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Friday April 3

GLOBAL REGULATION OF CHROMATIN STATES

Chair: Peter Verrijzer

IL.10 08.30 – 09.15

MECHANISMS OF STOCHASTIC GENE EXPRESSION

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We present a model to explain the stochastic expression of the IFN-â gene from a single allele in a small population of cells. The choice of the allele to be expressed depends on interchromosomal associations with three identified distinct genomic loci. These loci bear non-consensus NF-kB binding sites within specialized Alu repeats. We show that these loci can immediately receive the limiting transcription factor NF-kB and "deliver" it through long-range chromatin interactions to the IFN-â enhancer, where it nucleates enhanceosome assembly and activation of transcription from a single allele. The probability of a cell to express IFN-â is dramatically increased when the cells were transfected with any of these genomic loci, a result consistent with the proposed model. Although, the IFN-â gene is expressed from a single allele of a small proportion of cells at the early stages of virus infection, the number of expressing cells and expressing alleles per cell are increased gradually, in response to IFN-â cytokine signaling. The secreted IFN-â protein induces high-level expression of the enhanceosome factor IRF-7, which in turn promotes enhanceosome assembly and IFN-â transcription from the remaining alleles and on other initially non-expressing cells. Thus, the IFN-â enhancer functions in a non-linear fashion by working as a signal amplifier.
We have identified additional NF-kB regulated genes whose expression requires a physical interaction with the loci. As it is the case with the IFN-â gene, these interchromosomal interactions are monoallelic and are required for expression. Remarkably, we showed that individual alleles from different genes simultaneously interact with these loci in a small percentage of cells. These results suggest that coregulated monoallelically expressed genes coexist in the nucleus to share limiting transcription factors.

O4 09.15 – 09.30

MOLECULAR ARCHITECTURE OF CTCF AND THE INSULATOSOME

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Multi-protein, sequence-specific, DNA-binding complexes regulate the chromatin structure and transcriptional activation of genes as autonomously functioning loci throughout the human genome. Genetic units are demarcated in part by insulator proteins that flank one locus to protect promoters from activation by enhancers of other loci. Such enhancer blocking is distinct from silencing of gene activity. Silencing is a genetic switch with one logical criterion, that the acting cis element is an enhancer. Insulation bears two criteria, not simply that any enhancer is blocked, but also that the acting cis element be in a certain spatial position, thus allowing a privileged enhancer to function. We have characterized the molecular architecture of human CTCF, an insulator protein known to bridge distal DNA elements on the same and even different chromosomes. By determining biophysical properties such as structure and oligomeric state, as well as mapping evolutionary conservation, we can propose a functional architecture. We surmise that CTCF serves as a scaffold for the assembly of other proteins on insulator elements. A histone chaperone, chromatin remodeling enzyme, and cohesin may also function in insulation. We have purified recombinant proteins and tested different potential cofactors for the ability to directly bind CTCF. In order to expand our study to proteins that do not contact CTCF but still assemble on insulator elements, we profiled the genome-wide DNA-binding sites of putative cofactors at high resolution by chromatin immunoprecipitation followed by deep sequencing. We

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discuss the aggregate data, which in sum moves toward defining the molecular architecture of a complex we refer to as the insulatosome.

O5 09.30 – 09.45

HORMONE INDUCED ESTROGEN RECEPTOR ALPHA MEDIATED TRANSCRIPTION OCCURS THROUGHOUT THE NUCLEUS IN NORMAL AND CANCEROUS HUMAN MAMMARY CELLS

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Nuclear organization of genes may impact their regulation; in particular, gene activation may induce changes in the positioning of specific loci relative to each other. We investigated the nuclear distribution of estrogen receptor alpha (ER) target genes in two human breast epithelial cell lines, the MCF10A normal-like diploid cell line and the MCF7 aneuploid tumor cell line. Nuclear positions of a subset of genes upon addition of estradiol were assessed quantitatively using 3D in situ hybridization techniques. Transcription activation was determined using RT-qPCR. An MCF7 derived stably expressing functinal ER-GFP cell line was created. In MCF10A cells, the two alleles of TFF1 and of GREB1, and in MCF7 cells, 6 copies of TFF1 and 4 copies of GREB1, are scattered throughout the nucleus with no particular relationship to each other. Expression levels of these genes were near background levels and insensitive to hormone addition in MCF10A cells as expected for this ER negative cell line. In MCF7 cells, transcription activation of these genes in the presence of estradiol, confirmed by quantitative RT-PCR, did not induce significant changes in their intranuclear organisation. The relative positions between the 10 gene loci imaged were independent. While some target genes shifted from an internal to a peripheral position relative to their chromosome territory, the overall nuclear organisation of different chromosome territories remained unaffected by hormone addition. Furthermore, TFF1 and GREB1 colocalized with hormone induced discrete foci of ER in stable clones of ER-GFP in MCF7 cells. Our results demonstrate that ER regulated genes are transcribed in multiple specialized transcription factories assembled around the ER.

IL.11 09.45 – 10.30

GENOMIC LANDSCAPE OF THE DEVELOPMENTAL REGULATOR PAX6 IN DEVELOPMENT, EVOLUTION AND DISEASE

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The spatio-temporally, and quantitatively correct activity of a gene requires the presence of intact coding sequence as well as properly functioning regulatory control. Gene regulation depends not only on the presence of the required transcription factors and associated complexes, but also on the integrity, chromatin conformation and nuclear positioning of the gene's chromosomal segment. The term 'position effect' is used to refer to situations where the level of expression of a gene is deleteriously affected by an alteration in its chromosomal environment, while maintaining an intact transcription unit. Due to their often complex and extended regulatory landscapes the class of 'developmental regulator' genes appears especially susceptible to position effect mechanisms. We have studied the regulatory landscape of the PAX6 genomic locus. PAX6 has an essential role in development of the eye, brain, pancreas and olfactory system. The required functional activity of Pax6 critically depends on correct levels of transcription, as haploinsuffiency leads to the congenital eye malformation aniridia in humans and the smalleye phenotype in the mouse. Analysis of aniridia position effect patients has led to the discovery of long-range enhancers. Continued analysis of the locus using DNAse HS site mapping and evolutionary sequence conservation has revealed the presence of a large number of cis-regulatory elements, often with overlapping tissue-specificity. Deletion of specific distal regulatory elements in YAC transgenics abolishes expression in certain tissues despite the presence of more proximal enhancers with overlapping specificity, strongly suggesting interaction between the control elements. This notion is reinforced by studies in zebrafish where duplication of the pax6 locus has led to subfunctionalisation through regulatory divergence.

References:

[1] Long-range control of gene expression: emerging mechanisms and disruption in disease. (2005) Kleinjan DA, van Heyningen V. Am J Hum Genet. 76(1):8-32.
[2] Long-range downstream enhancers are essential for Pax6 expression. (2006) Kleinjan DA, Seawright A, Mella S, Carr CB, Tyas DA, Simpson TI, Mason JO, Price DJ, van Heyningen V. Dev Biol. 299(2):563-81.
[3] Subfunctionalization of duplicated zebrafish pax6

genes by cis-regulatory divergence. (2008) Kleinjan DA, Bancewicz RM, Gautier P, Dahm R, Schonthaler HB, Damante G, Seawright A, Hever AM, Yeyati PL, van Heyningen V, Coutinho P. PLoS Genet. 4(2):e29.

10.30 – 11.00 TEA AND COFFEE

IL.12 11.00 – 11.45

THE RAPID AND LOCUS-WIDE TRANSITION OF A GENE FROM A POTENTIATED TO A HIGHLY ACTIVATED STATE IN VIVO

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Hsp70 loci/genes experience dramatic changes in their chromatin and transcription factor association during the seconds and minutes following heat shock gene activation. We are using multiphoton microscopy to examine the dynamics of GFP- and photoactivatable GFP-tagged Pol II and specific transcription factors at particular chromosomal loci of Drosophila in real time and in living cells. Transcription 'compartments' are gradually established at heat shock loci following induction, and these 'compartments' are defined by their ability to retain and recycle Pol II and other factors, including Spt6 and Topoisomerase I, that play a role in transcriptional activation. We are also using biochemical mapping methods to examine the kinetics and location of the changes in chromatin structure that occur at heat shock loci in response to gene activation. Interestingly, the dramatic loss in nucleosomes at activated heat shock loci precedes the movement of Pol II through the gene. Moreover, the region affected covers a domain that is much larger than the transcription unit, and extends to, but not beyond, the known insulator regions that flank the Hsp70 genes. This induced change is dependent upon the upstream activators, HSF and GAGA factor, as one might expect. Additionally, Poly(ADP)-Ribose Polymerase (PARP1) and its enzymatic activity are critical for this dramatic and rapid change in chromatin architecture. The mechanistic role of PARP1 and the poly(ADP)-ribose that it produces is being investigated.

O6 11.45 – 12.00

VEZF1 SITES MEDIATE THE ABILITY OF A VERTEBRATE BARRIER TO PREVENT DNA METHYLATION

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The HS4 element located at the 5' boundary of the chicken beta-globin locus is a paradigm vertebrate insulator element, harboring separable enhancer blocking and silencing barrier activities. Here we identify the essential transcription factor VEZF1 as a novel chromatin barrier protein. We show that three VEZF1 sites are required for HS4's ability to protect a linked promoter from de novo DNA methylation. Strikingly, we observe a modular division of labor at HS4 as VEZF1 activity is separable from those mediated by CTCF and USF proteins. Notably, the ability of HS4 to protect a transgenic promoter from de novo methylation is independent of the promoter's histone acetylation or transcription status. We also find that the DNA methylation of a transgene promoter is independent of the methylation state of the insulator. This suggests either that methylation might not be propagated via spreading from the chromosomal integration site, or that methylation of insulator and promoter are controlled by separate mechanisms. We propose that barrier elements in vertebrates must be capable of preventing DNA methylation in addition to blocking the propagation of silencing histone modifications, as either of these mechanisms is sufficient to direct the establishment of an epigenetically stable silent chromatin state. HS4 is a fully effective vertebrate barrier that combines both of these properties in a single multi-component element.

IL.13 12.00 – 12.45

LONG NONCODING RNAs: INSIGHTS INTO FUNCTION

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Transcriptomic studies have revealed that the genomes of all studied organisms are almost entirely transcribed, generating an enormous number of non-protein-coding RNAs (ncRNAs). These RNAs are often organized in overlapping and interlacing patterns with protein-coding genes. However, the functions of these RNAs remain largely unknown. One of the challenges in the functional characterization of ncRNAs is that, unlike proteins, their sequences are currently not predictive of function. Here we employ an integrated approach that combines genome-scale computational and experimental data to identify candidates for targeted functional studies. We applied this approach in mouse to examine long ncRNAs in mouse brain and during embryonic stem (ES) cell differentiation. In the mouse brain, we used high-resolution in situ hybridization data of more than a thousand long ncRNAs in conjunction with the genomic positions of ncRNAs relative to protein-coding genes of known function. This analysis predicted a subset of ncRNAs with likely roles in various neurological functions, including memory formation and neurogenesis (1). To identify ncRNAs involved in pluripotency and development, we analyzed expression profiles of ~3,000 long ncRNAs during ES cell differentiation in combination with genome-wide chromatin state maps, genomic context and relative positions of promoter binding sites (2). Chromatin immunoprecipitation studies of two high-confidence candidates predicted by this bioinformatic analysis indicated these ncRNAs had a role in epigenetic regulation of homeotic loci during ES cell differentiation. Most recently, we have applied our long ncRNA analyses to whole transcriptome sequencing data from mouse liver, muscle, brain, and ES cells. These studies provide the first genome-wide insight into long ncRNA distribution, splicing, expression and regulation. In summary, our examination of long ncRNAs in mouse brain and ES cell differentiation suggest many of these transcripts function intrinsically as RNAs and are important in a diverse range of biological processes (3).

References:

[1] Mercer, T. R., M. E. Dinger, S. M. Sunkin, M. F. Mehler, and J. S. Mattick. 2008. Specific expression of long noncoding RNAs in the adult mouse brain. Proc Natl Acad Sci U S A 105: 716-721.

[2] Dinger, M. E., P. P. Amaral, T. R. Mercer, K. C. Pang, S. J. Bruce, B. B. Gardiner, M. E. Askarian-Amiri, K. Ru, G. Solda, C. Simons, S. M. Sunkin, M. L. Crowe, S. M. Grimmond, A. C. Perkins, and J. S. Mattick. 2008. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Res 18: 1433-1445.

[3] Mercer, T. R., M. E. Dinger, and J. S. Mattick. 2009. Long noncoding RNAs: insights into function. Nat Rev Genet 10: in press.

12.45 – 13.45 LUNCH

Chair: John Lis

IL.14 13.45 – 14.30

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TRANSCRIPTION COMPLEXES IN EPIGENETICS AND GENOME FUNCTION

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No abstract available

O7 14.30 – 14.45

EPIGENETIC CONTROL OF HETEROCHROMATIN REPLICATION

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Constitutive heterochromatin exhibits a unique epigenetic profile characterized by high levels of DNA methylation, histone hypoacetylation and H3K9m3 accumulation. These modifications have been proposed to promote the high condensation levels and late replication timing observed for this subset of the genome. To investigate this question, we disrupted the stereotypical epigenetic profile of constituitive heterochromatin using i)Trichostatin A treatment of mouse embryonic fibroblasts (MEFs) to inhibit HDACs, causing histone hyperacetylation; ii)Suv39h1/2-/- MEFs, deficient in H3K9m3; iii)dnmt1 0/0 MEFs with significantly decreased DNA methylation. The levels of different histone modifications were assessed by Western Blot analysis. DNA methylation levels were determined by pyrosequencing of major satellites after bisulfite treatment. Structural changes of chromocenters were analyzed by in situ DNA staining. Replication pattern distributions were quantified by immunodetection of modified nucleotides. Confocal and deconvolution

microscopy were used in conjunction with immunoFISH to quantify the colocalization of chromocenters with acetylated histones and early replication foci. Special emphasis was put on developing a software system for interactive image analysis that combines an efficient, fully automated quantification with the possibility of fast visual inspection. Finally, live cell microscopy was performed to follow the progression of replication foci at chromocenters over time. While the loss of H3K9m3 had no influence in the replication pattern distribution, hyperacetylation of constitutive heterochromatin and its concomitant decondensation correlated with a decrease in the frequency of late replication patterns and increased colocalization of heterochromatic regions with early replication foci. Loss of DNA methylation, observed exclusively in dnmt1 0/0 MEFs, was followed by hyperacetylation and decondensation of chromocenters and showed equivalent results regarding late replication. Hyperacetylation was sufficient to cause disruption of late replication and found in all cases thereof. Hence, we propose that the level of histone acetylation plays a key role in modulating the replication timing of constitutive heterochromatin.

O8 14.45 – 15.00

THE POLYCOMB REPRESSIVE COMPLEX 1 IS REQUIRED TO MAINTAIN COMPACT CHROMATIN STRUCTURE AT MAMMALIAN HOX LOCI

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In contrast to our knowledge about histone modifications, mechanisms that mediate changes in higher-order chromatin structure are poorly understood. Here we investigate whether specific factors are required to maintain the compact chromatin state of silent Hox loci in murine embryonic stem (ES) cells. Hox loci are broadly decorated by H3K27 tri-methylation, the histone modification laid down by the polycomb repressive complex PRC2, particularly over the regulatory and non-genic portions of the loci. This mark is preferentially lost from the 3' end of Hox loci upon ES cell differentiation. Here we show that the absence of either PRC2 or PRC1 activity in ES cells decondenses Hox loci. This is specific to Hox loci and is not seen at control loci. Decondensation is shown to be due to the absence of PRC1, rather than PRC2, since Hox loci are decondensed in Ring1b null cells that still have H3K27 methylation. We conclude that PRC1 plays a direct role in compacting higher-order chromatin structure in vivo and we suggest that this acts to block steps of transcription at genes poised for rapid activation during early development.

O9 15.00 – 15.15

DNA METHYLATION IN PLURIPOTENT CELLS CONSTITUTES AN EPIGENETIC GROUND STATE THAT DIVERSIFIES WITH DIFFERENTIATION

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DNA methylation is an important epigenetic modification that has been shown to correlate with transcriptionally active euchromatin and to play a role in the development of several diseases. DNA methylation of CpG islands has been successfully predicted for several fully differentiated adult tissues, based on DNA sequence features. Since the genome sequence is (almost) identical for all cells within an organism, the consistency of results obtained for different tissue types point to a low level of tissue specificity of DNA methylation, which is confirmed by initial experimental studies. A high-resolution, genome-scale analysis of tissue-specific DNA methylation has become possible only recently, utilizing bisulfite sequencing data for ~5% of all CpG dinucleotides in the mouse genome that have been generated for more than a dozen cell types. We analyzed and predicted DNA methylation profiles of several sets of functional genomic regions (i.e. CpG islands, promoters, most conserved elements) across all available cell types. Applying a novel toolkit for (epi-) genome analysis - EpiGRAPH - we showed that DNA methylation can be predicted with accuracies of above 75%, across a wide range of tissues and developmental stages and for various genomic regions. Interestingly, prediction accuracies were consistently higher in pluripotent cells than in terminally differentiated cells, consistent with a recent hypothesis that DNA methylation of embryonic cells is coded in the DNA sequence and may form an epigenetic ground state that increasingly erodes as differentiation progresses.

O10 15.15 – 15.30

HETEROCHROMATIN DOMAINS IN CANCER

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Transcription can be affected by genetic or epigenetic alterations in cancers. Genetic events take place at a single locus or over an entire chromosomal domain. Regarding epigenetic alterations, they are known to affect the expression of isolated genes and recently regional epigenetic silencing has also been characterized in colon, bladder and breast cancers. To understand to which extent regional epigenetic alterations take part in tumor progression, we had developed an original large-scale analysis of these alterations by comparing transcriptome and genomic data for a bladder tumor set (Stransky et al, Nat Genet 2006). With this bioinformatic approach, we had isolated 28 regions candidate for regional epigenetic control, where the expression of the genes is correlated independently of any genetic alteration. By studying the expression data for all those regions in tumor samples versus normal urothelium samples, we have now identified the regions undergoing a global down-regulation in subsets of tumor samples (n=10), i.e. potentially affected by regional epigenetic silencing. We have studied these candidate regions in vitro, using a cell line CL1207, which was derived with few passages from one of the invasive tumors, other bladder cancer cell lines and normal human urothelial cells. We found that seven of these regions were indeed epigenetically silenced in association with H3K9 hypoacetylation and H3K9 and K27 hypermethylation in cancer cells specifically, mimicking the formation of facultative heterochromatin domains. DNA methylation was not found in these regions. Trichostatin A enabled gene re-expression as well as a reversal of repressive histone marks, clearly suggesting that they take part in the silencing process.

15.30 – 16.00 TEA AND COFFEE

IL.15 16.00 – 16.45

DECIPHERING THE REGULATORY ARCHITECTURE OF MAMMALIAN GENOMES WITH CHROMOSOMAL ENGINEERING

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Vertebrate genomes are composed by large syntenic genomic loci, containing multiple genes with distinct functions and expression profiles, regulated by a plethora of cis-regulatory elements. The specific architecture of a locus appears to contribute substantially to the translation of these intermingled arrays of genes and regulatory elements into distinct gene-specific expression programs, as illustrated in a dramatic manner in several human genetic disorders. Similarly, the widespread structural variations found in humans could play a role in phenotypic diversity by modulating the expression profile of surrounding genes. However, the molecular and regulatory mechanisms associated with this dimension of the genome are mostly unknown. To get a better understanding of the regulatory role of genome organization and identify the underlying elements, we focused on the Pax2-Fgf8-Pitx3 interval. This 1.5 Mb region comprises several developmental genes with complex expression patterns as well as other genes with rather "housekeeping" functions. Importantly, chromosomal duplications disrupting this otherwise evolutionary conserved region have been associated with inherited human limb malformations. We have initiated a large-scale characterization of the regulatory elements that control the specific activities of the multiple genes present in this interval, and used chromosomal engineering to create a series of complementary deletions and duplications to reshuffle its organization and model the human duplications found in ectrodactylous patients. This complementary approach reveals the intricate regulatory organization of this locus and provides insights into the mechanisms controlling the functional and specific interactions between genes and cis-regulatory elements.
To facilitate and extend these studies to other regions, we have recently developed an in vivo transposon-based system from which we can isolate mouse lines with unique insertions distributed all over the genome. We will discuss how we can use this approach to probe the regulatory permissiveness/potential at given genomic positions and establish maps of regulatory domains along the chromosomes, exposing the functional organization of mammalian genome.

O11 16.45 – 17.00

A METHOD FOR THE HIGH-RESOLUTION IDENTIFICATION OF ACTIVE CIS ELEMENTS FOR ANY GIVEN GENE AND ITS APPLICATION TO THE HUMAN ALPHA GLOBIN LOCUS

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Cis-acting elements (promoters, enhancers, silencers, locus control regions, boundary elements) can often be identified via their conserved, non-coding DNA sequences. In addition, when active, they may have characteristic chromatin signatures and are bound by transcription factors and polymerase II. Such features can now be identified across the entire genome by chromatin immunoprecipitation (using ChIP on chip and ChIPseq). However, cis-elements (e.g. promoters and enhancers) may be located 100s or even 1000s kb apart and therefore it is often not clear which regulatory sequence (e.g.enhancer) interacts with which promoter or additional regulatory element. The chromosome conformation capture (3C) technique was designed to analyse physical interactions between specific, previously characterised, widely separated DNA elements and it has been shown that such interactions are an inherent feature of their function. We have adapted the 3C protocol and developed a method which is capable of identifying all DNA elements interacting with a selected sequence (e.g. promoter) without any prior knowledge of these elements. To validate this approach, we have initially applied this method to the human a globin locus in which the interacting cis-elements have been previously characterised in detail. Using this modified chromosome conformation capture technique we can simultaneously identify the known regulatory elements with high resolution and have shown that they occur in a tissue-specific manner. The method can be applied to the entire genome and is easily analysed using tiled micro-arrays or by high-throughput sequencing which provides additional information on the nature of individual interactions.

O12 17.00 – 17.15

CONSOLIDATION OF THE CANCER EPIGENOME TO REGIONS OF UNIFORM CHROMATIN

Clare Stirzaker; Jenny Z Song; Aaron L. Statham; Mark Robinson; Paul Lacaze; **Marcel Coolen**; Carlos S. Moreno; Kaplan Warren; Susan J. Clark.
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Gene silencing in cancer occurs by both genetic and epigenetic processes but the underlying mechanism is unclear. Here we show that contiguous regions of gene suppression commonly occur in cancer cells due to a process that involves long range epigenetic silencing (LRES). We identified LRES regions in clinical prostate cancer samples using a sliding window analysis on multiplatform expression array datasets and combined these results with epigenome tiling array analysis on prostate cancer cell lines for histone H3 K9ac, K9me2, K27me3 and DNA methylation marks. Our analysis reveals that in LRES regions, adjacent genes are commonly changed to the same epigenetic silencing state, resulting in a consolidation of the epigenome. This phenomenon was also observed ouside LRES regions, indicating this to be a common mechanism of epigenetic deregulation in cancer.

O13 17.15 – 17.30

INTRA-CHROMOSOMAL LOOPS UNCOUPLE METHYLATION AND IMPRINTED EXPRESSION IN CANCER

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Introduction: Genomic imprinting results in reciprocal silencing of the maternal IGF2 allele and the paternal H19 allele. These adjacent genes are 100kb apart and are regulated by enhancers located downstream of H19 and an insulator situated upstream of H19. CTCF binds to the insulator when it is unmethylated (maternal allele) and insulates IGF2 promoters from the enhancers. Methylation of the insulator (paternal allele) blocks CTCF binding and allows the IGF2 promoters to access the enhancers. Hypermethylation at the insulator results in loss of imprinting (LOI) and congenital growth disorders. In many cancers there is LOI, but without the hypermethylation that one would predict for LOI, suggesting that different mechanisms lead to LOI in the germ line and in somatic cells.

Methods & Materials: Recently cohesin was shown to localise to the same genomic sites as CTCF, suggesting that cohesin may play a role in long range gene regulation. We used chromosome conformation capture (3C) and RNAi techniques to determine whether cohesin has a role in looping structures at the IGF2 locus. We examined a panel of human cell lines with normal and abnormal imprinting for interactions between the promoter and enhancer elements and for interactions between the insulator and differentially methylated regions (DMRs) in the IGF2 gene. *Results:* Enhancer interactions with the IGF2 promoters were found on the paternal allele in normal cell lines. These interactions did not require CTCF or cohesin. Interactions between the insulator and IGF2 DMR0 occurred in normal cells but these interactions were lost when cohesin was knocked down. Cancer cell lines with hypermethylation at the insulator and IGF2 DMRs showed no interaction between the insulator and DMR0. However, cancer cell lines with hypomethylation at the insulator unexpectedly also showed a loss of interaction between the insulator and the DMR0.
Conclusion: In cancer, aberrant looping interactions may uncouple imprinting and methylation.

17.30 – 19.30 **POSTER SESSION II (Number 46 -)**

1930 – 20.00 **COACH TO DINNER**

20.00 – on **DINNER**