypomethylated compared to the wild type female germ cells for two crucial meiotic genes. In conclusion, we propose that Wnt-4 signalling may be involved in the regulation of DNA methylation in female germ cells.

O-16

Oral Presentation

Pennings, Sari

Centre for Cardiovascular Science, Queen's Medical Research Institute

Wongtawan T, Chebotareva T, Thakrar S, Wongtawan B, Taylor J, Wilmut I, Pennings S

Epigenetic control in model systems and development

Epigenetics research has benefited from the use of cell and animal model systems. The epigenetic dynamics during early development are commonly represented by the mouse model featuring rapid loss of 5mC in the early embryo and during PGC development. Furthermore, several chromatin modification changes are associated with developmental stages. The role of DNA methylation and histone modifications in early developmental cell differentiation is not fully understood, however. Epigenetic diversity between different animal models suggests different routes to conserved functional development. Our studies of the epigenetic role of heterochromatin in early cell lineages showed that mature heterochromatin is not established until late mouse foetal development, whereas it is present in ES cells derived from embryos. Repressive histone methylation systems are nevertheless essential for development. These findings suggest a role for heterochromatin in maintenance rather than in the setting up of epigenetic states directing gene repression, as well as the influence of culture conditions on embryos and cells. Studies in mouse, rat and zebrafish indicate that comparative studies of DNA methylation and histone dynamics in mammalian and non-mammalian organisms are necessary to reveal common mechanisms governing the diverse developmental beginnings.

Oral Presentation

Peters, Antoine
Friedrich Miescher Institute for Biomedical Research

Erkek S, Hisano M, Posfai E, Kunzmann R, Brykczynska U, Albert M, Stadler M, Peters A

Parental epigenetic control of embryogenesis: a balance between inheritance and reprogramming?

In mammals, totipotent embryos are formed by fusion of differentiated gametes. Acquisition of totipotency concurs with remodeling of chromatin at parental genomes, changes in maternal transcriptome and proteome, and zygotic genome activation. It is, however, unknown to what extent inheritance and/or reprogramming of chromatin states at distinct genome regions between generations is required for the establishment of totipotency in the early embryo. Our recent studies suggest the existence of intergenerational transmission of epigenetic information encoded by modified histones through the female and possibly the male germ line (Puschendorf et al., 2008; Brykczynska et al., 2010; Posfai et al., 2012; Erkek et al., 2013). Current studies are aimed at testing the hypothesis whether expression of genes in pre-implantation embryos is controlled by specific chromatin configurations inherited from previous generations. In the seminar, I will review the extensive chromatin remodeling events that take place during the development of “progenitor germ cells” into mature oocytes and spermatozoa and in pre-implantation embryos (Gill et al., 2012). I will discuss our work addressing the contribution of chromatin in epigenetic inheritance and reprogramming during gametogenesis and early embryogenesis.

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Oral Presentation

Rotlland, Josep
Marine Research Institute, Spanish National Research Council (CSIC)

Torres E, Perez-Figueroa A, Moran P, Rotllant J

Transcriptional regulation of Sparc (Osteonectin) gene by DNA methylation in teleosts

Sparc (Osteonectin) is an evolutionary conserved matricellular protein that modulates cell-matrix interaction and cell function. Within all vertebrates, osteonectin is expressed in a temporally and spatially specific manner with strong expression during embryogenesis in developing tissue such as the notochord, somites and embryonic skeleton and a marked reduction in osteonectin expression occurs once adulthood is reached. However, the precise function of osteonectin and the regulatory elements required for its temporally and spatially specific expression in particular during early embryogenesis is largely unknown. We report here the analyses of Sparc expression using Sparc-egfp transgenic zebrafish. Sparc-Egfp transgenic zebrafish were generated using the 0.2-kb Sparc promoter and its 5'-flanking sequence 7 kb upstream of the translated exon II. GFP expression was found in the notochord, otic vesicle, fin fold, somites, intermediate cell mass, olfactory bulb, skeletal and cardiac muscles of Sparc-egfp transgenic embryos. In situ hybridization confirmed Sparc mRNA expression in these tissues, suggesting that the expression of the Sparc-gfp transgene recapitulated that of the endogenous Sparc gene. To understand the molecular mechanisms regulating Sparc gene expression, we performed a functional characterization of the Sparc promoter. Deletion analyses on the 5' end of the promoter region, excluded the functional importance of proximal promoter in the transcriptional regulation of the gene and revealed that intron removal resulted in a complete reduction of promoter activity. Computer-based analysis found a number of cis-acting transcription factor binding sites and also identified a CpG island immediately proximal to the translation start site within the intron sequence. DNA specific methylation assays revealed that CpG dinucleotide specific demethylation can confer a 3-4 fold increase in Sparc gene transcriptional activity.

O-19

Oral Presentation

Sagi, Amir

Department of Life Sciences, Ben Gurion University

Sagi A

Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture

The androgenic gland (AG) is the key regulator of masculine sexual differentiation in crustaceans. Recent discoveries of AG-specific insulin-like peptides (IAGs) have deepened our understanding of the gland's mode of action. Temporal IAG knockdown, using RNA interference, in the prawn M. rosenbergii has recently enabled the alteration of the phenotypic sex of genetic males into functional 'neo-females' capable of producing an all-male progeny. All-male monosex culture is a desirable practice in prawn aquaculture since males grow faster and reach a larger size at harvest than females.

The intervention through RNAi does not require the use of hormones or other exogenous chemicals. It is temporal, applied at the youngest broodstock stage, and does not pose genetic modifications (non-GMO). This is the first instance of an aquaculture commercial use of a monosex population derived from a single gene silencing-induced sex reversal.

Oral Presentation

Slawinska, Anna

Department of Animal Biotechnology and Histology, University of Technology and Life Sciences

Slawinska A, Siwek M, Niesyn E, Vieira S, Rutkowski A, Bednarczyk M

Pre- and synbiotics injected in ovo stimulate performance traits and immune responses in adult chickens

Bioactive compounds such as pre-, pro- and synbiotics account for a healthy alternative to antibiotic growth promoters in chickens. Administration of those substances at early stages of chicken embryo development (12th day of incubation) modifies not only the microflora, but also physiological responses of the host. Hereby, we conducted the study to analyze the effects of in ovo injection of prebiotics alone or in a synergistic combinations with probiotic bacteria (synbiotics) on performance traits and immune responses in chickens. For in ovo injections three types of synbiotics were used: two in-house developed strains of lactic acid bacteria (LAB) in combination with lupin seed extract (RFO, raffinose family oligosaccharides) and a commercial synbiotic including two strains of LAB and lactose. We studied the effects of in ovo treatment at 6th week after hatch, at the level of production traits related to growth performance and nutrients digestibility, as well as at the molecular level of the expression of the genes involved in immune-related pathways. In ovo injection with pre- and synbiotics increased body weight gain, nutrient digestibility and nitrogen retention. However, it also increased overall feed intake, which resulted in a higher feed conversion ratio. With respect to the gene expression, in ovo treatment caused up-regulation of the IFNβ, IFNβ, IL6 and IL18 genes in the spleen and IL18 and CD80 genes in the caecal tonsils. The results indicate the positive impact of the single in ovo treatment with pre- and synbiotics at both performance and molecular levels in the adult chicken.
The research was supported by the National Science Centre in Kraków (Poland), grant no. N N311 623938 and 011/01/B/NZ9/00642

Oral Presentation

Soen, Yoav
Department of Biological Chemistry, Weizmann Institute of Science

Soen Y

Non-Mendelian Mechanisms of Inheritance of a Response to Toxic Stress

Studies of gene environment interactions often focus on programs of response to common environmental conditions such as starvation, temperature changes etc'. Most of these studies also concentrate on the exposed individual, ignoring the ability of parental environment to affect the development and health of their non-exposed progeny.

To investigate transgenerational implications of exposure to a novel environmental condition, we confront the development of the fly, D. melanogaster, with artificial distributions of toxic stress that are not expected to occur during fly development. Survival of the flies in this system depends of their ability to modify their development.

We found that this stress modifies the otherwise robust patterns of development, resulting in changes in gene expression as well as in the rate of larval development and adult morphology (in some of the cases). We show that part of this response is enabled by stress mediated suppression of Polycomb group genes (PcG), which leads to de-repression of developmental regulators and their expression in new domains, hence the change in developmental patterns. Some of the induced developmental changes were non-genetically inherited by subsequent generations of non-exposed offspring suggesting that the stress also modifies the germline of the flies. In support of this hypothesis, we provide evidence indicating that the inheritance is maintained by a combined action of germline and microbial-mediated mechanisms of transgenerational transfer.

These findings begin to portray distinct, epigenetic and symbiotic modes of transgenerational influence by which induced response to stress in parents can be transferred to the offspring. This highlights the importance of parental environment to the development and health of their offspring

Oral Presentation

Szyf, Moshe
Pharmacology Department, McGill University

Szyf M

Epigenetic processes mediating genome adaptation to experience and exposure

Experience during early-life is known to have long-lasting impact on the phenotype of the offspring. What are the mechanisms that mediate between exposure in early life and long-term changes in the phenotype? We have been testing the hypothesis that system wide DNA methylation changes early in life in response to social stress occur in both humans and animals. These are proposed to be “adaptive genomic” mechanisms that prepares life-long genome programming to the anticipated life-long environment based on signals received during gestation and early life. Data from nonhuman primates and humans show overlapping genes that are altered in response to both prenatal and postnatal stress in multiple tissues; placenta, the immune system and the prefrontal cortex. A fraction of these alterations in the methylome remain in a gender specific way into adulthood. We have evidence from a study of a natural disaster in humans that objective stress is associated with changes in DNA methylation that are detectable in T cells and remain into adolescence. We will discuss the hypothesis that stress hormones might be mediating the genome wide and system wide response of the methylome to adverse experience. Glucocorticoids as well as other hormones might act as 'integrators' that translate the social stress signals during gestation to genome wide methylation changes across multiple systems.

Oral Presentation

Turner, Bryan
Institute of Biomedical Research, University of Birmingham

Turner B

The nucleosome as a transducer of environmental signals

This talk will examine the proposition that the nucleosome, the basic unit of chromatin structure in eukaryotes, is primarily a signalling module by which changing environmental and metabolic conditions can influence genomic functions. The many enzyme-catalysed, post-translational modifications (PTM) of the core histones are closely involved in this signalling process and their roles will be critically assessed. In general PTMs can influence nucleosome structure, and hence genomic function, in three ways. First, modification of amino acids within the globular domains of the core histones (ie. inside the DNA wrapped around the histone octamer) can exert a direct affect on nucleosome conformation; second, acetylation of multiple lysines on the histone N-terminal tails reduces their net positive charge and it has been proposed that the consequent reduction in DNA binding loosens higher order chromatin packaging; finally, and perhaps most importantly, modified amino acids can be recognised and bound, often with exquisite specificity, by proteins carrying one of several binding domains. These highly conserved binding domains, singly or as protein complexes, exert multiple effects on genomic function, often by mediating the effects of drugs, metabolites or environmental agents on histone PTMs. I will discuss the current status of the nucleosome signalling paradigm and how it might be exploited to change cellular or organismal behaviour for practical ends.

Oral Presentation

Valente, Luisa

Nutrition, Growth and Quality of Fish Group, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR)

Valente LMP, Campos C, Canada P, Engrola S, Conceicao LEC, Fernandes JMO

Programming of muscle growth in fish: Epigenetic regulation of development and growth in Senegalese sole (Solea senegalensis)

Environmentally-induced imbalances during early life stages of fish are implicated in the genesis of skeletal deformities and impaired growth, which are considered as one of the most significant quality and welfare problems of the aquaculture sector. These problems are partly related to inadequate environmental and, particularly, nutritional factors that affect nutrient utilization and induce poorly developed muscular systems of fish larvae. Despite the abundant information on larval morphological development with age, there is a lack of reliable methods and parameters to measure physiological changes experienced by developing larvae at key stages. Recently developed molecular tools brought up new information on the functional ontogeny of growth in the flatfish Senegalese sole. Changes in water temperature, applied at different stages of this species early development (embryos and larvae), affected growth performance and muscle cellularity of larvae and post-larvae. Low temperatures decreased protein absorption and retention and increased DNA methylation levels of myog putative promoter in skeletal muscle resulting in reduced larvae size and developmental delay. Nutritional supply is known to affect skeletal muscle development in several vertebrates with possible log-term consequences on growth potential. In particular, the availability of certain amino acids seems to play a major role in stimulating protein accretion and skeletal myofibre numbers. In Senegalese sole, the substitution of fish protein hydrolysate by crystalline free amino acids from 2 to 51 DAH showed a negative effect on larval size without major differences on retention and catabolism of peptides. The proliferative capacity of myogenic cells and the growth potential of these larvae remained unaffected after metamorphosis (25 DAH). More studies are required to understand the influence of early nutritional programming on muscle differentiation and growth, and its relation to fry quality and size variation in species with importance for aquaculture.

O-25

Oral Presentation

Vaquerizas, Juanma

Regulatory Genomics, Max Planck Institute for Molecular Biomedicine

Vaquerizas JM

Computational approaches to study epigenetic regulation: Drosophila dosage compensation as a model system

Dosage compensation is a process that balances the expression of sex-linked genes in species that have evolved unequal numbers of sex chromosomes. In Drosophila, this involves hyperactivation of the single male X chromosome to equalise for the combined transcriptional activity of both female X chromosomes. The two-fold increase in expression is regulated by the MSL complex and involves extensive chromatin modifications and chromosomal organisation. Therefore, dosage compensation constitutes a prime example of epigenetic regulation. In this talk, I will present some of our latest results in the characterisation of this process. In particular, I will focus on a genomic analysis of the MSL complex and I will show how it regulates the recruitment of RNA polymerase II to expressed genes.

Oral Presentation

Wojciechowicz, Bartosz
Department of Animal Physiology, University of Warmia and Mazury in Olsztyn

Wojciechowicz B, Franczak A, Kolakowska J, Kotwica G

Endometrial steroidogenesis during peri-implantation period in pigs - state of the art

Steroids are involved in majority of known reproduction-related mechanisms in pregnant pigs, including the maternal recognition of pregnancy. In addition to porcine embryos, the uterine tissues are capable of steroids synthesis. This study describes the steroidogenesis in the endometrium and indicates factors potentially important for its regulation. The transcriptional profile of the endometrium was investigated in pigs from days 15-16 of pregnancy. Furthermore, the expression of genes encoding selected enzymes of steroidogenesis pathway (HSD3B1, CYP17A1, HSD17B1 and CYP19A3), the presence and activity of selected enzymes (3βHSD, 17βHSD and P450arom) in the endometrium of pigs from days 10-11, 12-13 and 15-16 of pregnancy and 10-11, 12-13 and 15-16 of the estrous cycle were examined. In addition, the effect of LH, FSH, PRL, EGF, IGF-I, RU 486 and DL-A on P4, A4, T, E1 and E2 production by endometrial explants incubated in vitro was studied. It was found that 10 genes encoding for steroidogenic enzymes are up-regulated in pregnant endometrium. Gene expression, the relative amount and activity of the enzymes of steroidogenesis pathway encoded by these genes were found to be dependent on both the physiological status of the examined gilts, and on days of pregnancy or the estrous cycle. Used experimental factors regulate the rate of steroids production by endometrium in a manner depended on the day of early pregnancy or the estrous cycle. Interestingly, during maternal recognition of pregnancy (days 12-13), LH, FSH and PRL did not stimulate the E2 secretion whilst these hormones increased endometrial E2 release during remaining examined days of pregnancy and all days of the estrous cycle. In conclusion, this study confirmed the presence of active steroid hormones biosynthesis and metabolism pathway in the endometrium of early pregnant and cyclic gilts (days 10-11, 12-13, 15-16 of the studied periods).
Funded by NCN, grant N N311 526940

P-27

Poster Presentation

Aguirre-Lavin, Tiphaine

Developmental and Reproduction Biology, French National Institute for Agricultural Research (INRA)

Aguirre-Lavin T, Adenot P, Lehmann G, Boulesteix C, Debey P, Beaujean N

3D-FISH analysis of embryonic nuclei in mouse highlights several abrupt changes of the nuclear organization during preimplantation development

Background: Embryonic development proceeds through finely tuned reprogramming of the parental genomes to form a totipotent embryo. Cells within this embryo will then differentiate and give rise to all the tissues of a new individual. Early embryonic development thus offers a particularly interesting system in which to analyze functional nuclear organization. When the organization of higher-order chromatin structures, such as pericentromeric heterochromatin, was first analyzed in mouse embryos, specific nuclear rearrangements were observed that correlated with embryonic genome activation at the 2-cell stage. However, most existing analyses have been conducted by visual observation of fluorescent images, in two dimensions or on z-stack sections/projections, but only rarely in three dimensions (3D).

Results: In the present study, we used DNA fluorescent in situ hybridization (FISH) to localize centromeric (minor satellites), pericentromeric (major satellites), and telomeric genomic sequences throughout the preimplantation period in naturally fertilized mouse embryos (from the 1-cell to blastocyst stage). Their distribution was then analyzed in 3D on confocal image stacks, focusing on the nucleolar precursor bodies and nucleoli known to evolve
rapidly throughout the first developmental stages. We used computational imaging to quantify various nuclear parameters in the 3D-FISH images, to analyze the organization of compartments of interest, and to measure physical distances between these compartments.

Conclusions: The results highlight differences in nuclear organization between the two parental inherited genomes at the 1-cell stage, i.e. just after fertilization. We also found that the reprogramming of the embryonic genome, which starts at the 2-cell stage, undergoes other remarkable changes during preimplantation development, particularly at the 4-cell stage.

Poster Presentation

Allon, Guy
National Center for Mariculture, Israel Oceanographic and Limnological Research

Allon G, Koven B, Bitan A, Tandler A

Electroporation as a novel method for delivering epigenetic agents into fertilized fish eggs

As fish embryos develop externally they are affected by environmental conditions and specific compounds having an effect on gene expression. This means that the ability to introduce therapeutic genomic and proteomic agents into embryos at the egg stage, at specific developmental windows, could serve as a useful model for studying epigenetic effects. The present study investigates electroporation as a viable platform for high throughput delivery of molecules of interest into fish eggs. This novel approach has been used to deliver the beta amino-sulfonic acid taurine into gilthead sea bream (Sparus aurata) fertilized eggs. Taurine has critical roles in bile salt synthesis, appetite stimulation, muscle growth, photoreceptor protection and as an antioxidant. Approximately 60 gilthead sea bream fertilized eggs at primary organ formation stage (PO1-PO3) were loaded on to 0.4cm cuvettes with Hank's solution. Optimum electroporation conditions were set to insure high egg hatchability and larval survival 24h after hatching. In addition a protocol for post-electroporation handling and the most effective taurine concentration (4.2%) were developed. This novel electroporation approach successfully increased more than 6 times the level of taurine found in the fertilized eggs of the gilthead sea bream. The elevated taurine levels in egg and freshly hatched larvae appear to have greatly reduced urinary calculi, a major cause of larval mortality in a number of species. Furthermore the success in introducing this molecule of high physiological value testifies to the potential of electroporation as a platform for epigenetic manipulation.

Poster Presentation

Dias, Jorge
Aquaculture Research Group, Centre of Marine Sciences (CCMAR)

Rocha F, Dias J, Gavaia PJ, Geurden I, Panserat S, Dinis MT, Engrola S.

14C-Glucose metabolism in zebrafish juveniles conditioned with high glucose levels during embryogenesis

Nutritional programming in mammals has demonstrated the possibility to change specific metabolic pathways or functions in adulthood based on early nutritional stimulus. Altering the nutritional value of yolk reserves during fish embryogenesis can be a valuable approach to understand why some fish show a low ability to use dietary carbohydrates as energy substrates. Relying on the use of 14C-Glucose as a metabolic tracer, a study was undertaken to assess the long-term effects of yolk fortification with glucose (early stimulus) on the carbohydrate metabolism of zebrafish juveniles challenged with either a low or high carbohydrate diet. A pool of fertilized eggs was microinjected at 1 dpf (day post-fertilization) with either glucose or saline solutions. Throughout the trial, fish were reared under standardized conditions and up to 25 dpf, juvenile fish from both glucose and saline injected group were fed with a low carbohydrate/high protein diet. From 25 to 35 dpf, half of each group was submitted to a dietary challenge with a high carbohydrate/low protein diet. The metabolic fate of dietary carbohydrates in these four distinct experimental treatments was assessed by a capillary-feeding method, using 14C-Glucose as a tracer. Zebrafish juveniles subjected to an early glucose stimulus showed significantly lower ($P<0.05$) retention of glucose in visceral tissue (but not in muscle tissue) and consequently a higher glucose catabolism in comparison to the control group. However, the glucose stimulus exerted at an early embryonic stage had no marked effect on the ability of zebrafish juveniles to catabolize glucose under conditions of high and low carbohydrate intake. Despite this high metabolic plasticity of zebrafish to cope with different levels of dietary carbohydrates, our data suggests that an early nutritional stimulus has the potential to alter some of the carbohydrate metabolic pathways in zebrafish juveniles.

Poster Presentation

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Dziekonski M, Zmijewska A, Okrasa S, Franczak A, Kotwica G

The expression of MI OPIOID Receptor (MOR) gene in endometrium of cyclic and early pregnanat pigs; a potential effect of Interleukin 1β, TNF-α and Interleukin 6

It is well known that endogenous opioid peptides (EOP) may affect reproduction in females by acting at all levels of the hypothalamus-pituitary-gonadal axis. In previous studies, the expression of proopiomelanocortin and the presence of β-endorfin in porcine uterus were demonstrated. Thus, the aim of this study was to determine the expression MOR gene in non-treated and interleukin 1β (IL-1β), TNF-α or interleukin 6 (IL-6) treated endometrial tissue, collected from cyclic (Days 10-11, 12-13 and 15-16; n=3×5) and pregnant (Days 10-11, 12-13 and 15-16; n=3×5) gilts. Slices of endometrium (100±10 mg) were pre-incubated (18 h) in 2 ml of Medium199 under an atmosphere of 95% O2+5% CO2 at 37° C and then incubated for the next 12 h with or without the addition of IL-1β (10 ng/ml), TNF-α (10 ng/ml) or IL-6 (10 ng/ml). After incubation, the total mRNA was isolated from the tissue. The expression of MOR gene was estimated using a semi-quantitative RT-PCR method. Changes in basal expression of MOR gene in endometrial tissue were observed only during the estrous cycle with the highest values on Days 10-11 (18,25±7,94 vs. 0,92±0,21 and 1,27±0,29 on Days 12-13 and 15-16 respectively; $p<0.05$). IL-1β significantly decreased the expression of MOR gene during Days 10-11 of the cycle (from 3,56±2,62 to 0,11±0,03; $p<0.05$), but did not affect the expression of this gene during pregnancy. TNF-α significantly increased the expression of MOR gene on Days 15-16 (from 1,27±0,29 to 15,99±7,57; $p<0.05$) but tended to decrease on Days 10-11 of the estrous cycle ($p=0.059$) and increase it on Days 15-16 of pregnancy ($p=0.06$). IL-6 increased the expression of MOR gene on Days 15-16 of the cycle (from 1,27±0,29 to 19,65±8,31) and pregnancy (from 3,27±2,37 to 160,77±73). In conclusion, this study indicate potential role of opioid peptides acting through MOR in the regulation of endometrial functions during the estrous cycle and pregnancy as well as the influence of tested cytokines on this process.

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Poster Presentation

Fresard, Laure

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Fresard L, Leroux S, Gourichon D, Dehais P, Servin B, Cristobal MS, Marsaud N, Bed'hom B, Beaumont C, Zerjal T, Vignal A, Morisson M, Lagarrigue S, Pitel F

Transcriptome-wide investigation of genomic imprinting in chicken

Our project deals with the question of the imprinting evolution in vertebrates and its existence in birds, not definitely answered yet. Genomic imprinting is an epigenetic modification leading to parent-of-origin-specific expression of several genes. It has been observed in eutherians mammals and marsupials, but not in monotremes or birds. So far, the allelic expression of several genes orthologous to mammalian imprinted ones has been analyzed in chicken, without any reliable evidence of imprinting in this species. Our main objectives are to detect genes for which variation in expression is observed according to the allele, either because of an allele-specific expression or a parent-of-origin dependent expression. We thus screen the entire genome for allele-specific differential expression on whole embryonic transcriptomes by using high-throughput sequencing. We chose 4.5 days-old embryos, an interesting developmental time point to study functional features of the genome expression: the embryo is already confronted to external environment putatively influencing the future gene expression, but has not undergone yet important steps of development, such as the gonad differentiation between sexes.

Two chicken lines were used, as inbred as possible and as genetically distant as possible, to unquestionably identify the parental origin of each observed haplotype. Two families were produced, coming from two reciprocal crosses. Transcripts from 20 embryos (4.5 days) have been tagged and sequenced through 6 HiSeq2000 lanes. About 200 Gb have been generated and their analysis allowed the detection of 79 candidate SNPs. Validation by pyrosequencing was performed on 17 candidates but none of them could be confirmed. The study being conducted on the whole embryo at a precise stage of development, this could prevent from detecting tissue and stage specific genomic imprinting. However, these results come together without any a priori with previous statements assessing that genomic imprinting seem to be absent in birds.

Poster Presentation

Morisson, Mireille
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Morisson M, Basso B, Bernadet MD, Cornuez A, Sellier N, Leroux S, Lessire M, Pitel F, Brun JM

Effects of maternal methionine-restricted diet on 'foie gras' production in force-fed ducks

In France, male mule ducks which are sterile offspring of common duck females (Anas platyrhynchos) and Muskovy drakes (Cairina moschata) are bred for the production of fatty liver also called 'foie gras'. The force-feeding leads to a hepatic steatosis, with a tenfold increase in liver weight within two weeks. In mammals, dietary methyl donors are linked with fetal programming and liver disorders such as hepatic steatosis. In this context, we assumed that the production of 'foie gras' in mule duck could depend on early availability of methyl-donors. This early nutritional modulation was achieved by methionine restriction applied to the mule duck's mothers. Three levels of methionine content were designed: 4.2 g/kg (control, C), 2.6 g/kg (maximum restriction, Rm) and an intermediate level (Ri). The dams were fed the experimental diets from the age of 10 weeks until the conception of offspring of both sexes which were force-fed from the age of 12 weeks and slaughtered. The traits studied were body weight at 4, 8, 12 and 14 weeks of age, carcass weight at slaughter, 'foie gras' weight, pectoral muscle ('magret') weight and subcutaneous fat of the magret. Data were analysed by analysis of variance with the fixed effects of the sex, of the maternal diet and their interaction. The effect of the diet was significant for 12 weeks body weight (Rm=Ri>C, $p=0.06$) and for magret fatness (Rm>Ri>C, $p=0.09$). The most striking results concerned 'foie gras', exhibiting a significant sex by diet interaction ($p<0.01$): the ranking of the diets was Rm>Ri=C in males and C>Ri=Rm in females. This influence of maternal nutrition on the production of 'foie gras' is of great interest as the male mule ducks from dams fed the Rm diet exhibited a 20% increase in 'foie gras' weight that could allow reducing the duration of force-feeding.

Poster Presentation

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The developing pig embryo modulates the microRNA expression profile of the endometrium

Background: Although an optimized maternal endocrine and paracrine milieu of the secretory endometrium in the implantation window provide a crucial background platform to support implantation, it is the developing blastocyst that initiates a final decisive cascade of mutually synchronized events that culminate in its successful implantation. MicroRNAs are significant intracellular regulators at the transcriptional and post -transcriptional levels. They have the potential to target hundreds of transcripts simultaneously.

Hypothesis: A dynamic change in endometrial microRNA expression profile is an essential component in orchestrating its response to the developing embryo.

Methodology: Sows were laparoscopically inseminated with semen in one oviduct and with diluent on the contralateral oviduct. An affymetrix GeneChip miRNA 3.0 microarray was used to compare the microRNA expression profiles of the endometrium exposed to unfertilised oocytes vs developing embryos.

Results: 9 porcine microRNAs were consistently down-regulated and 3 were up-regulated in the endometrium exposed to developing embryos compared to oocytes. 3278 predicted target transcript to these microRNAs were functionally annotated and cluster to 44 defined cellular pathways. Predicted target sequences were further compared to known transcripts that are known to be differentially expressed by the pig endometrium in response to contact with embryos. 21 predicted target transcripts were verified as differentially regulated. Functional annotation and pathway analysis showed that Focal Adhesion Pathway as being a candidate for microRNA - mediated differential regulation in response to contact with developing embryos.

Conclusion: Developing embryos alter micro RNA profile of the pig endometrium. The endometrium can differentiate between embryos and unferitized oocytes. MicroRNAs probably constitute a mechanistic link in the embryo - endometrial dialogue, serving to prepare the endometrial microenvironment and embryonic epigenome during the process of implantation.

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Poster Presentation

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Pendzialek SM, Schindler M, Gurke J, Haucke E, Plosch T, Fischer B, Navarrete-Santos A

Effects of an insulin dependent diabetes mellitus on the maternal and embryonic cholesterol metabolism during the preimplantation period: Insights from the rabbit model

Metabolic disorders as diabetes mellitus are common complications of human pregnancies, occurring in 3-10% of cases. Both forms of diabetes mellitus, the insulin-dependent (IDD) and the gestational diabetes, are associated with an increased prevalence of neonatal macrosomia and metabolic diseases in offspring later in life. Human studies indicate a link between dyslipidemia of diabetic mothers and an altered lipid metabolism in embryos and fetuses. Recently, we have shown that blastocysts from diabetic rabbits (experimentally-induced IDD; expIDD) generate considerably more lipid droplets in embryoblast and trophoblast cells.

Here we describe the impact of diabetes mellitus on the maternal and embryonic cholesterol metabolism during early pregnancy in the rabbit model.

In the uterine fluid of diabetic rabbits cholesterol levels were increased, accompanied by changes in the lipoprotein composition of VLDL, LDL and HDL in blood serum. Additionally, the mRNA expression of HMGCR, LDLR, VLDLR, SREBP-2, Insig-1 and CYP7A1 was altered in hepatic and adipose tissue. VLDLR protein amount was significantly decreased in adipose tissue of diabetic rabbits.

In the rabbit embryo HMGCR, LDLR and SREBP-2 were expressed from day 3 p.c. onwards, whereas VLDLR and Insig-1 were first present at the blastocyst stage. CYP7A1 was not detectable until day 8 p.c. Blastocysts from diabetic rabbits showed an increased SREBP-2-to-Insig-1-ratio and an increase in LDLR mRNA expression. In vitro experiments revealed a glucose- but not insulin-dependent HMGCR expression in blastocysts.

We demonstrated that rabbit blastocysts are capable of cholesterol synthesis and uptake as well as expression of important cholesterol metabolism regulators. Diabetic changes in maternal metabolism do not disturb the embryonic regulation of the cholesterol target genes, indicating that the preimplantation embryo has the capacity for an autonomous cholesterol homeostasis.

This work was supported by EU (FP-7 Epihealth 278418) and the Wilhelm Roux Programme of the MLU Faculty of Medicine.

Poster Presentation

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Effects of an insulin-dependent diabetes mellitus on maternal and embryonic cholesterol metabolism during the preimplantation period: Insights from the rabbit model

Diabetes mellitus is a common complication of human pregnancies, occurring in 3-10% of cases. Both forms of diabetes mellitus, the insulin-dependent (IDD) and the gestational diabetes, are associated with an increased prevalence of neonatal macrosomia and metabolic diseases in offspring later in life. Human studies indicate a link between dyslipidemia of diabetic mothers and an altered lipid metabolism in embryos and fetuses. Recently, we have shown that blastocysts from diabetic rabbits (experimentally induced IDD; expIDD) generate considerably more intracellular lipid droplets in embryoblast and trophoblast cells.
Aim of current study was to analyze cholesterol metabolism and the impact of diabetes mellitus on the maternal and embryonic cholesterol metabolism during early pregnancy in expIDD rabbits.
Cholesterol levels were increased in the uterine fluid of diabetic rabbits, accompanied by changes in the lipoprotein composition of VLDL, LDL and HDL in blood serum. The mRNA expression of HMGCR, LDLR, VLDLR, SREBP-2, Insig-1 and CYP7A1 was altered in hepatic and adipose tissue.
In the embryo, HMGCR, LDLR and SREBP-2 were expressed from day 3 p.c. onwards, whereas VLDLR and Insig-1 were first present at the blastocyst stage. CYP7A1 was not detectable until day 8 p.c. The single alterations observed in blastocysts from diabetic rabbits were an increased SREBP-2-to-Insig-1 ratio and an increase in LDLR mRNA expression. In vitro experiments revealed a glucose- but not insulin-dependent HMGCR expression in blastocysts.
In conclusion, we demonstrate that rabbit blastocysts are capable of cholesterol synthesis and uptake and express important cholesterol metabolism regulators. Diabetic changes in maternal metabolism do not disturb the regulation of the cholesterol target genes in embryos, indicating that the preimplantation embryo has the capacity for an autonomous cholesterol homeostasis.
This work was supported by the EU (FP-7 EpiHealth No 278418) and the Wilhelm Roux Programme of the MLU Faculty of Medicine.

Poster Presentation

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Impact of embryonic heat exposure on bird methylome

Climate change, economic constraints and social concern for a sustainable agriculture strongly influence animal production systems. Improving the adaptation of poultry - a major source of proteins for human food - to variable conditions of climate and feed is thus a necessity, particularly in countries with a hot climate. Meat-type chickens have long been selected on growth performance traits, but the significant improvement in body weight has been associated with a reduced ability to cope with extreme environmental temperatures. In order to improve temperature tolerance and welfare without impairing growth, a method of early exposure to heat during egg development has been shown to improve chicken thermotolerance in later life. This treatment affects the expression level of several genes in the muscle, involved notably in energy metabolism, possibly resulting from altered DNA methylation patterns. The goal of this study was to perform a genome-wide DNA methylation analysis using high-throughput sequencing of sodium-bisulfite-treated DNA on heat-exposed birds.

To date, few studies have investigated the genome-wide distribution of methylation in chicken. We first performed a pilot study to provide a single-base resolution CpG methylome map of chicken genomic DNA in 4.5 days old embryos. The methylation pattern observed showed common features of methylation in animals, with a high prevalence of CpG methylation compared to non CpG contexts, and a higher methylation level in exons than in upstream regions of the genes associated to a low methylation level in CpG islands. We then analysed differential methylated regions between muscle tissue from adults that were early-exposed to heat and from control birds. Preliminary results will be presented.

Funding: INRA Research Divisions `Animal Physiology and Livestock Systems' and `Animal genetics' (Epitherm and Elasetic projects) and Agence Nationale de la Recherche, Project ANR-09-JCJC-0015-01 THERMOCHICK.

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Poster Presentation

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Development of an in vivo system in zebrafish to investigate the effects of altered DNA methylation during development

Little is known on DNA methylation in fish. Previous studies in our lab showed sex-related differences in the methylation levels of the promoter of cyp19a1, a gene essential for female development. The aim of this study was to develop a suitable in vivo system in which DNA methylation could be altered by the most common DNMT inhibitor, 5'-aza-cytidine (5-'aza-C), and determine the effects of dose, duration and timing of administration. Several dose-response experiments were carried out (0 -75 μM) from 2 hours to 20 days after fertilization (dpf). To examine the biological effects, hatching, survival, presence of teratologies, growth during early development, sex ratio, gene expression and global DNA methylation levels were measured. Results showed that treatment with 5-'aza-C altered development in early stages with delayed hatching and in increased mortality. Larval growth was affected with the higher doses but fish recovered from the treatment when adults. Interestingly, in some experiments an increase in the number of females was observed, suggesting that methylation of key genes related to gonadal sex differentiation might be altered by the treatment. Further, gene expression analysis revealed a significant inhibition of dnmt3 of treated fish. However, preliminary global DNA methylation assays at early stages of development (6 dpf) showed no consistent results. Currently, more studies on gene expression of key reproductive genes are carried out. Together, our results show that the in vivo inhibition of DNA methyltransferase enzymatic activity can represent a powerful tool to modulate gene expression of reproduction-related genes as well as other functions in the zebrafish model.

Supported by Spanish Government grant AGL2010-15939 (“Epigen-Aqua”) to F.P.

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Poster Presentation

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Effects of butyrate-supplemented diet on histone acetylation status in European seabass (Dicentrarchus labrax)

The expansion of aquaculture has been accompanied by an increase in fish meal demand, the base component in feed formulation for aquatic animals. The decline in worldwide supplies of fish meal has led to replace it with other protein source in diets of commercially important fish. Currently soybean meal is the foremost candidate to supply fish meal protein. Likewise the ingestion of soybean meal triggers metabolic dysfunction in fish liver, intestinal inflammation and reduced protein deposition. In this context butyrate, a short chain fatty acid, has received particular attention as a nutritional supplement in fish feed for its multiple beneficial effects on the intestinal tract and hepatic metabolism. The mechanism of action of butyrate is mainly related to its regulatory effects on gene expression. As an epigenetic factor, butyrate regulates the transcription via influencing core histone acetylation. Indeed, butyrate is a histone deacetylase inhibitor and induces histone hyperacetylation.

The present study aimed to evaluate the epigenetic effects of butyrate used as a feed additive in European seabass as well as butyrate beneficial effect on intestinal inflammation and liver. Two groups of 35 seabass juveniles were fed for three months with a diet containing 40% soybean meal with 2% of sodium butyrate added to the diet of only one group. At the end of the feeding trial four fish from each group were sampled and their liver and intestine were dissected out for molecular and histological analysis. Histones were isolated from liver cell nuclei and acetylation of hepatic core histones was screened by western blotting. Results showed a significant reduction of intestinal inflammation in fish receiving butyrate. Additionally, butyrate-supplemented diet caused hyperacetylation of histone H2A, and H4, whereas no changes were monitored in the acetylation state of H2B and H3.

Poster Presentation

Teperek, Marta
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Teperek M, Gaggioli V, Allen G, Simeone A, Kwon T, Marcotte E, Miyamoto K, Bradshaw C, Gurdon J, Jullien J

Sperm is programmed to support transcription of developmentally-important embryonic genes

Sperm was originally believed to be solely a carrier of genetic material at fertilisation. However, recent findings suggest that important information for subsequent embryonic development is encoded (programmed) in sperm. The nature of this programming is elusive. Here we have developed an experimental system in Xenopus laevis in which the developmental ability of embryos generated with sperm is directly compared to those generated with a spermatid, immediate precursor of sperm. We find that sperm-derived embryos develop significantly better than spermatid-derived embryos. Benefiting from unique tools available in Xenopus, such as egg extracts and haploid embryos, we show that the sperm programming is not related to its ability to undergo efficient DNA replication, but to regulation of embryonic gene transcription. Our finding therefore provides experimental evidence supporting the hypothesis that the sperm is not merely a carrier of generic material, but also provides important information on embryonic gene expression. This proper gene regulation is likely associated with histone H3 lysine 27 trimethylation on developmentally-important genes.

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Poster Presentation

Terova, Genciana

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Influence of exposure of rainbow trout embryos to methionine on embryonic development and epigenetics

It has been documented that methylation of DNA molecules is important in regulation of gene expression during embryonic development. Genome of the oocyte is hypomethylated in comparison to hypermethylated sperm. We aimed to establish experimentally how important de/re-methylation is as an epigenetic modifier. Unfertilized rainbow trout eggs from 4 females (Troutlodge Inc., Sumner, WA) and sperm (London State Fish Hatchery, London, OH) from 2 males were used. Fertilized embryos were divided into two groups and treated with identical solutions at two different embryonic stages-fertilization (exp 1) and neurula stage
(exp 2). Immediately after fertilization embryos were distributed between 12 containers (4 treatments x 3 replications) with 10 and 100 fold higher than the physiological concentration of L-Met, or 10 fold level of DL-ethionine (Met antagonist), and system water bath as control. It was designated as M10 and M100, E10 and CON, respectively. All treatments were incubated with intense aeration for 1 h (8.7°C). The eyed stage was reached after 189 degree days (°D) followed by the first hatching at 345°D. Met-treated embryos at the fertilization stage (exp 1) resulted in increased fractional survival between fertilization and hatching in comparison to CON (Table 1). However, there was no difference in survival from hatching and swim-up or in the deformity at swim-up stage (Table 1). No beneficial influence of Met enrichment was observed in embryos treated at neurula stage (exp 2) or weight gain after 8 weeks feeding trial in juveniles treated at fertilization stage .

Poster and Oral Presentation

Franczak, Anita
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Transcriptomic profile of the porcine myometrium - novel players during peri-implantation period

The porcine myometrium is responsible not only for maintaining the shape and motility of the uterus, but also possesses an endocrine activity. It has been previously established that the myometrium may secrete prostaglandins and steroid hormones in response to oxytocin (OT) and cytokines (e.g. IL1β, IL6 and TNFα). Therefore, the myometrium possesses potential role in early pregnancy maintenance in pigs. The specific role of this tissue during periimplantation period in pigs is still not well understood. In the current study, the DNA Microarrays were used for determination changes of transcriptomic profile of the myometrium obtained from pigs on days 15-16 of pregnancy on the background of the estrous cycle. Four two-color arrays were used in balanced block design with dye swap. Among 619 differentially expressed (FC > 1.1) and accurately annotated genes in pregnant myometrium 333 were up-regulated and 286 were down-regulated. The constructed interaction network revealed potential significant roles of several genes with HOXA13, toll-like receptor 9 (TLR9) and interleukin 13 receptor type A2 (IL13RA2) among the most interesting. Other authors have found that HOXA13, similarly to HOXA10 may affect uterine development and remodeling required for successful implantation and placentation. It is also known that TLR9 is involved in an innate immune response and IL13 can stimulate growth factors production. The changes in genes expression encoding for these factors in pregnant porcine myometrium suggest their role in regulation of processes important for successful implantation, placentation and maintenance of pregnancy. For this reason, the spatial and temporal expression as well as the function of above-mentioned factors should be investigated in the future to provide a more detailed insight into the embryo-maternal interface and into the role of the myometrium during periimplantation period in pigs.
This research was funded by National Science Centre, Poland; grant N N311 526940 (2011-2013)

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Poster and Oral Presentation

Johnsen, Hanne

Production Biology, Norwegian Institute of Food, Fishery and Aquaculture (NOFIMA)

Johnsen H, Tveiten H

Influence of embryonic stress on HPI axis development and functionality in Atlantic salmon (Salmo salar L.)

In fish, development of endocrine organs and factors involved in regulation of the HPI axis (hypothalamus-pituitary-interrenal axis) take place during embryogenesis and post hatch stages. Genes involved in control of the HPI-axis include paralogs of crh (corticotropin-releasing hormone), paralogs of pomc (pro-opiomelanocortin), mc2r (melanocortin 2 receptor), mr (mineralocorticoid receptors) and paralogs of gr (glucocorticoid receptor). In mammals, exposure to stress during embryonic and early life may alter stress coping capacity during juvenile and adult stages. The influence of stress during early development on later stress coping ability may be positive or negative dependent on the dose and stage of exposure. Information on the influence of stress on the development and functionality of the HPI axis in fish is limited. To this end, groups of Atlantic salmon (Salmo salar) embryos were exposed to bouts of stress (cold-chock), either during embryogenesis or during yolk-sac stages, or both. Genes related to HPI-axis development and functionality was mapped during normal ontogeny and in stress treated groups. To compare HPI-axis functionality, stress tests were undertaken just prior to start feeding and during juvenile stages. This study will provide knowledge about how stress during early life may alter gene expression related to HPI-axis development in teleost fish, and if such changes may alter stress functionality during later life. Methylation analyses of specific gene promoters may provide information about molecular and epigenetic mechanisms involved in long term changes in gene expression.

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Poster and Oral Presentation

Jullien, Jerome

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Teperek M, Gaggioli V, Allen G, Simeone A, Kwon T, Marcotte E, Miyamoto K, Bradshaw C, Gurdon J, Jullien J

Sperm is programmed to support transcription of developmentally-important embryonic genes

Sperm was originally believed to be solely a carrier of genetic material at fertilisation. However, recent findings suggest that important information for subsequent embryonic development is encoded (programmed) in sperm. The nature of this programming is elusive. Here we have developed an experimental system in Xenopus laevis in which the developmental ability of embryos generated with sperm is directly compared to those generated with a spermatid, immediate precursor of sperm. We find that sperm-derived embryos develop significantly better than spermatid-derived embryos. Benefiting from unique tools available in Xenopus, such as egg extracts and haploid embryos, we show that the sperm programming is not related to its ability to undergo efficient DNA replication, but to regulation of embryonic gene transcription. Our finding therefore provides experimental evidence supporting the hypothesis that the sperm is not merely a carrier of generic material, but also provides important information on embryonic gene expression. This proper gene regulation is likely associated with histone H3 lysine 27 trimethylation on developmentally-important genes.

Poster and Oral Presentation

Kradolfer, David
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An imprinted gene underlies postzygotic reproductive isolation in Arabidopsis thaliana

Postzygotic reproductive isolation in response to interploidy hybridization is a well-known phenomenon in plants that forms a major path for sympatric speciation. The phenomenon has been termed “triploid block”, describing the difficulty of obtaining viable triploid seeds by diploid-tetraploid crosses. The endosperm is a key determinant for the success of interploidy hybridizations and abnormalities in the growth and structure of the endosperm are the source of the triploid block. In most angiosperms the endosperm is a triploid tissue derived after fertilization of the homodiploid central cell with a haploid sperm cell. The endosperm serves to support and nurture the growing embryo, similar to the role of the placenta in mammals. Reciprocal hybridizations of plants that differ in ploidy have reciprocal effects on endosperm development; whereby pollinations of maternal plants with pollen donors of higher ploidy will increase endosperm proliferation, the reciprocal cross will suppress proliferation. The sensitivity of the endosperm to changes in parental genome balance led to the hypothesis that imprinted genes are causal for the response to interploidy hybridizations.
In a genetic screen for suppressors of triploid seed abortion, we have identified the paternally expressed imprinted gene ADMETOS (ADM). Here, we present evidence that increased dosage of ADM causes triploid seed arrest. Our study supports the theory that deregulated imprinted genes underpin dosage sensitivity of the endosperm and generates the molecular basis for our understanding of postzygotic hybridization barriers in plants.

Poster and Oral Presentation

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Epigenetic control of phenotypes relevant for aquatic species

Diseases caused by opportunistic bacteria belonging to the Vibrio spp. have been a serious problem for sustainable aquaculture development. Finding novel strategies for conditioning economically important brood fishes/crustaceans resulting in robust offspring (disease resistant and fast growing) is highly necessary to meet the growing demand of high quality food fish/shellfish. The different breeding strategies that are being applied, such as cross breeding and selective breeding, for producing healthy and quality seeds are very much labor intensive and require large investments. It has therefore become imperative to find novel ways, which are effective in terms of both cost and time, to produce healthy and disease-resistant seeds for sustainable aquaculture production. Very recently, epigenetic has been considered as an approach for producing healthy and stress-resistant animals. Epigenetic is the study of heritable changes in gene expression and function that cannot be explained by changes in DNA sequence. The exact molecular bases of these epigenetic changes are slowly being unraveled. Epigenetic modifications are responsible for activation and suppression of (certain) genes which can get inherited across generations. The molecular mechanisms behind epigenetics modifications and across generation inheritance of phenotypes can be studied more easily in populations with genetically identical individuals. Parthenogenetic Artemia exhibits an excellent model organism for these types of studies since there is a possibility for producing a clonal culture in a short period of time. In this study, phenotypic responses of the Artemia clonal culture offsprings, whose parents were treated with heat shocks, are presented.

Poster and Oral Presentation

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Seahorses: A new and unique epigenetic model

Environmental influences (including diet and feeding conditions) experienced during development modify animals' phenotype, in a way possibly 'predictive' of future conditions, and may be essential for their survival and reproduction. With their particular reproductive behaviour, where males not only become pregnant but also support spermatogenesis, seahorses constitute an excellent model. The aims of this work were to (1) investigate the hypothesis that parental nutrition in adult seahorses (Hippocampus reidi) affects the growth and development of offspring, much as in mammals, and (2) validate the proposal that seahorses may be a valuable and effective epigenetic model.
The experimental design involved using either wild or commercial mysids to influence the peri- and post-conception environment. Sixteen seahorse breeding pairs were divided into separate aquariums. We manipulated the male's and female's diets separately prior to mating, obtaining offspring from animals from all combinations of dietary treatments (male x female x wild x commercial) over 5 months. Approximately 10,000 offspring were born.
In the first experiment brood pairs in which males received commercial and females received wild diet, produced significantly larger offspring than controls (males and females both receiving wild diet). Offspring produced by commercial-fed females were consistently smaller than the controls, suggesting that maternal nutrition directly affects offspring development. These results demonstrated that the growth and survival of offspring is dependent on the quality of diet received by both parents prior to conception. It is of interest that commercial-fed males (commercial diet is lower in polyunsaturated fatty acids) had abnormally large offspring. The results support the hypothesis that the male's brood pouch does more than simply provide a suitable environment that sustains embryonic growth and supports the hypothesis that seahorses could be used as an experimental model for studying the relationships between parental nutrition and offspring fitness.

Poster and Oral Presentation

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Transgenerational epigenetic effect of parental methyl supplements in Zebrafish diets, project description and preliminary results

During the last decade there has been a gradual change from marine to plant based alternatives in fish feed for the aquaculture industry. This also change the ratios of essential nutrients and influence the metabolism of folate, amino acids, and vitamin B12, all of which are important for cellular methylation reactions. Among these methylation reactions is the methylation of DNA. DNA methylation is the most studied epigenetic mechanism and is a biochemical process whereby a methyl (1C) group is added to cytosine and hereby influence the potential for gene expression. The plant based feed alternatives have previously shown to influence both growth performance and flesh quality, but how the fish feed composition affect the epigenetic regulation of gene transcription during the different life stages and across generations, remains an important and unexplored field. These epigenetic changes are also sensitive to environmental factors like nutrition. We have designed a project to study how plant based feed enriched with methyl donors regulates gene expression patterns by DNA methylation, directly and across generations in fish. A transgenerational (F0-F3) zebrafish feeding trial is currently ongoing, using plant based raw materials. Two diets has been designed, one that has high and one that has low levels of the nutrients important for methylation reactions. Embryos at 1.5 DPF, larvae, ongrowing and dissected samples of mature zebrafish from each generation are currently being sampled. Livers from mature fish (F0 and F1) will be sampled for methylome and metabolom studies. Preliminary results indicate lower folate concentration in F0, a significant decreased weight at 3 months and lower fecundity in the low 1C group, reflecting the general effects observed for plant based diets used in aquaculture settings. qPCR results show that mRNA expression of genes involved in the 1C cycle have lower level of expression in F1 if sufficient methyl supplements are added in the diet to the F0 generation.

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