NUCLEAR ORGANISATION
Chair: Barbara Panning

IL.2  08.30 – 09.30

INSIGHTS INTO INTERPHASE LARGE-SCALE CHROMATIN STRUCTURE FROM ANALYSIS OF ENGINEERED CHROMOSOME REGIONS

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How 10 and 30 nm chromatin fibers fold into mitotic and interphase chromosomes has remained a difficult question for many years. This has been as much a problem of experimental methods for visualizing specific sequences as a problem of limitations of resolution and spatial dynamic range of available microscopy methods. Over a number of years we have used engineered chromosome regions tagged with lac operator repeats as a means of visualizing the structure of specific chromosome regions in live cells and cells fixed under conditions which preserve large-scale chromatin structure while also facilitating a reductionist experimental approach. We have progressed from gene amplified chromosome regions to regions created by multi-copy integration of plasmids, and now to engineered chromosome regions formed by multi-copy BAC repeats carrying 100-200 kb of defined genomic loci. We also recently have developed a novel in vivo immunogold labeling procedure to visualize these chromosome regions at the ultrastructural level without perturbation induced by exposure to detergents and buffer conditions which might alter chromatin conformation.

Here we summarize insights into interphase large-scale chromatin folding and dynamics using these engineered chromosome regions. In particular, we focus on the visualization of the interphase chromosome structure of actively transcribing chromosome regions. Our recent results with the multi-copy BAC constructs suggest that transcription at levels approaching within several fold the level of the corresponding endogenous gene locus occurs on a condensed template which, however, undergoes a global, long-range several fold decondensation with transcripational induction. Experiments examining engineered chromosome regions in mouse ES cells suggest a surprising degree of plasticity in large-scale chromatin structure during differentiation, while maintaining similar levels of transcription. Multi-copy BAC inserts create "mini-territories" which self-organize such as to reproduce certain features observed for real chromosome territories. Finally, multi-copy BAC transgenes reproduce gene-specific nuclear compartmentalization, which will facilitate future dissection of nuclear targeting mechanisms.

References:
[1] Chuang, C., Carpenter, A.E., Fuchsova, B., Johnson, T., de Lanerolle, P., Belmont, A.S., Long-range directional movement of an interphase chromosome site. Curr. Biol. 16:825-831 (2006)
[2] Kireev, I, Lakonishok, M., Liu, W., Joshi, V.N., Powell, R., A.S. Belmont, In vivo immunogold labeling confirms large-scale chromatin folding motifs, Nat Methods 5:311-313 (2008)

BREAST CANCER DIAGNOSTICS BASED ON INTERPHASE SPATIAL GENOME POSITIONING

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Introduction: Genomes are non-randomly organized within the three-dimensional space of the cell nucleus. The nuclear position of many genes and genomic regions changes during physiological processes such as proliferation, differentiation and, importantly, disease. We have exploited the changes in gene positioning patterns to develop a novel diagnostic tool in the detection of breast cancer.

Methods & Materials: To identify genes which occupy distinct intra-nuclear positions in normal and malignant cells we visualized gene loci by fluorescent in situ hybridization either in an established human mammary epithelial 3D cell culture model of early breast cancer or in a panel of seven normal and eleven invasive ductal carcinoma human breast tissues. The radial position of a gene signal, normalized to the size of the nucleus, was
determined. 88-220 nuclei per sample were analyzed and combined to generate cumulative distributions for each gene in a tissue, which were then statistically compared to other tissues using the 1D-Kolmogorov-Smirnov test. **Results:** Using the 3D cell culture model, we identified several genes which specifically reposition during tumourigenesis. We extended these studies to human tissues and using an unbiased screening approach, we identified several genes whose nuclear positions are robustly altered in breast cancer as compared to normal tissue. These markers, used either singularly or in combination, are able to detect cancer tissues with high accuracy in a retrospective analysis. The changes in positioning are not the consequence of global spatial genome reorganization in cancer cells since we find a gene-specific repositioning behaviour. Moreover, the repositioning events are specific to cancer and do not occur in non-cancerous breast disease. We are currently validating our marker genes in larger sample set and are identifying positioning markers to distinguishing different cancer sub-types. **Conclusions:** These results establish spatial genome organization as a novel diagnostic strategy in cancer detection.

**IL.3 09.45 – 10.30**

**FUNCTIONAL INTERACTIONS BETWEEN NUCLEAR PORE PROTEINS AND ACTIVE GENES INSIDE THE NUCLEOPLASM**

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Nuclear pore complexes (NPCs) mediate bidirectional nuclear transport and have been proposed to also promote mRNA export by attracting active genes. Many NPC components (nucleoporins) also localize inside the nucleoplasm, with hitherto unknown function. We analyzed genomic interactions of nucleoporins Nup98, Nup50 and Nup62 in Drosophila cells and compared these with genomic interactions of nucleoplasmic and NPC-tethered Nup98. We found that wild-type nucleoporins predominantly interacted with chromatin inside the nucleoplasm. These interactions occurred on active genes and enhanced gene expression. At the NPC, nucleoporins did not preferentially associate with active genes, suggesting nucleoporin-chromatin interactions do not generally function to attract active genes to the NPC. Instead, our data indicate that NPC components directly stimulate gene expression inside the nucleoplasm. **References:**

[1] Bernike Kalverda, Michael D. Roling and Maarten Fornerod (2008). Chromatin organization in relation to the nuclear periphery. FEBS Lett. 582:2017-2022.  
[2] Bernike Kalverda and Maarten Fornerod (2007). The nuclear life of nucleoporins. Dev Cell 13:164-165.  
[3] Helen Pickersgill, Bernike Kalverda, Elzo de Wit, Wendy Talhout, Maarten Fornerod, Bas van Steensel (2006). Characterization of the Drosophila genome at the nuclear lamina. Nature Genet 38:1005-1014

**10.30 – 11.00 TEA AND COFFEE**

**IL.4 11.00 – 11.45**

**NUCLEAR ARCHITECTURE STUDIED BY MICROSCOPY: WHERE THE FIELD STANDS AND WHERE TO MOVE NEXT**

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The dynamic, functional topography of nuclear components, such as chromosome territories, chromatin loops and domains, genes, nuclear bodies, splicing speckles, as well as molecular machineries for transcription, co-transcriptional splicing, DNA-replication and repair is not well understood. Profound differences between present models of nuclear architecture point to the huge gap of knowledge, which must still be bridged to achieve an integrated understanding of nuclear structure and function from the molecular level to the level of higher order organization. This gap can now be closed using new methods for the multicolor visualization of nuclear structures in living and fixed cells in combination with new possibilities of quantitative imaging. New types of laser microscopy, which surpass the resolution limit of conventional light microscopy down to the nanometer scale have recently been developed. The sequential use of 4D (space and time) live cell imaging, 3D light optical nanoscopy and 3D electron microscopy provides unprecedented possibilities for correlative microscopy and will allow studies of the dynamic organization of nuclei with unprecedented detail.

**O2 11.45 – 12.00**

**MITOSIS UPDATED - PICH AND THE ANAPHASE THREADS**
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The process of mitosis has been illustrated in more or less unchanged ways in textbooks for decades and still resembles the original observations made by Flemming back in 1882. However, PICH (Plk1-interacting checkpoint helicase) was recently identified as an essential component of the spindle assembly checkpoint and shown to localize to kinetochores, inner centromeres, and most interestingly, it decorates thin threads connecting separating sister-chromatids even until late anaphase. PICH-positive threads evolve from inner centromeres as they stretch in response to tension reaching up to 15 µm in length. With the discovery of DNA threads connecting sister-chromatids until late anaphase a new level of cell cycle regulation seems likely. The properties of the PICH protein lead to the hypothesis that it associates with catenated centromeric DNA, where it may act as a tension sensor to monitor the bipolar attachment of sister kinetochores and thus ensuring accurate chromosome segregation. Indeed, we could recently demonstrate that these threads are in fact mainly constituted of stretched alphoid centromeric DNA and that topoisomerase activity is required during anaphase for the resolution of PICH-positive threads. Additionally, knock-down as well as over-expression of the PICH protein result in severe chromosomal mis-segregation. These data indicate that the complete separation of sister chromatids occurs later than previously assumed as well as that PICH and the alphoid centromeric DNA repeats are part of an additional mechanism to safeguard the genomic integrity.

IL.5 12.00 – 12.45

GENE ASSOCIATIONS: A REAL ATTRACTION OR A CHANCE MEETING?

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As erythroid precursors differentiate into red blood cells, the alpha and beta globin genes switch on and transcribe rapidly over the course of a few days in order to fill the developing erythroblasts with haemoglobin. We observed that the active globin genes were often in close association during this period. Could such physical organisation be a functional requirement for the proper regulation of gene expression? We broadened our study and found that other genes upregulated during erythropoiesis could also be found in association with each other, but that the frequency was variable. We demonstrated that the degree of associations between active genes was dependent on the surrounding chromatin environment of each gene, where genes embedded in regions of decondensed chromatin with high coding density were more likely to be found in association with other active genes. By replacing an endogenous mouse alpha globin gene (which associates to a much lesser degree) and cis-regulatory elements with its human counterpart, we found that the frequency of association reduced substantially to the level of the normal mouse and yet there was no effect on the level of transcription or the proper developmental program of expression. We also demonstrated that genes in close association are not commonly occupying the same transcription factories. We conclude that the associations we observe in a percentage of active genes are not functionally relevant to transcription. We did find however that associating genes were located at the same nuclear speckles. We propose that active genes on decondensed stretches of chromatin can be brought together in a probabilistic manner by the nucleation of splicing-associated proteins into nuclear speckles.

References:
[1] Coregulated human globin genes are frequently in spatial proximity when active. Brown JM, Leach J, Reittie JE, Atzberger A, Leeprudhoe J, Wood WG, Higgs DR, Iborra FJ, Buckle VJ. J Cell Biol. 2006 Jan 16;172(2):177-87.
[2] Association between active genes occurs at nuclear speckles and is modulated by chromatin environment. Brown JM, Green J, Pires das Neves R, Wallace HA, Smith AJ, Hughes J, Gray N, Taylor S, Wood WG, Higgs DR, Iborra FJ, Buckle VJ. J Cell Biol. 2008 Sep 22;182(6):1083-97.
[3] Gene associations: true romance or chance meeting in a nuclear neighborhood? Lawrence JB, Clemson CM. J Cell Biol. 2008 Sep 22;182(6):1035-8.
CHROMATIN ASSOCIATED DISEASES
Chair: Andy Belmont

IL.6 13.45 – 14.30

CHROMATIN DYNAMICS AND TRANSCRIPTION CONTROL DURING CELL DIFFERENTIATION

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Maintenance of gene transcription status plays a critical role in development and disease. The evolutionary conserved Polycomb- (PcG) and trithorax group (trxG) proteins respectively maintain the repressed or activated status of many developmental and disease-associated genes. These opposing regulators function by changing the structure of chromatin through ATP-dependent chromatin remodelling and covalent post-translational histone modifications. We will discuss a number of novel biochemical mechanisms through which dynamic changes in chromatin status mediates developmental gene control. Firstly, we will present results suggesting a PcG-dependent higher-order chromatin structure controlling coordinate and gene-selective expression of the INK4/ARF tumor suppressor locus during aging, cell differentiation and cellular senescence. Secondly, we will present recent results concerning the antagonistic functions of histone H2A and H2B ubiquitylation. Whereas histone H2A ubiquitylation is associated with gene silencing, histone H2B ubiquitylation correlates with transcription activation. To gain more insight in the enzymatic network underpinning PcG silencing, we characterized dRING and its associated factors. Although it is a bona-fide PRC1 subunit, we found that a significant fraction of dRING is part of a separate assemblage, we named dRAF. We identified dKDM2 as a key dRAF subunit that plays a pivotal role in a trans-histone pathway involving the removal of an active histone H3 methyl mark and formation of the repressive H2Aub mark during PcG silencing. New results on the functioning of dRAF and related complexes will be presented. Finally, we will discuss developmental gene regulation by the GMP synthetase - Ubiquitin Specific Protease 7 (USP7-GMPS) complex, which directs histone H2Bub de-ubiquitylation. Together, the metabolic enzyme GMPS and the deubiquitylating enzyme USP7 play remarkably specific roles in developmental transcription regulation. Our results uncovered a direct link between basic cell metabolism and gene expression control. We will discuss the impact of histone ubiquitylation and de-ubiquitylation on genespecific transcription control.

References:
[1] Lagarou A. et al (2008) dKDM2 couples histone H2A ubiquitylation to histone H3 demethylation during Polycomb group silencing. Genes Dev. 22: 2799-2810.
[2] Kheradmand Kia et al. (2008) SWI/SNF mediates polycomb eviction and epigenetic reprogramming of the INK4b-ARF-INK4a locus. Mol Cell Biol. 28: 3457-64.
[3] Van der Knaap et al. (2005). GMP Synthetase Stimulates Histone H2B Deubiquitylation by the Epigenetic Silencer USP7. Mol. Cell 17: 695-707.

O3 14.30 – 14.45

IMPRINTED MAMMALIAN SNORNA CLUSTERS UNDERGO CHROMATIN DECONDENSATION DURING NEURONAL MATURATION

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Introduction: Imprinting, non-coding RNAs, and chromatin organization are three modes of epigenetic regulation that modulate gene expression and are necessary for mammalian neurodevelopment. We have found visual and experimental evidence of these three epigenetic mechanisms working in concert to transcriptionally regulate the only two mammalian loci containing neurally expressed imprinted clusters of small nucleolar RNAs (snoRNAs): SNRPN through UBE3A(15q11-13/mouse 7qC) and GTL2(14q32.2/mouse 12qF1), disruption of which cause at least five distinct human neurodevelopmental disorders with overlapping phenotypes.

Methods & Materials: DNA fluorescence in situ hybridization (FISH) was performed for a series of highly expressed and epigenetically regulated loci in mouse and human brain including a series of developmental timepoints, mouse primary neurons, patient brain samples and mice with genetic Snrpn-Ube3a alterations. RNA FISH and qRT-PCR was also used to determine RNA levels from these tissues.

Results: DNA FISH of Snrpn-Ube3a revealed an eightfold allele-specific chromatin decondensation of the paternal allele in mammalian neurons. Paternal allele
decondensatin was specific to neurons, occurred during the first two weeks of postnatal life, and correlated with nucleolar size and nucleolar snoRNA accumulation. Localizing to euchromatin and reaching a packing ratio similar to a 30 nm fiber, 43% of the paternal decondensed alleles appeared to loop back on to their imprinting center, while their maternal inherited counterparts remained compact and at the edge of heterochromatic foci. Gtl2 DNA FISH exhibited similar allele-specific characteristics. Mouse brain with genetic alterations of the maternal and paternal imprinting center of the Snrpn-Ube3a locus demonstrated the requirement for transcriptional activity for chromatin decondensation. Conclusions: These results provide an unprecedented example of endogenous chromatin decondensation in developing neurons relevant to neuronal snoRNA expression and nucleolar maturation. These results are also expected to be relevant to the understanding of the pathogenesis of multiple neurodevelopmental disorders.

IL.7  14.45 – 15.30
ROLE OF THE SUMO PATHWAY IN VIVO

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Modification by the ubiquitin-related SUMO modifier regulates a wide range of fundamental cellular processes. The elaboration of cellular and animal models deficient/attenuated for sumoylation should provide valuable tools for the elucidation of specific roles of this pathway in normal and disease-related processes. We had shown previously that inactivation of the murine Ubc9 gene that encodes the SUMO E2 enzyme leads to early embryonic lethality together with major defects in nuclear architecture and chromosome segregation indicating a major role for sumoylation in nuclear structure and function. By contrast, Ubc9+/- mice show no overt phenotype but a reproducible 10% growth deficit with respect to their wild-type littermates. These weight differences affect all tissues to similar degrees suggesting possible alteration of the somatotropic axis. To circumvent the early embryonic lethality, we have generated a series of mice in which expression of Ubc9 is reduced in a conditional and graded fashion from normal to zero by the use of various combinations of wild-type, hypomorphic and knock-out alleles (Ubc9+/- ? Ubc9+/H ? Ubc9+/- ? Ubc9+/-?H ? Ubc9+/-?H ? Ubc9+/-). The Ubc9+/-?H animals show a peri-natal lethality associated with a striking 50% harmonious size reduction, a phenotype resembling that of the Ubc9+/- mice, though much more pronounced. The phenotype of the Ubc9-insufficient MEFs have been analyzed for their ability to immortalize and transform in combination with Ras in vitro and in vivo. Preliminary experiments indicate a reduced propensity to transform for MEFs with low amounts of Ubc9. Moreover, Ubc9 haploinsufficiency significantly reduces tumour formation in a mouse model of colorectal tumours. Our findings suggest that Ubc9 expression is required for tumorigenesis and that perturbations in global sumoylation may have important impact on tumour development. Therefore targeting the SUMO pathway might represent an effective approach for cancer treatment..

15.30 – 16.00  TEA AND COFFEE

IL.8  16.00 – 16.45
GENOMIC APPROACHES TO HUMAN DEVELOPMENTAL BIOLOGY

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Congenital malformations in humans are now the most common reason for death in children under 5 years-of-age in developed countries making the etiology of these disorders an important area of scientific investigation. The identification of genes that cause specific malformations is beginning to have a significant impact on our understanding of normal developmental processes. My lab has concentrated on characterizing a particular group of mutations associated with human malformations; de novo structural chromosomal anomalies. We have several examples of de novo apparently balanced chromosomal rearrangements (ABCRs) producing a phenotypic effect via genetic mechanisms that are analogous to either mouse transgene-driven ectopic expression or tissue-specific conditional knock-outs in mouse embryos. I will describe our studies in identifying and characterizing very distant cis-regulatory mutations affecting SOX9, which are associated with a particular type of cleft palate known as Pierre Robin syndrome. The size of the regulatory domain revealed by these disruptions has relevance to the size of the "target regions" used in clinical mutation analysis. The process of elucidating the
mechanism of this mutation was considerably helped by a close liaison between human geneticists, chromatin biologists, bioinformaticians and developmental biologists.

**IL.9 16.45 – 17.30**

**THE GENETIC AND EPIGENETIC CHARACTERISTICS OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY**

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Autosomal dominant Facioscapulohumeral Muscular Dystrophy (FSHD) is the second most common myopathy in adults. FSHD is mainly characterized by progressive weakness and wasting of the facial, shoulder and upper arm muscles, often in an asymmetric fashion. Genetically, FSHD is associated with a contraction of the D4Z4 macrosatellite repeat in the chromosome 4q subtelomere in >95% of patients. In healthy individuals this polymorphic repeat can vary between 11-100 D4Z4 units, each unit being 3.3kb in size. Patients with FSHD typically carry one allele with a D4Z4 repeat of 1-10 units.

D4Z4 repeat contractions are associated with DNA hypomethylation. This hypomethylation is restricted D4Z4, which suggests that a local change in chromatin structure at D4Z4 is underlying FSHD. In support for this hypothesis we observed that in FSHD phenocopies without contractions of this repeat (FSHD2), D4Z4 is also hypomethylated. In addition, D4Z4 contraction-linked FSHD (FSHD1) and FSHD2 also have other changes in the chromatin structure in common at D4Z4 strongly suggesting that both conditions share a common epigenetic disease mechanism.

D4Z4 contraction alone is insufficient to cause FSHD as it needs to occur on a specific genetic background. At least nine different haplotypes of 4qter exist. These haplotypes differ by small sequence variations in and close to D4Z4. Contractions of D4Z4 in only one of these haplotypes (4qA161), are associated with FSHD while contractions in at least two other 4qter haplotypes are phenotypically neutral. FSHD2 patients carry at least one hypomethylated 4qA161 allele suggesting that FSHD1 and FSHD2 also share a genetic commonality. The immediate downstream consequences of D4Z4 chromatin changes are currently not well understood but there is increasing evidence for a local change in transcriptional activity that may involve the candidate genes DUX4 and FRG1, although expression studies in affected muscle are inconclusive. DUX4, which is located within D4Z4 encodes for a putative nuclear transcription factor while the function of the FRG1 gene product is not known. FRG1 also encodes a nuclear protein that is enriched in spliceosome complexes suggesting a role in RNA biogenesis. It is expected that functional studies of both candidate genes should elucidate their role in FSHD.