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P1
NUCLEAR DYNAMICS AND EPIGENETIC MARKS OF TRANSCRIPTION IN MANTLE CELL LYMPHOMA

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Mantle cell lymphoma (MCL) is a malignant proliferation of B cells in the mantle zone of lymphoid follicles. Cytogenetic analyses have revealed that MCL is closely associated with the t(11;14)(q13;q32). This translocation juxtaposes Ig heavy chain gene (IGH, 14q32) sequences with the BCL-1 locus, leading to an overexpression of a number of genes including the cyclin D1gene (CCND1). However, CCND1 overexpression does not seem sufficient for hematopoietic transformation since mice transgenic for cyclin D1 do not develop lymphoma. Recent transcriptome studies have revealed that several genes located in the vicinity of the breakpoint on chromosome 11 are overexpressed in MCL cells. This general transcription upregulation might be due to epigenetic processes. Chromosome 11 is located in a largely heterochromatic and pripheral region of the nucleus, while chromosome 14 is found in a more central region. We studied the nuclear dynamics of these regions using 3D-immuno-FISH on control and MCL B-lymphocytes. We propose that the t(11 ; 14)(q13 ; q32) translocation induces the transposition of the 11q13 locus from an heterochromatic to an euchromatic region of the nucleus. This movement could then cause the overexpression of the genes located on 11q13. We are currently studying the biochemical mechanisms that underlie these genes overexpressions.

P2
NUCLEAR 3D CONFIGURATION OF CANDIDATE CDH-GENES

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Congenital Diaphragmatic Hernia (CDH) is a severe birth defect with an incidence worldwide of 1 in 3000 births and a mortality-rate up to 40%. Its multi-factorial aetiology is still poorly understood. An important role for the retinoïc-acid pathway is suggested by the identification of human chromosomal hot spots that harbour crucial vitamin-A pathway genes and by the existence of various KO-and teratogenic-animal models. However, sequence analysis of these candidate Vitamin-A pathway genes in human CDH cases has yielded a disappointing number of mutations so far. Therefore, an alternative patho-epigenetic mechanism is hypothesized in which the coordinated expression of Vitamin-A pathway genes during diaphragm development and/or upon RA induction is altered. In this proposed model, the nuclear spatial organization of Vitamin-A pathway genes is different (or changes differently upon RA induction) in CDH cases as compared to normal controls. To test this model we performed multicolour 3D-FISH experiments in cell-lines of CDH patients with an abnormal karyotype to explore the impact of structural chromosomal changes (e.g. translocations) on the nuclear organisation of these gene-loci. In control and del15q26 cell lines from CDH patients we have investigated the nuclear 3D configuration of candidate CDH-genes in relation to each other, their chromosome territory or the lamina and in future studies we will explore the effect of RA induction.

P3
H3K27ME3 FORMS BLOCS OVER SILENT GENES AND INTERGENIC REGIONS AND SPECIFIES A HISTONE BANDING PATTERN ON A MOUSE AUTOSOMAL CHROMOSOME

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Introduction: In mammals broad domains of repressive histone modifications are present on pericentromeric and telomeric repeats and on the inactive X chromosome. Only a few autosomal loci (Hox gene clusters) have been shown to lie in broad domains of repressive histone

modifications.
Methods & Materials: MEF cells, ChIP-chip, ChIp-Seq, RNA-chip, Bioinformatic analyses.
Results: Here we present a ChIP-Chip analysis of the repressive H3K27me3 histone modification along Chr.17 in mouse embryonic fibroblast cells using an algorithm named BLOCs (Broad Local enrichments), which allows the identification of broad regions of histone modifications. We also performed a low-resolution analysis of whole mouse Chr.17, which revealed that H3K27me3 is enriched in megabasepair sized domains that are also enriched for genes, SINEs and active histone modifications. These genic H3K27me3 domains alternate with similar sized gene-poor domains that are deficient in active histone modifications as well as H3K27me3, but are enriched for LINE and LTR transposons and H3K9me3 and H4K20me3.
Conclusions: Our results, confirmed by BLOC analysis of a whole genome ChIP-Seq data set, show that the majority of H3K27me3 modifications form BLOCs rather than focal peaks. H3K27me3 BLOCs modify silent genes of all types plus flanking intergenic regions, and their distribution indicates a negative correlation between H3K27me3 and transcription. Additionally, a mouse autosome can be seen to contain alternating chromatin bands that predominantly separate genes from one retrotransposon class, which could offer unique domains for the specific regulation of genes or the silencing of autonomous retrotransposons.
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P4
REGULATED INDEPENDENT EXPRESSION OF 3' UNTRANSLATED REGIONS IN MAMMALS

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3' untranslated regions (3\'UTRs) regulate eukaryotic mRNA localization, translation and stability. Here we report that large numbers of 3'UTRs are not only linked to but are also expressed separately from their associated protein-coding sequences. This phenomenon is widespread throughout the genome, and is supported by recent genome-wide CAGE, RNA-seq and microarray analysis (1). These 3'UTR derived transcripts, which we term utRNAs, contain specific sequence motifs and structure. In addition to these genome-wide studies, in situ hybridizations demonstrate that 3'UTR-derived transcripts can be expressed as part of mRNAs or discordantly in a developmentally regulated and cell-specific manner, and in some cases nuclear localized. We also provide evidence that chromatin remodelling and transcription factor networks are involved in regulating utRNA expression (2). These observations suggest that 3'UTRs not only function in cis to regulate protein expression but also in trans as noncoding RNAs, a conclusion supported by genetic studies dating back over a decade and prompt a re-evaluation of 3'UTR biology and the complexity of genome architecture in mammals (3).
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P5
CHROMATIN CONDENSATION IN CORNELIA DE LANGE SYNDROME

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Introduction: Cornelia de Lange Syndrome (CdLS; OMIM 122470) is a multi-system genetic disorder caused by mutations in components of the cohesin complex and its loader NIPBL. The cohesin complex is known to hold replicated sister chromatids together prior to their separation at mitosis, however recent evidence suggests that it also has many roles in gene expression in animals. In humans cohesin associates with CTCF, the insulator protein, and may regulate gene expression by anchoring CTCF loops together. In CdLS, loss of these CTCF/cohesin mediated loops may result in aberrant gene expression, giving the complex phenotypes of the disorder.
Methods and Materials: I am studying the misregulation of CTCF/cohesin looping in CdLS cells compared to wildtype by FISH. This allows us to measure the physical distance between two points of known genomic

distance at different regions of the genome. This was studied at regions with rich and poor CTCF/cohesin binding, at different cell cycle stages, in 2D and in 3D. *Results:* CdLS cells have larger nuclei than wildtype cells. On top of this, chromatin regions of high CTCF/cohesin binding appear to become less condensed in CdLS compared to wildtype cells, even when normalised to the larger nuclear volume. Regions of low CTCF/cohesin binding remain equally condensed in CdLS compared to wildtype, however appear more condensed when normalised to the larger nuclear area. This effect does not alter through the cell cycle. *Conclusions:* Chromatin in CdLS is generally decompacted compared to wildtype. This decompaction is due to the loss of tightly regulated CTCF/cohesin mediated loops, which control the expression patterns of many genes.

P6

MOLECULAR CHARACTERIZATION OF THE 12Q13.3-14.1 RECURRENT BREAKPOINT REGION ON RING CHROMOSOMES 12 IN ATYPICAL LIPOMATOUS TUMORS

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Ring chromosomes represent a common cytogenetic hallmark of genomic amplification in several of bone and soft tissue tumors. Particularly, they have been observed in a subgroup of mesenchymal tumors of low grade or borderline malignancy that include atypical lipomatous tumors (ALT). Several studies demonstrated that these structures, with few exceptions, contain amplified material from the central part of the long arm of chromosome 12, mainly 12q12?15, but also material from other chromosomes. All the amplified genes, including CDK4, DYRK2, TSPAN31, MDM2 and HMGA2 can play a key role in cancer development. A previous study by our group mapped in detail such amplified sequences using tiling resolution microarray, encompassing 32.433 bacterial artificial chromosome clones (BAC). Notably, more than 60% of the analyzed soft tissue tumors showed two narrow peaks of amplification, i.e. a 0.42 Mb segment in 12q13.3-14.1 containing 17 genes (including SAS and CDK4), and a 0.75 Mb genomic region in 12q15 comprising 16 genes (including MDM2). Interestingly, the first region disclosed a shared proximal border corresponding to BAC clone RP11-571M6. As very little is known about the mechanism behind ring formation in cancer, in the present study we used BAC array CGH, fluorescent in situ hybridization (FISH), Real time quantitative polymerase chain reaction (RQ-PCR) and Vectorette PCR, in order to perform a fine mapping of this "hotspot" breakpoint region in a series of 12 ALT cases. Our study aims to disclose if a particular genomic architecture at this region could play a pivotal role in the genesis of chromosome 12 ring chromosomes in ALTs.

P7

RECONSTRUCTION AND COMPARISON OF NUCLEOTIDE SEQUENCES IN THE GENES OF EMX, OTX, MEIS AND PHOX INVOLVED IN THE EVOLUTION OF THE BRAIN AND TUMOURS ALTERATIONS (MEDULLOBASTOMAS AND NEUROBLASTOMA), IN THE SPECIES HOMO, PAN, MACACA, PONGO AND MARMOSET

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The information on the selected genes were derived from the database stored at the University of California at Santa Cruz (UCSC, http://genome.ucsc.edu), referring to the published version of the human genome (http:hgdownload.cse.ucsc.edu/goldenPath/hg18). In particular, it is utilized table containing all refGene and non-coding genes coding for proteins annotated collection of RNA NCBI reference sequences. In this way it was possible to obtain detailed information about human genes, such as chromosome, start position and end transcription start position and end of the coding, number of exons, positions of exons. The annotations of genes related to the other 4 species (Chimpanzee, Pan, Rhesus, Marmoset) were derived using the algorithm litfOver (http://genome.ucsc.edu/cgi-bin/hgLiftOver). This tool allows you to convert coordinate genomic and genomic annotations between different versions of genomes in the database of UCSC. In this way the positions of genes on the human genome, it was possible to derive the corresponding coordinates on the 4 other genomes. Note the positions, their genomic sequences were downloaded from the database and for each gene were aligned globally sequences of different species using the algorithm ClustalW

(http://www.ebi.ac.uk/Tools/clustalw2). An analysis of the alignments of each gene has produced the positions of nucleotide sequence variation containing between 5 species. The coordinates of the beginning and end of exons in the human genome has been mapped alignment so as to deepen the analysis. The presence of elements stored is verified using the information contained in table phastConsElements28way database UCSC. This table contains the predictions of the items stored in the program phastCons products based on a comprehensive alignment genomes of 28 species. The positions were reported alignment. The alignment is represented graphically by creating an HTML page showing all the information above. All operations, from research of genomic information to create were performed by mathematical statistical software R (http://www.r-project.org/).

P8

TRANSCRIPTION-DEPENDENT REMODELING OF HIGHER ORDER CHROMOSOME STRUCTURES

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Cellular transcription in mammalian cells is influenced by the conformation of the chromatin fibre. To investigate the relationship between transcription and higher levels of chromatin folding we analysed the chromatin structure of transcriptionally active and inactive gene loci on the female X chromosome. By measuring the compaction of the interphase chromatin fibre from active and inactive X chromosome loci we find that gene-rich regions are decondensed two-fold upon transcriptional activation whilst gene poor regions are remodelled into a more condensed state. Live cell imaging shows the inactive X chromosome periodically moves to the nucleolus but disruption of the nucleolus does not influence the location of the inactive X chromosome. In contrast transcriptional inhibition influences the location of the active X chromosome. To investigate whether these higher-order changes are driven by alterations in the conformation of the 30 nm fibre we have also analysed the 30 nm chromatin fibre structure of the two X chromosomes. Globally, gene dense regions are enriched in disrupted chromatin but the structure of transcriptionally active and inactive loci are similar suggesting that transcription is unable to disrupt 30 nm chromatin fibre structures but determines interphase chromatin structures. Our results now indicate a direct relationship between transcriptional processes and higher-order genome architecture.

P9

IDENTIFICATION OF UNDERLYING MECHANISMS IN X-CHROMOSOME DISORDERS

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Non-allelic homologous recombination (NAHR), nonhomologous end joining (NHEJ), and recently Fork Stalling and Template Switching (FoSTeS) have been implicated as the main mechanisms for the creation of genomic disorders. In order to investigate the mechanisms responsible for the creation of X-chromosome disorders we analyzed 70 cases bearing cytogenetically visible X-chromosome abnormalities using whole genome tiling path BAC arrays and custom designed targeted ultra-high resolution oligo-arrays. Using whole genome tiling path BAC CGH, we were able to accurately map the breakpoints of 35 cases bearing isochromosomes of the long arm of chromosome X at a resolution of 150kb. Fifteen of these had breakpoints at the centromere and were considered monocentric. The remaining 20 were isodicentric and had breakpoints in proximal Xp in ChrX:51500000-

58500000. This region of chromosome X is rich in segmental duplications and contains some of the largest and most homologous inverted repeats in the human genome. Based on the BAC-array CGH findings, we designed custom oligo-arrays which cover this region in ultra-high resolution (44K and 385K oligos in 7Mb region of interest) and feature enhanced coverage of segmental duplications. Screening the isodicentric cases with these ultra-high resolution arrays enabled us to identify previously undiscovered breakpoint complexity in 45% of the isodicentrics and demonstrate that they are formed by NAHR, facilitated by specific highly homologous inverted repeats. Twenty two percent of the isodicentrics were mapped within repetitive sequences and 33% have simple breakpoints that do not coincide with segmental duplications and are probably mediated by a nonhomologous recombination mechanism.

P10

A ROLE FOR DNA METHYLATION IN REGULATING MACRO NON-CODING RNAS

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Introduction: Macro non-coding RNAs (ncRNAs) are common regulators of gene expression. Several macro ncRNAs are controlled by DNA methylation of their promoters. Recently human tumors were found to express macro ncRNAs, which can cause the repression of tumor suppressor genes and therefore contribute to tumor progression. DNA methylation, especially of repetitive elements, is perturbed in tumors. Therefore it is tempting to hypothesise that many repressed ncRNAs, whose promoters are located in these methylated repetitive elements, become derepressed in tumors. To address this question normal human fibroblasts will be treated by DNA methylation inhibitors and the transcriptome will be analyzed for ncRNAs by large scale RNA-chip analysis.
Methods & Materials: DNA methyltransferase inhibitors: 5-aza-2'deoxycytidine and Zebularine Methods: DNA-blot, qRT-PCR, RNA-chip.
Results: Preliminary data shows that: 1. Known ncRNAs can be detected by RNA-chip 2. Human foreskin fibroblasts (Hs-27 cell line) show normal methylation of known methylated regions 3. Repetitive elements of human foreskin fibroblasts can readily be demethylated by Zebularine and 5-aza- 2'deoxycytidine treatment.
Conclusions: We have set up a system to analyze the involvement of DNA methylation in the repression of macro ncRNAs in the human genome. By combining 5-aza-2'deoxycytidine or Zebularine treatment and RNA-chip we might be able to identify crucial and/or diagnostic ncRNAs that might be involved in tumor development.

P11

SEX-DEPENDENT DYNAMICS OF H3K9ME2 IN HUMAN MEIOTIC PROPHASE: PRELUDE TO POST-PACHYTENE GERM CELL DEVELOPMENT?

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Introduction: Core histones (H2A, H2B, H3, H4) are subject to various modifications that participate in different nuclear processes. Dimethylation of histone H3 lysine 9 (H3K9) is essential for heterochromatin formation and transcriptional gene silencing. Moreover, pericentric location of H3K9me2 was recently shown to be important for proper chromosome segregation in yeast and this function is conserved throughout evolution. Against this background, however, sex specific differences in H3K9 methylation have been reported in mouse meiotic prophase, implying differences in dimethylated histone dynamics between spermatocytes and oocytes. We were interested in extending these studies to humans and, in this report, for the first time summarize results from analyses of prophase stage human spermatocytes and oocytes. Our results suggest that there are, indeed, sex-specific differences in H3K9 dimethylation in human meiosis.
Methods & Materials: Immunofluorescence was used to study the localization patterns of H3K9me2 in prophase meiocytes of two men with obstructive azoospermia and in four fetal ovarian samples obtained from elective terminations.
Results: In general, the distribution of H3K9me2 signals was similarly homogeneous in both males and females at the earliest stages of meiotic prophase. However, pronounced sex-specific differences were obvious by late pachytene. Most importantly, more than 50% of spermatocytes showed the expected pericentric location

of the modified histone, whereas in females a homogenous distribution was invariably maintained until at least early diplotene ($p<0.001$).
Conclusions: The pattern of H3K9me2 localization appears to be regulated differently in human male and female meiosis. Possibly, dimethylation of H3K9 in male germ cells is important for subsequent chromosome segregation at the first division; if so, loss of H3K9me2 over the prolonged period of oocyte arrest may contribute to the high rate of aneuploidy in human oocytes.

P12
PARENTAL-SPECIFIC DIFFERENCES IN CHROMATIN ARCHITECTURE AT THE IMPRINTED IGF2R LOCUS

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Introduction: The Igf2r imprinted gene cluster is located on mouse chromosome 17 and contains three maternally expressed genes: Igf2r (ubiquitously imprinted), Slc22a2 and Slc22a3 (imprinted in extra-embryonic tissues). The long ncRNA Airn is expressed only from the paternal allele and silences Igf2r, Slc22a2 and Slc22a3 in cis (DNA methylation of the Imprint Control Element (ICE) silences Airn on the maternal allele). Two hypotheses explaining Airn mediated silencing have been proposed: (1), Airn RNA associates with Igf2r, Slc22a2 and Slc22a3 promoters and recruits repressive epigenetic modifications leading to silencing, (2), transcription of Airn overlaps Igf2r directly disrupting its promoter, and indirectly disrupts expression of the non-overlapping Slc22a2 and Slc22a3 genes by interfering with an interaction between their promoters and a putative Slc enhancer element. We plan to map higher-order chromatin interactions to determine if the parental-specific interactions predicted in hypothesis 2 can be detected.
Methods & Materials: To detect chromatin interactions within the Igf2r gene cluster we are using the Chromosome Conformation Capture technique (3C). In this technique chromatin interactions are captured by formaldehyde cross-linking, the intervening DNA is cut with a restriction enzyme and the remained DNA ligated together. The chromatin interactions are then quantified using a real-time PCR assay that detects the ligated DNA fragments.
Results: Using 3C we have confirmed published interactions at the Igf2/H19 locus. As a first step we have conducted an unbiased 3C experiment looking for interactions between the Airn promoter/ICE and elements within the 500kb Igf2r imprinted gene cluster. Using parental allele-specific cell lines we have detected chromatin interactions common to both alleles, and some unique to each allele, including a paternal-specific interaction within the Airn gene.
Conclusions: The Airn promoter shows some paternal-specific 3C interactions that may be related to expression of Airn or the un-methylated status of the ICE.

P13
COPY NUMBER ABERRATIONS IN POST-CHERNOBYL CHILDHOOD PAPILLARY THYROID CARCINOMA (PTC) DETECTED BY ARRAY-CGH

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An increasing incidence in childhood thyroid cancer is a main consequence of the Chernobyl accident in the contaminated regions. Previous studies revealed a distinct genetic heterogeneity of RET/PTC rearrangements in a subgroup of PTC. Since these findings suggest oligoclonal tumour development, we studied a post-Chernobyl childhood PTC-cohort to analyse whether other genes are involved in thyroid carcinogenesis. In particular, we aimed to identify candidate genes either interacting with RET/PTC or pointing to alternative routes of tumour development. 52 childhood PTC from the Chernobyl Tissue Bank were analysed. The cohort consisted of patients both exposed and not exposed to radioiodine fallout. In order to detect copy number aberrations (CNA) we applied array-CGH using 1-Mb BAC-Arrays. Cases with similar aberration patterns were identified by hierarchical cluster analysis (HCA). FISH analysis on FFPE tissue sections was applied to validate frequent CNA. HCA revealed two distinct tumour subgroups that correlate with the presence of RET/PTC rearrangements and tumour size. However, there was no correlation with radiation exposure. Statistical analysis identified significant differences in aberration patterns between the tumour subgroups which include DNA gains on chromosomes 1p and 14q and DNA losses on chromosomes 2p, 2q, 7q, 8p, 8q, 11p, 11q, 13q and 18q. Overall, we identified 98 tumour related candidate genes that are located in

commonly or cluster-specific altered regions and are involved in various molecular pathways (e.g. MAP-kinase-, PI3K-pathway). FISH confirmed amplifications of candidate genes HRAS, WNT1, CDK5 and CTTN. Our findings on pre- and postfallout tumours do not support the hypothesis of radiation-specific CNA. We detected distinct aberration patterns in RET/PTC-positive and -negative cases indicating different mechanisms of tumour development. The identification of new candidate genes in thyroid tumourigenesis and their involvement in different pathways may serve as a starting point for further expression and functional studies.

P14

CHROMOSOMAL CHANGES CHARACTERIZE HEAD AND NECK CANCER WITH POOR PROGNOSIS

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It is well established that genetic alterations may be associated to prognosis in tumour patients. We investigated chromosomal changes that predict the clinical outcome of head and neck squamous cell carcinoma (HNSCC) and correlate to characteristic clinico-pathological parameters. We applied comparative genomic hybridization (CGH) to tissue samples from 117 HNSCC patients scheduled for radiotherapy. Genomic aberrations occurring in more than five patients were studied for impact on locoregional-progression-(LPR)-free survival. P-values were adjusted by the Hochberg-Benjamini procedure and significant aberrations and clinical variables subjected to a stepwise backwards Cox proportional model. Significant alterations were further analyzed by array CGH and Fluorescence in situ Hybridization (FISH). In multivariate survival analysis gains on 1q and 16q predict reduced LPR-free survival after radiotherapy independently from known prognostic factors. Cluster analysis separated the patients into two clusters that are characterized by significant differences for imbalances in 13 chromosomal regions. Array-CGH pinpoints 16q24.3 to be the region of interest on chromosome 16 which was further verified by FISH analysis where an increased copy number of FANCA, a member of the FA/BRCA pathway, could be identified. Quantitative Real-Time-PCR confirmed increased FANCA expression on RNA-level. To analyze radio sensitivity of FANCA-over-expressing cells, two cell lines were stably transfected with the gene and tested for cell survival after irradiation with different doses of ionizing radiation. The findings demonstrate that chromosomal gains on 1q and 16q represent prognostic markers in HNSCC and that these alterations may explain to some extent the dismal course of a subgroup of patients after radiotherapy. Preliminary results of the cell survival experiments indicate reduced radiation sensitivity in FANCA-over-expressing cells.

P15

TRANSCRIPTIONAL AND EPIGENETIC ANALYSIS OF THE MOUSE HOMOLOGUE OF D4Z4

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder that is caused by contraction of the D4Z4 array located on 4q35. Although FSHD is generally believed to be a chromatin-associated disease, the downstream molecular mechanism is unclear. Although some models propose that the deletions alter expression of genes outside the array; recent studies point towards a direct role for a potential homeobox gene, DUX4, that has a copy in each D4Z4 repeat unit. Genetic studies have demonstrated the requirement of particular D4Z4 haplotypes in order for the deletions to cause FSHD, and expression of DUX4 has been reported to be upregulated in patient muscle. We recently identified a conserved DUX homeobox array in the mouse that may be functionally equivalent to D4Z4. While transcription of this repeat has been demonstrated, it is not clear whether all or only some of the DUX copies (40-50) are functional. We are investigating sequence variation along the mouse DUX array and comparing this to the levels of variation within the human D4Z4 array. A number of studies have suggested that D4Z4 deletions result in changes in the chromatin architecture of the 4q35 region in FSHD patients. It is therefore possible that any functional variants identified will affect epigenetic mechanisms in this region. It is intriguing that both the human and mouse DUX arrays are arranged as long tandem arrays.

Therefore, we are investigating the methylation status and other epigenetic modifications on the mouse DUX repeat in order to establish whether the mouse locus is under similar epigenetic influences to D4Z4. The aim of this work is to determine whether the human and mouse DUX loci have equivalent functions, with the long-term goal of producing a mouse model for FSHD.

P16

CYTOGENETIC CHARACTERISATION OF TRANSGENIC TRK-T1 AND RET/PTC3 MOUSE CASES

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Rearrangements of genes along the MAP-kinase-pathway are frequent alterations in papillary thyroid carcinomas. To investigate whether additional gene alterations are present in these tumours we are analysing tumours from RET/PTC3 and TRK-T1 transgenic mice for chromosomal imbalances. So far, 17 TRK-T1 and 7 RET/PTC thyroid tumours derived from transgenic mice were analysed by conventional CGH in order to identify chromosomal imbalances that may have impact on the development of thyroid cancer. The most common aberrations were deletions on chromosomes 3, 4, 12 and 14 and amplifications on chromosomes 1, 11 and 15. The deletions on chromosomes 3 and 4 as well as the amplification on chromosome 1 could be verified by interphase FISH. In one RET/PTC3 and two TRK-T1 tumours a deletion on 14E5 (RET/PTC) and 14E4-E5 (TRK-T1) has been detected by conventional CGH. This deletion has been further characterized by positional cloning using 26 BAC clones that confirmed a deletion of 1.6 Mb. Within this region SLITRK5 could be identified. Additionally, in three different TRK-T1 cases a heterozygous deletion of the gene was detected. Preliminary results indicate a down-regulation of SLITRK5 also in human tissue samples. Further studies, like FISH with BAC clones and RT-PCR are in progress. In addition, array CGH on 25 TRK-T1 and 11 RET/PTC3 cases have been performed on mouse 1 Mb BAC arrays provided by the Sanger Institute in Cambridge. The most frequent aberrations on TRK-T1 cases were amplifications on chromosome 5, 7, 10 and 15 as well as deletions on chromosome 4, 5 and 11. Numerical aberrations of chromosome 16 could be detected on RET/PTC3 cases. The findings indicate that additional gene alterations are present in RET/PTC3 and TRK-T1 positive tumours. Thus, it appears that multiple genetic changes are necessary for malignant transformation of epithelial thyroid cells.

P17

MALDI-TOF IMS REVEALS A DIFFERENT PROTEIN EXPRESSION PROFILE FOR PTC WITH AND WITHOUT METASTASIS

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Introduction: For the papillary thyroid cancer (PTC) there are already known important alterations like RET/PTC rearrangements or BRAF mutations. For an identification of of novel molecular markers in PTC the tumour proteome was investigatedfor aberrrant protein expressions.
Methods & Materials: Thirty cases of adult post-Chernobyl carcinoma were investigated using MALDI-TOF IMS. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) imaging mass spectrometry (IMS) is a powerful tool for investigating the distribution of proteins and small molecules within within tissue sections by the in situ analysis of frozen samples. In one single measurement MALDI-TOF IMS can determine hundreds of unknown compounds, so that an expression profile of the tumour tissue can be aquired while maintaining the cellular and molecular integrity. Confirmatory immunochemistry using specific antibodies against for candidate proteins was performed on FFPE-tissue-microarrays of PTC.
Results: In PTC class discriminating peaks were discovered, that were only present in normal or tumour tissue, but not in both. One candidate protein that was constantly overexpressed in tumour tissues was Calcyclin/S100A6 which was confirmed by immunochemistry. Unsupervised clustering of profile data over all cases resulted in two categories of tumours, consisting either of T2N0 tumours or of T2N1 tumours. Thus, metastasizing tumours revealed a different protein expression profile which is detectable by MALDI-TOF

IMS.
Conclusions: It could be demonstrated that the protein expression profile in PTC indicates metastasis. The overexpression of Calcylin and Calmodulin could be established as a marker of PTC which is in good agreement with arrayCGH data showing frequently gained region on 1q21. Similar histological tissue sections showed also a similar expression profile. So all results are in best accordance with the findings of established methods.

P18
PROGNOSTIC SIGNIFICANCE OF GREMLIN1 (GREM1) PROMOTER CPG ISLAND HYPERMETHYLATION IN CLEAR CELL RENAL CELL CARCINOMA

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Introduction: Gremlin1 (GREM1), a bone morphogenetic protein (BMP) antagonist and putative angiogenesis-modulating tumor suppressor gene, is silenced by promoter CpG island hypermethylation in several human malignancies. GREM1 promoter CpG island methylation in clear cell renal cell carcinoma (ccRCC) and its impact on tumor characteristics and clinical outcome has not yet been evaluated. *Methods & Materials:* Three loci (GREM1-i, -ii, -iii) within the GREM1 promoter CpG island were analyzed for CpG methylation by methylation specific PCR (MSP) and/or bisulfite sequencing in four ccRCC cell lines, and in tumor samples derived from two independent (n=150 and n=185), well characterized ccRCC patients series. *Results:* were correlated with clinicopathological and angiogenesis parameters. All statistical tests were two-sided. Results Bisulfite sequencing and MSP analysis of ccRCC cell lines showed dense methylation of the GREM1 promoter CpG island, which was associated with absence of GREM1 mRNA. Tumor specific GREM1 promoter CpG island methylation prevalence in ccRCC varied between the three loci: 55%, 24%, and 20% methylation for region i, ii, and iii, respectively. GREM1-iii methylation was significantly associated with increased tumor size (cm, p=0.02), stage (p=0.013), grade (p=0.04), tumor cell proliferation (ratio, p=0.001), endothelial cell proliferation (ratio, p=0.0001), and decreased mean vessel density (per mm2, p=0.001). In the univariate analysis, GREM1-iii methylated ccRCCs had a significant worse survival as compared to unmethylated ccRCCs (hazard ratio (HR) =2.35 (95% CI: 1.29-4.28)), but not in the multivariate analysis (HR=0.88, 95% CI: 0.45-1.74). In the validation series, GREM1-iii methylation was associated with increased Fuhrman grade (p=0.03) and decreased overall survival (p=0.001) in both univariate and multivariate analyses (HR= 2.32, 95% CI: 1.52-3.53 and HR=2.27, 95% CI: 1.44-3.59, respectively).
Conclusions: The strong correlation between GREM1-iii promoter methylation and increased malignancy in two independent series of ccRCCs as well as a correlation with active angiogenesis indicates a putative role for GREM1 in ccRCC carcinogenesis and tumor angiogenesis.

P19
GENE EXPRESSION DOWNREGULATION BY HAPLOINSUFFICIENCY MECHANISM IN CHRONIC MYELOID LEUKEMIA WITH DELETIONS ON der(9)

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Introduction: Genomic deletions flanking the breakpoint on der(9)t(9;22) occur in ~15% of patients with CML, have a variable extension and involve sequences on chromosomes 9 and/or 22. These deletions are associated with a poor prognosis on IFN-a therapy, whereas controversial data are available about their influence on the response to imatinib. The molecular mechanisms responsible for this unfavourable prognosis are still unclear. We report a gene expression study performed by qRT-PCR on 30 CML patients bearing genomic microdeletions on der(9) chromosome. Such an expression study of genes mapping within the deleted regions on der(9) has never been performed. *Methods & Materials:* 334 CML patients in chronic phase were analyzed by FISH experiments. Sixty (18%) showed genomic deletions on der(9) chromosome; a detailed characterization of deletions extension was performed by using specific BAC contigs. 47 genes with known functions were found located inside the regions most frequently deleted; among all selected genes, 37 resulted to be homogeneously expressed in normal bone marrow samples and efficient primers pairs were

validated for 28 of them. Gene expression was evaluated in 30 CML cases bearing der(9) deletions and compared to a pool of samples derived from 10 CML patients without microdeletions.
Results: All the 28 analyzed genes were found downregulated with respect to CML cases without deletions. However, the difference was statistically significant only for six: PKN3, SH3GLB2, PPP2R4, ASB6, USP20, and TOR1B. These six genes are located on chromosome 9q, centromeric to the ABL gene, and are implicated in crucial cellular pathways.
Conclusions: We have showed for the first time an expression downregulation of genes located on der(9) chromosome in CML patients bearing genomic microdeletions. These findings support the haploinsufficiency hypothesis, suggesting that one allele is always not sufficient to ensure an adequate gene expression dosage. However, the biological meaning and clinical implications of this gene downregulation remain to be investigated.

P20
IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF GENOMIC DELETIONS ON 7p12 IN IKZF1 GENE AND 9p13 IN PAX5 GENE, IN A GROUP OF BCR-ABL1-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA

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The BCR-ABL1 fusion gene defines the subgroup of acute lymphoblastic leukemia (ALL) with the worst clinical prognosis. To identify, at submicroscopic level, oncogenic lesions that cooperate with BCR-ABL1 to induce ALL, we studied by Affymetrix Genome-Wide Human SNP arrays (250K NspI and SNP 6.0) and genomic PCR 106 cases of adult BCR-ABL1-positive ALL. The deletions on 7p12 in IKZF1 gene were the most frequent somatic copy number alterations in BCR-ABL1-positive ALL, which encodes the transcription factor Ikaros identified in 68/106 patients (64%). Two major deletions occurred: type A characterized by loss of exons from 4 to 7 and type B, that we cloned, removing the exons from 2 to 7. The extent of type A and B deletions correlated with the expression of dominant-negative isoforms with cytoplasmatic localization and oncogenic activity. Moreover, a variable number of nucleotides (patient-specific) were inserted at the conjunction and maintained with fidelity at the time of relapse. The IKZF1 deletion was identified also at the progression of chronic myeloid leukemia (CML) to lymphoid blast crisis, but never in myeloid blast crisis and chronic phase CML, and acute myeloid leukemia patients. Finally, we have also characterized, by means of FISH and RQ-PCR, PAX5 and CDKN2A deletions, frequently found in IKZF1 deleted Ph+ ALL patients, as they have never been investigated at sequence resolution, in order to find out common features to IKZF1 rearrangements. Category: Long Range Control of Gene Expression.

P21
TUMOR RADIO SENSITIVITY DURING RADIOTHERAPY: DOSE DISTRIBUTION

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Introduction: Ionizing radiation remains one of the most effective tools in the therapy of cancer, but the tumor resistance to radiation usually becomes a serious issue at the certain point during the therapy. The aim of our research is to determine how much sensitivity to radiation of the tumor cells is affected by dose distribution and what molecular mechanisms are involved.
Methods & Materials: To monitor the dynamics of sensitivity of experimental tumor we chose the lung cell line LLC and observed their radio sensitivity in vivo and in vitro. Cells were injected into the CBA mice to form tumors, which were irradiated according to the experimental scheme with 2Gy in different time periods. In order to find out the dynamics of radio sensitivity, we measured DNA damage by the Comet assay and Colony assay.
Results: Data we observed show that irradiation of tumor cells (LLC) with gamma radiation causing DNA damage, reduces subsequent sensitivity of cells to gamma radiation in vivo and in vitro. By comet assay we can see the amount of damaged DNA gets lower after each irradiation if there the intervals between irradiation are 24 hours or less. If intervals are prolonged time period the amount of damaged DNA is prominently higher. By a colony assay we may notice the same tendency of radio sensitivity in LLC cells.

Conclusions: Sensitivity to irradiation restores, after damaged DNA is repaired, that's why irradiation should be performed after the repair of DNA damage. We hypothesize that changes in tumor sensitivity to treatment could be associated to genomic changes, which, when determined, could be used as molecular markers to adjust the individualized radiation strategy for specific tumor type and patient. Genomic alterations causing cellular sensitivity/resistance to irradiation could be determined using developed experimental model and aCGH and expression array technique.

P22

QUANTITATIVE ANALYSIS OF CHROMATIN HIGHER-ORDER ORGANIZATION IN LIVING CELLS

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Introduction: The regulation of the higher-order chromatin structure, including the condensation of mitotic chromosomes, is a highly dynamic process essential for most genomic functions. Due to technical difficulties, not least the resolution available using light microscopy, it has been difficult to reliably resolve and quantitate the level of chromatin condensation in live cells, and few studies have therefore investigated chromatin condensation levels in vivo at the molecular scale (~10 nm), i.e. at the nucleosomal array level. *Methods & Materials:* To measure chromatin condensation levels in live mammalian cells, we developed a quantitative FRET assay based on Multiphoton Fluorescence Lifetime Imaging Microscopy (FLIM) of human cells stably co-expressing core histone H2B fused to either EGFP or mCherry fluorescent proteins, respectively.
Results: We established and characterized a double stable HeLa cell line: HeLaH2B-EGFP/mCherry-H2B co-expressing histone H2B tagged to either EGFP or mCherry proteins. Then, by measuring FRET by the FLIM approach in living interphasic HeLaH2B-EGFP/mCherry-H2B cells, we detected, quantified and spatially mapped the location of distinct FRET efficiency territories reflecting different condensed chromatin structures throughout nuclei. By analysing cells at different stages of mitosis, we showed that the maximal compaction of the entire set of chromosomes is reached in late anaphase. Interestingly, from a detailed quantification of the FRET measurements at each stage, we observed that, at the molecular level, the nucleosome environment remains similar during metaphase and anaphase. By treating cells using the acetyl transferase inhibitor trichostatin A (TSA), we identified bulk regions of interphase chromatin where FRET signals decreased, reflecting a general decondensation of chromatin. However, we found that specific chromatin domains were not affected to the same extent by changes in the histone acetylation level, with remaining regions of condensed heterochromatin structures at the nuclear membrane and surrounding nucleoli. Conversely, after depletion of ATP in cells, we measured in vivo a massive increase in global chromatin condensation by FLIM/FRET, as well as confirming this by electron and light microscopy. Furthermore, the effect of ATP depletion was not an indirect of blocking ongoing transcription because neither the large increase in FRET signal nor the appearance of clumps of condensed chromatin in the EM were evident after using transcription inhibitors. Importantly, the effect of ATP-depletion was fully reversible.
Conclusions: We have developed a FRET-based assay using FLIM technology to measure and map the higher-order organization of chromatin in living cells at the scale of nucleosome arrays.

P23

SEQUENCE EVOLUTION IN CENTROMERES: NEW INSIGHTS FROM MARMOSET (CALLITHRIX JACCHUS)

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a-satellite DNA is the major component of centromeric DNA and forms the centromere central domain of both human and all studied primate chromosomes, ranging in size up to 5Mb. It is the most studied repeated DNA family and is made up of a basic 171bp unit tandemly organized and was first described in the african green monkey as a 340bp repeated unit consisting of two 171 bp different monomers. Human alphoid DNA is organised into higher order structures and suprachromosomal subfamilies that hybridize to a distinct subset of chromosomes. Besides higher order repeats a-satellite in HSA can organize into monomeric

repeats which are likely to be emerged first during karyotype evolution. a-satellite pancentromeric localization clearly suggests its functional involvement in an active centromere. These long homogenous stretches of repetitive DNA could work as a spacer to protect the centromeric locus from transposable sequences that would disrupt centromeric activity rather than having a sequence-mediated specific role. In order to gain information about the evolutionary history of a-satellite DNA using several experimental approaches we cloned, sequenced and characterized a-satellite sequences from a New World monkey, the common marmoset. Sequencing data and PCR analysis showed very high sequence divergence between apes and NWM. Further our results prove that despite HSA and apes, marmoset a-satellite DNA lacks higher order structures or subfamily organization; besides, sequence and Southern blot analyses demonstrate that marmoset a-satellite is made up of 342bp units. We propose a model for primate satellite evolution involving genomic amplification, unequal crossover and sequence homogenization. According to this model two 171 bp units were amplified as single unit thus forming an ancient dimeric repeat unit (~342 bp) in the Platyrrini lineage so that shared by all the New World monkeys.

P24

CHROMOSOMAL ABERRATIONS ASSOCIATED WITH PROGRESSION CHARACTERISTICS OF LOW-GRADE GLIOMAS

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Introduction: Detection and delineation of copy number alterations (CNAs) allow a deeper insight into the biology of a variety of tumors. Classical examples of CNAs in solid tumors include amplification of established oncogenes, such as ERBB2 and MYC, and inactivation of tumor suppressor genes, such as PTEN and CDKN2A. Array comparative genomic hybridization (aCGH) is currently the method of choice for the detection of CNAs. For a wide variety of tumor types CNAs have been mapped genome-wide and led to better cancer classifications, prognosis and outcome prediction. However, for low-grade gliomas (LGG), which constitute about 25% of the glioma patient population, the information is sparse. LGG's can be distinguished into slow growing genuine LGGs, where the tumor shows no signs of progression during several years after diagnosis and higher graded pseudo LGGs, which have a much more aggressive behavior. Currently, we have no means to predict the progression characteristics, such that choice and timing of treatment of LGG after initial surgical biopsy or resection are a matter of continuous debate. Treatment would be considerably improved when we would be able to distinguish genuine LGGs from pseudo LGGs. We hypothesized that specific CNAs can make this distinction.
Methods & Materials: Therefore, we analyzed 25 LGG samples by means of aCGH. This samples were split in two groups; one group of 9 pseudo LGGs with median overall survival of 8.2 years, ranging from 4.5-9.6 years and one group of 16 genuine LGGs with long survival of more than >10 years including patients that are still alive.
Results: Overall frequency analysis of CNAs revealed similar loss at 1p and 19q, and gain at 9q, 14p and 17q. Interestingly, one small region of 5 Mb at 3p21.1 was present in 60% of the genuine LGGs which was not detected in pseudo LGGs, which entail a p-value of 0.012 after multiple testing correction with a FDR of 0.22.
Conclusions: In summary, aCGH profiling has proven to be an effective technology for better classification, prognosis, and prediction of outcome in cancer. Here we show that LGG specific alterations seem to be associated with survival. Larger studies are required to validate our findings, but these results are an encouraging first step towards better selection of pseudo LGG patients whom will benefit of early therapy, and genuine LGG patients whom will benefit of a 'wait-and-scan' approach.

P25

NUCLEAR ARCHITECTURE AND POSITIONING OF HIV INTEGRATION SITES IN DIFFERENT HUMAN CELL TYPES

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It has been over 25 years since the human immunodeficiency virus (HIV)-1 was identified as the causal pathogen for AIDS. Since that time researchers have been trying to develop a vaccine but until now without the final success. Meanwhile another and putative beneficial side of the virus is being investigated. In the field of gene therapy HIV and other retroviral derivates are commonly used gene vectors. Various trials

using retroviral vectors already showed promising results. One of the problems using this type of vector is the rather arbitrary position where the genetic material is integrated into the host genome. Several studies have investigated preferential integration site selection of HIV and other retroviruses on the molecular level of the linear genome. The three-dimensional nuclear organization, large-scale chromatin structure and possible dynamics of integration sites of retroviruses and retroviral gene vectors, however, have not been studied so far. We investigate cell lines with one or a small number of retroviral integration sites with identified genomic localization. We visualize the integration sites together with their immediate chromosomal neighborhood and the corresponding chromosome territory by fluorescence in situ hybridization (FISH) and record high resolution 3D image stacks with confocal microscopy. Quantitative image analysis is applied to measure 3D positions of integration sites relative to the nuclear space and colabeled structures and to compare the spatial characteristics of the integration site with the respective genomic site without integration on the homologous chromosome. We show that, depending on the cell type, there may be indeed a change of the nuclear position upon integration of the provirus.

P26
GENOMIC ABERRATIONS IN RELATION WITH UNEXPLAINED MENTAL RETARDATION IN ESTONIAN PATIENTS

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Mental retardation (MR) affects 1-3% of population. This heterogeneous disorder is caused by genetic, epigenetic and environmental factors solely or in their combination. Despite extensive investigations, the underlying reason(s) remain unknown in approximately half of the MR patients and molecular basis of the pathogenesis is still poorly understood. In order to find out causative factors in Estonian patients with idiopathic MR and to help to shed light on underlying molecular mechanisms, we have collected a cohort of 250 unexplained MR patients and unaffected members from 90 families. To screen genomic aberrations potentially related to the clinical phenotype we have applied Infinium Human370CNV SNP arrays (Illumina Inc). Acquired data has been analysed for DNA copy-number variations (CNV) and all relevant results have been confirmed using qPCR and/or FISH. Also whole-genome detection of inconsistencies of mendelian inheritance as indicative of potential uniparental disomy (UPD) events has been performed. In 14 out of 76 families (18%) analysed to date we have found aberrations with putative clinical significance, ranging in size from 0.5 to 8.3 Mb. These either affirm recently described microdeletion (like 15q13, 17q21.3) or microduplication (for example 7q11.23) syndromes or are less characterized regions with potential clinical significance. In some of these regions a number of dosage sensitive genes are already known, for which gene copy number directly influences expression level. Our further aim is to characterize how genomic alterations in our patients effect gene expression, possibly revealing additional dosage sensitive genes and also regulatory elements that can alternate expression levels. More detailed characterization of these genomic regions as well as investigation how these imbalances influence expression in relations to etiology of MR is currently our main interest.

P27
TRANSCRIPTION-DEPENDENT DIFFERENTIAL HIGHER ORDER CHROMATIN ORGANIZATION OF THE HUMAN SCL GENE LOCUS

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Today we have the linear sequence information of our genome along with genome- wide localization maps of various transcription factors and histone modifications. However, we know little of how the genome is organized within the three-dimensional space of the nucleus. Recent technological advancements in chromatin biology are now shedding light on the higher order chromatin perspective of the organization of our genes and the genome. The stem cell leukemia (SCL) gene encodes a bHLH transcription factor with a pivotal role in haematopoiesis and vasculogenesis, and its expression is controlled by multiple cis-acting regulatory elements. Here we studied the higher order chromatin relationships between various these regulatory elements and their relationships to the state of SCL gene transcription. As a pilot study, we used high resolution improved chromosome conformation capture on chip (i4C) methodology on a genomic tiling array covering 256kb of human SCL locus. We used K562 (SCL expressing

cell type) and HPB-ALL (non- expressing cells type) cell lines for this study. We found differential higher order organization of the human SCL gene locus in expressing and non- expressing cell types. Positive (enhancer) and negative (repressor) SCL regulatory elements are in close proximity to the human SCL gene promoters in a transcription-dependent manner. Furthermore, these 3-dimensional relationships fit in well with the known functional information for cis-regulatory elements of SCL. We are currently extending our analysis using Solexa sequencing, in order to understand how the SCL locus interacts in trans with other gene loci with the human genome.

P28

GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: Chromosomal abnormalities are independent prognostic indicators in patients with chronic lymphocytic leukaemia (CLL). However, their role in disease initiation, progression, transformation and response to treatment remains unclear. To answer these clinical questions, we have implemented a large genome-wide study of the genetic alterations in 200 CLL patients. *Methods & Materials:* We are using a state-of-the-art genomic profiling platform (Affymetrix SNP 6.0), which includes 1.8 million probes optimized for SNP and copy number analysis. Where available, normal DNA matched to each patient was used as a control. Copy number alterations (CNA) and LOH events were mapped to the human genome sequence using standard approaches. Confirmation was performed with FISH and PCR. Associations with clinical characteristics were ascertained with standard methodologies.
Results: We have currently processed 100 tumor DNA samples. A total of 278 CNA were identified, ranging from 0-16 per patient. Deletions were more common then gains (78% vs 21%). CNA targeting chromosomes 11q and 13q were mapped at high resolution, showing common regions of deletion (CRD) of 0.5Mb (containing 6 genes) and 0.08 Mb (containing a single gene) respectively. Additional aberrations target 2p (n=19), 6q (n=15), 12 (n=10, trisomy 12 (n=21)), 17p (n=7) and 18 (n=14). The smallest aberration detected was 1Kb. Additionally 11 copy number neutral LOH events targeted 11q, 13q, 17p and 18q. *Conclusions:* We have identified novel recurrent genomic imbalances in our patients and accurately mapped those previous characterized. We show the CLL genome is more complex than previously thought. Further confirmation and clinical correlations are currently ongoing. It is hoped that new genetic loci identified in this study will improve clinical management of patients with CLL.

P29

EVOLUTIONARY-NEW CENTROMERES PREFERENTIALLY EMERGE WITHIN GENE DESERTS

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Evolutionary-new centromeres (ENCs) result from the seeding of a centromere at an ectopic location along the chromosome during evolution. The novel centromere rapidly acquires the complex structure typical of eukaryote centromeres. This phenomenon has played an important role in shaping primate karyotypes. A recent study on the evolutionary-new centromere of macaque chromosome 4 (human 6) showed that the evolutionary-new centromere domain was deeply restructured, following the seeding, with respect to the corresponding human region assumed as ancestral. It was also demonstrated that the region was devoid of genes. We hypothesized that these two observations were not merely coincidental and that the absence of genes in the seeding area constituted a crucial condition for the evolutionary-new centromere fixation in the population. To test this hypothesis, 14 primate ENCs were analyzed in order to ascertain the presence of novel segmental duplications around the ENC suggestive of a restructuring process and to survey the gene density in the seeding regions. Reiterative FISH experiments with corresponding human BAC clones were performed in nonhuman primate metaphases in order to precisely map these ENCs. We carefully analyzed the human genome (used as reference for non-human ENCs) and the macaque genome (used as reference for the human ENCs) for annotated genes mapping within or in proximity of ENC seeding regions. No RefSeq genes were identified. In order to assess the statistical

significance of gene depletion in the regions where ENCs were seeded, we performed a gene/exon density simulation for 14 ENC regions. Our study strongly supports the hypothesis that the evolutionary fate of a repositioned centromere is largely dependent upon a low gene density of the seeding region. The absence of genes, which was found to be of high statistical significance, appeared as a unique favorable scenario permissive of evolutionary-new centromere fixation in the population.

P30

EPIGENETIC CONDITIONING ON HEMATOPOIETIC STEM CELL (HSC) CHROMATIN DOMAINS

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In recent years, the availability of the human genome sequence disclosed novel opportunities to study biological processes from a wider perspective. The concurrent advance in bioinformatics, as well as in high-throughput technologies for the analysis of gene expression allowed deepening the knowledge of genome structure and function. We recently developed a new computational approach for the analysis of gene expression data, then applied to the analysis of human normal myelopoiesis. In particular, the integration between expression data and functional characteristics of genes allowed to analyze genomic distribution of constitutively expressed /silent genes during myelopoiesis, as well as genomic distribution of genes differentially expressed during myeloid commitment. Data obtained showed the existence of a positional regulation of gene expression, characterized by the presence of co-regulated gene clusters. In particular, we identified that in hematopoietic stem cells there are specific regions of chromatin where silent genes, mainly related to development or function of other non-hematopoietic tissues, are clustered. These evidences suggest that tissue-specific gene clusters could be selectively activated or repressed, thus defining a pattern of tissue-specific silent region able to induce cell differentiation plasticity. However, the mechanisms underlying coordinated patterns of gene expression within physical clusters, as well as their biological role are still not clear. Epigenetics could regulate gene expression within chromatin domains but also more complex mechanisms concerning intra nuclear interactions between distinct portions of the genome could be implicated. Indeed there are evidences that chromosomes have non-random distribution within interphasic nuclei. The aim of the project is to investigate the mechanisms regulating transcription within clusters of co-regulated genes. In particular we will examine both the role of epigenetic mechanisms, including HDACs, Dnmts and Polycomb repressive complexes activity, and the role of intra-nucleus three-dimensional interactions between co-regulated chromatin domains, as well as their interactions with specialized nuclear domains.

P31

THE INTERPLAY BETWEEN GENOME ORGANIZATION AND NUCLEAR ARCHITECTURE OF PRIMATE EVOLUTIONARY NEO-CENTROMERES

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An Evolutionary Neo-Centromere (ENC) is a centromere that emerged in an ectopic region of a chromosome during evolution, concomitantly to the inactivation of the ancestral centromere. Since the first clear examples of this phenomenon were reported in human chromosome 9 orthologs, numerous examples have been reported from primates and non-primate mammals. We investigated if the ENC formation affects the 3D nuclear positioning of the old and/or the novel pericentromeric region, or if centromere repositioning is favoured in chromosomal regions with similar genomic properties and hence conserved nuclear localisation For several ENCs, we have established comparative three-dimensional nuclear topology maps by fluorescence in situ hybridization to morphologically preserved interphase nuclei. Specifically, we determined the spatial arrangement of BAC clones flanking the ancestral centromere and the neocentromere, with respect to the nucleus, the chromosome territory and to each other. We have observed a preferential radial nuclear localisation of active centromeres, which however differed between chromosomes. In species showing the derived state, no major nuclear repositioning of ENC pericentromeric regions have been observed compared species that preserved the ancestral state, although the presence of a centromere appeared to impose an increased geometric constrain to the involved region. The radial positioning of BAC clones within the respective chromosome territories was found largely conserved between the analysed

species pairs. On the other and we have also compared this situation with the overall 3D chromosome territory conformation changes caused by an inversion. We found that, on the contrary of the formation of a ENC, this kind of rearrangment deeply affects the 3D conformation. Collectively, our results indicate that a gene-density correlated nuclear positioning of orthologous loci is maintained despite centromere (in)activation. We conclude that ENCs are preferentially seeded in chromosome regions with similar genomic properties, which require no relocalisation to a different functional nuclear compartment.

P32

IN-VITRO FUNCTIONAL STUDIES OF THE LTA+80A LEPROSY RISK FACTOR

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Introduction: Leprosy is a disease caused by Mycobacterium leprae that affects an estimated 300,000 new cases each year. The LTA+80 A promoter allele, a strong leprosy risk factor (Alcaïs A. et al., 2007), has been shown to facilitate binding of the ABF1 transcriptional repressor in human B-cells (Knight J. et al., 2004). Direct studies on the impact of LTA+80 genotypes on LTA expression levels following exposure of human cells to M. leprae are missing. *Methods & Materials:* In vitro analysis of expression levels were determined by employing a dual luciferase assay. LTA expression levels in M. leprae stimulated and un-stimulated samples were quantitated by RT-PCR in RNA samples extracted from whole blood obtained from 70 unrelated Vietnamese subjects. Allele specific expression in LTA+80 heterozygous cells was determined by a cross-linked chromatin pull down assay. Quantification of the allelic ratios was done by sequencing, using genomic samples not cross-linked as the reference.
Results: Using a transient transfection assay, the LTA+80 A allele was shown to exert significantly reduced activity relative to the C allele ($P<0.00001$) in a Ramos B-lymphocyte cell line. These results follow the hypothesis that the LTA+80A allele has a repressing effect on expression. However, when the alleles were co-transfected with ABF1, there was no alteration of baseline expression levels, suggesting that ABF1 is not responsible for differential ability of the LTA promoter alleles to drive luciferase activity. Comparative analysis of LTA expression in M. leprae stimulated and un-stimulated human blood samples revealed that LTA expression patterns did not differ between genotype groups. Observed LTA expression differences show a variable pattern within genotype groups suggesting a complex mechanism of action. Analysis of allele-specific expression in heterozygous samples produced inconsistent results when comparing mRNA:DNA sequence peak height ratios. Furthermore, analysis of allele-specific expression in heterozygous cell lines using cross-linking experiments indicated that expression was not LTA+80 allele-specific as the allelic ratios were consistent.
Conclusions: We failed to detect a simple relationship between LTA+80 genotypes and LTA expression levels.

P33

MicroRNA EXPRESSION PROFILING AND ITS IMPLICATIONS FOR THE DIAGNOSIS AND CLASSIFICATION AND THE PREDICTION OF PATIENT OUTCOME IN COLORECTAL CANCER

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MicroRNAs (miRNAs) are small non-coding RNAs of 19-24 in length that have been shown to regulate gene expression during crucial cell processes such as apoptosis, differentiation and development. Findings over the past five years have shown a significant number of differentially expressed miRNAs in normal and tumor tissues from cancer patients. Alterations in miRNAs expression have been associated with tumor suppressor and oncogenic pathways, and metastasis and poor prognosis in human colorectal cancer (CRC). In the present study we examine the expression profile of some oncogenic miRNAs such as miR21, miR106a, miR106b, and miR372-373, and the tumor suppressor miRNAs miR34a, miR34b and miR34c in tumor specimens and adjacent colon mucosa from colorectal cancer patients with complete data on DNA mismatch repair (MMR) deficiency or sufficiency. We correlated these data with clinicopathological features, with TP53 gene mutational status, and with the immunohistochemical expression profiles of p53 protein and its effectors/ or partners such as MDM2, LATS2, and p21/WAF1 proteins. We hypothesized that; the expression profile of these

miRNAs may improve the diagnosis and classification of CRCs, and in some cases accurately predict the outcome of colorectal cancer patients.

P34
CHANGES OF CHROMOSOME ARRANGEMENTS IN CYCLING CELLS: THEODOR BOVERI'S MODEL (1909) REVISITED

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In 1909 Theodor Boveri (1862-1915) published embryos of the horse roundworm Parascaris equorum during the first two cell cycles. His observations led him to several seminal conclusions: (1) Chromosomes occupy distinct chromosome territories (CTs) in the cell nucleus. (2) CT order is stably maintained during interphase. (3) Changes of chromosome neighbourhoods occur during mitosis, in particular during prometaphase, when chromosomes become attached to the spindle and move toward the metaphase plate. (4) The rather symmetrical movements of both sets of chromatids during anaphase lead to rather symmetrical arrangements of CTs in the two daughter nuclei. As a consequence of profound changes of chromosome order during prometaphase neighbourhood arrangements of CTs can differ greatly from one cell cycle to the next. To test Boveri's claims with a state-of-the-art experimental design, we performed life-cell experiments with cultured human cells that stably express H4-photoactivatable-GFP. We activated GFP fluorescence in selected areas of interphase and early prophase nuclei, as well as in prometaphase, metaphase, anaphase and telophase by laser microirradiation (440 nm). Using time-lapse, spinning disc confocal microscopy, we traced labelled chromatin through the remaining part of mitosis and analyzed the distribution of labelled chromatin in the daughter nuclei. Our results are in clear concordance with the predictions derived from Boveri's one hundred year old studies.

P35
CHROMATIN NATURE OF HUMAN NEOCENTROMERES

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Introduction: Neocentromeres are fully functional centromeres that occur at ectopic genomic locations, contain no tandemly repeated DNA that resides at typical human centromeres, and have been shown to bind all essential centromeric proteins, such as CENP-A and CENP-C (Marshall OJ, et al., Am J Hum Genet 2008, 82:261). In this study human neocentromeres are analysed as models to investigate the structure and the molecular mechanisms that underlay the formation and the maintenance of normal centromeres. *Methods & Materials:* The chromatin of three human lymphoblastoid cell lines, each carrying a neocentromere located on chromosomes 3, 6 or 9 (HL-neo3, HL-neo6 and HL-neo9, respectively), was immunoprecipitated with antibodies against CENP-A, CENP-C and H3K4me2. The immunoprecipitated DNA was amplified and hybridized to a NimbleGene custom tiling array (average resolution of about 100 bp). DNA binding peaks were identified by using a statistical model and the enrichment of ChIP DNA was validated by Real-Time PCR. Results ChIP-on-chip analysis demonstrated that CENP-A and CENP-C co-localize in the three neocentromeres. In HL-neo3, CENP-A/CENP-C-associated chromatin is discontinuous, consisting of three domains, while in HL-neo6 and HL-neo9, it is continuous and composed of a single domain. Sequence and RepeatMasker analysis in the three neocentromeres failed to find significant deviations from the genome average, in terms of various centromere motifs or repetitive elements. In HL-neo3, H3K4me2 binds to neocentromeric domains in three different sites. In HL-neo6, the neocentromere contains the BTN3A2 gene and its level of expression is very similar to other lymphoblastoid cell lines taken as reference. *Conclusions:* This work represents the highest resolution and most detailed description to date of chromatin domains across human neocentromeres. These results provide further lines of evidence that centromere specification is determined by an epigenetic rather than a sequence-specific mechanism and that centromeric structure is largely irrelevant to gene transcription.

P36
EPIGENETIC CHANGES AND DNA REPLICATION IN ICF SYNDROME

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ICF (Immunodeficiency, Centromeric instability, Facial anomalies) syndrome is a rare recessive disease caused by mutations in the DNMT3B gene (DNA methyltransferase 3B). ICF patients suffer from a severe immunodeficiency and often die at early age of chronic respiratory and gastrointestinal infections. A constant trait is centromeric instability: the juxtacentromeric heterocromatin of chromosomes 1, 9 and 16 is markedly undercondensed, tends to break, and gets involved in chromosome rearrangements and multiradiate associations. This chromosomal instability correlates with a severe hypomethylation of the classical satellites 2 and 3, the major components of juxtacentromeric heterochromatin. We had previously observed that heterochromatic juxtacentromeric genes undergo DNA hypomethylation and escape silencing in cancer; in this study we asked whether the same process occurs in ICF syndrome. To test our hypothesis, we analysed DNA methylation by bisulfite conversion and genomic sequencing in the juxtacentromeric region of chromosome 21, and we further analysed gene expression by qPCR. We showed that heterochromatic genes are hypomethylated in ICF patients and some of them escape silencing. We are currently analysing histone modifications in the same region by native chromatin immunoprecipitation (ChIP). Factors others than DNA hypomethylation could determine genomic instability in ICF syndrome. One candidate factor is DNA replication. To test whether DNA replication is altered in ICF syndrome, we used DNA molecular combing and we found a significant increase in replication fork speed in some ICF patients compared to controls.

P37
RELEVANCE OF HETEROCHROMATIN ORGANIZATION IN GENOME SILENCING DURING DIFFERENTIATION

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Cytologically, two types of chromatin can be distinguished; hetero- and euchromatin, with the latter containing actively expressed genes. Studies in yeast, Drosophila, and in mammals provided strong evidence for a role of nuclear topology, in particular heterochromatin proximity, in transcriptional silencing. We have recently shown that large scale heterochromatic reorganization is induced during differentiation. We are now investigating whether this reorganization may play a general role in the changes of gene expression patterns occurring during differentiation. We have performed a genome-wide transcriptional profiling and identified the genes that are up or down regulated during myogenesis. The position of each gene is visualized by FISH (Fluorescent in vitro hybridization) within 3D-preserved cells. The distance of the genes to the nearest chromocenter surface is then measured and further correlated with its change of expression during differentiation. In addition, we have measured the chromocenter content within certain radii of the gene of interest. Several normalization procedures are being implemented to account for the volume, chromocenter distribution as well as random gene positioning in each individual cell nucleus. We will present our methodological approach and controls as well as discuss the outcome of our analysis.

P38
POLY(ADP-RIBOSYL)ATION OF MeCP2 REGULATES CHROMATIN ORGANIZATION

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Introduction: In mammals, methylation of cytosine residues at the dinucleotide CpG is proposed to play an important role in regulation of genome organization as well as expression and is recognized by a family of methyl-CpG binding proteins (MBDs) with MeCP2 as the founding member. Expression of MeCP2 is increased during differentiation and induces large scale chromatin reorganisation. Mutations within the MECP2 gene have been linked to Rett syndrome, a neurological disorder. Recently, several post-translational modifications of MBD proteins have been identified, one of them phosphorylation of MeCP2 that alters its DNA binding ability. Here we investigated the functional role of post-translational poly(ADP-ribosyl)ation of MeCP2 in chromatin organisation and disease.

Methods & Materials: Protein-interactions were tested performing Co-Immunoprecipitations in vivo as well as pull down assays with recombinant full lenght MeCP2 and truncations derived from insect cells. Post-translational modification of MeCP2 was checked using antibodies against PAR and via in vitro ribosylation assays with [32P]NAD+. Chromocenter clustering was assessed by imaging of cells transfected with fluorescently tagged proteins. For quantification an image analysis programme was developed providing a fully automated segmentation and quantification of chromocenters.
Results: Our results demonstrate the physical and direct interaction of MeCP2 with PARP-1 and its poly(ADP-ribosyl)ation in vivo and in vitro. MeCP2 mediated clustering of chromocenters is enhanced both by PARP inhibition as well as by deletion of the poly(ADP-ribosyl)ated regions within MeCP2.
Conclusions: We will discuss the role of poly(ADP-ribosyl)ation in MeCP2's induced chromatin higher order structure and its relevance in disease.

P39
QUANTIFYING LOCALITY OF CIS-REGULATORY MECHANISMS BASED ON ChIP-Seq DATA

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Introduction: New experimental methods, such as ChIP-on-Chip and ChIP-Seq, have enabled genome-wide mapping of epigenetic modifications and transcription factor binding sites, providing us with comprehensive maps of functional elements and regulatory regions in the human genome. However, the functional relevance of these data is difficult to assess. In particular, high-throughput mapping of functional elements usually provides little information about which genes are affected by which regulatory regions.
Methods & Materials: The majority of gene regulatory mechanisms appear to act in cis and have a relatively local scope. We have devised a bioinformatic method for systematically assessing the average locality of gene regulatory mechanisms. It relies on the assumption that most regulatory mechanisms target a subset of genes with characteristic biological functions. Based on a genome-wide map of DNA regulatory elements, such as binding sites of a transcription factor or a chromatin remodeling complex, we estimate the typical distance on the DNA strand between the regulatory elements and their target genes. A distinguishing feature of our method is that it takes chromosome structure into account, modeling the effect of insulators and epigenetic boundary elements.
Results: We derived a biologically plausible estimate of the locality of Polycomb Repressive Complex 2 binding in human. We are currently extending this work to several transcription factors. We have implemented our approach as a software toolkit (Regions2Genes, http://bioinf.mpi-inf.mpg.de/regions2genes/).
Conclusions: Many gene regulatory mechanisms - including the binding of transcription enhancers, suppressors and chromatin remodeling complexes - primarily affects genes in a local neighborhood surrounding the binding sites. Under the assumption that regulators preferentially target a subset of genes specialized in certain biological processes, we are able to quantify the typical locality of a given regulatory mechanism. Based on this knowledge, we can derive more realistic predictions of which genes will be affected by which genomic binding events.

P40
COPY NUMBER VARIATIONS OF TUMOR SUPPRESSOR GENES IN GLIOBLASTOMA MULTIFORME

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Introduction: DNA copy number variations (CNVs) are ubiquitous and significant source of inherited human genetic variation. However, the importance of CNVs to cancer susceptibility and tumor progression has not yet been discovered. Brain tumors exhibit the most dramatic prognosis in all cancer types. Glioblastoma multiforme (GBM) is the most common primary malignant neoplasm of the central nervous system in adults. Most of primary brain tumors present a broad range of biological and clinical manifestations and thus have the potential to cause problems for neuro-oncologists in diagnosing and treating the condition. We aimed to evaluate DNA copy number variations among MGMT, p53, PIKE, PTEN, Rb1 and RUNX3 genes in GBM.
Methods & Materials: DNA was isolated from explant cell cultures of brain tumors obtained during surgery (13

Female-16 Male; average age: 53.03±13.82) by using high pure PCR template preparation kit. Real-time quantitative PCR analyses of CNV of genes were performed. To quantify the DNA copy number of the genes, we constructed a calibration curve of GAPDH. *Results:* There were no significant differences between the mean of copy number of PTEN, PIKE and RUNX3 genes in GBM. On the other hand, the mean of copy number of MGMT gene was significantly lower when compared to PIKE, PTEN, Rb1, RUNX3 and p53 genes and this result was evaluated as loss in the gene region 10q26 which MGMT localized ($p<0.05$). The mean copy number of Rb1 gene was found moderately low ($p<0.05$). When compared to all other genes, the mean copy number of p53 was found significantly higher. *Conclusions:* Loss of MGMT gene region may cause dysregulation of DNA repair system. Despite the amplification of p53 tumor suppressor gene, the oncogenes localized in chromosome 17 might be amplified in brain tumors. According to these results, MGMT and p53 gene regions might play an important role in GBM.

P41

THE TMPRSS2-ERG FUSION ACTIVATES THE WNT SIGNALING PATHWAY IN PROSTATE CANCER

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Oncogenic gene fusions between a prostate-specific gene (most frequently TMPRSS2), and one of the 4 ETS transcription factors ERG (>90% of the cases), ETV1, ETV4, or ETV5, occur in approximately 50% of all prostate cancers (PCa's). Here, we experimentally explored the functions of ERG in PCa. We developed strategies for the targeted knock-in or knock-down of ERG in normal prostate epithelial or PCa cell lines, respectively. A functional ERG-GFP vector construct was transfected into the non-transformed prostate epithelial cell line RWPE1, resulting in transient over-expression of the ERG-GFP fusion protein or the GFP-only control. Second, siRNA and lentivirus-mediated shRNA strategies were used to knock-down ERG expression in the VCAP line. Ten different siRNAs targeting ERG were tested, with only one showing an effective knock-down of the ERG protein. Five different lentiviral constructs targeting ERG from the BROAD Institute's MISSiON shRNA library were acquired, and scrambled-siRNA vectors as control. Infected cells were selected with puromycin for several weeks. Only two stable cell lines with consistent ERG knock-down could be established. RNA from transiently transfected RPWE1 (ERG-GFP vs. GFP only) and VCaP cells (with siRNAs) was harvested 48 and 72h after transfection, and analyzed on Illumina bead arrays. RNA was also extracted from the 2 stable ERG knock-down lines and compared to the scrambled-control shRNA clone. Knock down of the TMPRSS2-ERG fusion protein in VCaP by siRNA and shRNA decreased cell proliferation significantly, while transient over-expression of ERG-GFP expectedly increased proliferation in RWPE1 cells. Stable ERG knock-down caused a number of morphological changes in VCaP cells. Most prominently, ERG knock-down cells showed greatly increased cell adhesion, very high expression of E-Cadherin, and active ß-Integrin 1 signalling. Our gene expression studies, using gene set enrichment (GSEA) and MetaCore analysis, revealed a number of gene ontology terms and pathways to be affected in opposing directions, depending on the activity of ERG (knock-in versus knock-down). This included, most prominently, the WNT and Notch signalling pathways, Vitamin D, Estrogen and Glucocorticoid receptor signal transduction, cell adhesion, epigenetic mechanisms, and regulation of angiogenesis. These findings were in line with our concomitant in silico analysis of gene expression patterns in > 500 clinical Normal and PCa samples, showing that the Wnt pathway is strongly activated in ERG+ PCa's versus those lacking ETS oncogenes. The fifteen top-ranking genes from both the in silico and our Illumina studies were successfully validated by quantitative real-time RT-PCR (qRT-PCR), comparing 9 ERG+ and 7 ERG- clinical PCa samples. For most of the 15 genes, expression patterns in clinical samples matched the experimental cell lines data. Last not least, an inducible TCF/LEF-responsive GFP reporter assay confirmed that TMPRSS2-ERG knock down decreases Wnt signalling in PCa cells. In summary, we can show that ERG expression in PCa is associated with the loss of cell-cell adhesion in PCa, activation of Wnt signaling, and a large number of cancer-relevant pathways.

P42

ARE THE NUCLEOID-ASSOCIATED HU PROTEINS OF STREPTOMYCES COELICOLOR REGULATED BY POST-TRANSLATIONAL MODIFICATION?

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Despite lacking a membrane-bund nucleus, Streptomyces coelicolor A3(2) is able to differentiate into several cell types through complex regulation of its large (8.7Mb) genome. Streptomyces is an economically important genus, being the source of two-thirds of antibiotics and also being able to cause disease in plants (potato scab; Streptomyces scabies) and, rarely, humans (mycetoma; Streptomyces somaliensis). Several histone-like DNA-binding proteins affect the global structure of the bacterial nucleoid: mutation of the protein HU caused excessive compaction of the genome, which corresponded with global changes in gene expression[1]. Also, the DNaseI sensitivity of several S. coelicolor genes corresponds positively with level of transcription[2]. While homologs of eukaryotic histones are found in Archaea, they lack flexible tails. An unrelated nucleoid-associated protein, Alba, is reversibly modified at the lysine contained in the motif P(X6)GK by an antagonistic HAT/HDAC pair, affecting Alba's affinity for DNA[3]. S. coelicolor possesses two class III HDACs (sirtuins) and one similar to class I/IV HDACs. Their substrates are not yet known, but we hypothesize that one may act to reversibly modify HU in S. coelicolor, perhaps at the lysine contained in the motif P(X6)GK in the lysine-region domain of SCO5556. Preliminary tBLASTn searches have not detected homologs of proteins found in eukaryotic chromatin-modifying complexes, nor of proteins containing bromodomains/chromodomains. We found that deleting the gene SCO5556 alters the bacterium's secondary metabolism and its response to sodium butyrate, a HDAC inhibitor, although this does necessarily indicate any direct interaction between SCO5556 and a HDAC. We are currently attempting to purify a myc-tagged form of the two HU genes which will be treated with chemical acetylating and methylating agents, then sequenced using MALDI-TOF to detect modifications.

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P43

REPAIR OF DNA SINGLE-STRAND BREAKS IN NEURONS: IMPLICATIONS ON HUMAN HEALTH

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Oxidative stress is an etiological factor in several spinocerebellar ataxias and is a major source of DNA single-strand breaks (SSBs), which are the commonest type of DNA damage arising in cells (tens of thousands per cell/per day). Recently, we and others have implicated defects in the repair of SSBs in spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) and ataxia oculomotor apraxia-1 (AOA1), two hereditary neurological diseases characterized by progressive spinocerebellar ataxia. SCAN1 is associated with mutations of tyrosyl DNA phosphodiesterase (TDP1), which removes stalled Topoisomerase1 from the 3'-end of DNA. Aprataxin on the other hand resolves abortive ligation intermediates by removing AMP form the 5'-terminus of DNA, mutation of which leads to AOA1. Here, the molecular defect in these diseases will be discussed with emphasis on transcription in primary neural cells.

P44

GENOMIC IMPRINTING IN MAMMALS & QUALITY TRAITS IN CATTLE

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Genomic imprinting is an epigenetic parent-of-origin effect whereby imprinted genes exhibit differential expression levels in the offspring, depending on whether the allele is of maternal or paternal origin. Genomic imprinting has been identified for ~100 mammalian genes, primarily in humans and mice. Many such imprinted genes play a significant role in the growth and development of the mammalian foetus and placenta. Many imprinted genes have been identified in humans and mice, suggesting that they could be evolutionarily conserved in other mammals but further research is needed to confirm this. We take the known mammalian imprinted genes as a starting point to determine whether the orthologous genes are also imprinted in cattle and horses and whether homologous systems of imprinting regulation are in operation between humans, mice and other mammals. Also, many imprinted genes are known to affect traits that are sexually selected, we hypothesise that selective animal breeding has operated on some imprinted genes to generate the agronomic traits (e.g. muscle, milk production etc) that are the production basis

of the most specialised cattle breeds. To determine whether agronomic traits in cattle are associated with particular haplotypes at imprinted loci, we have identified single nucleotide polymorphisms (SNPs) in the bovine genome, and are conducting SNP-based association studies to identify associations with performance data such as growth, carcass and meat quality traits. The overall objective is to identify economically relevant polymorphisms (ERPs) at imprinted loci in the bovine genome.

P45

CHROMOSOME SHATTERING IS DIRECTLY LINKED TO MITOSES WITH CONDENSATION FAILURE - AN OBSERVATION BY LIVE CELL MICROSCOPY

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Chromosome shattering has previously been described as a special form of cell death resulting from severe DNA damage. The dynamics of this process, the correlation to its immediate "counterpart" in the living cell and mechanisms that induce chromosome shattering remained speculative so far. We used V79 cells stably transfected with H2B-mRFP and induced chromosome shattering after UV-irradiation and caffeine posttreatment. By live cell observation we demonstrate that chromosome shattering directly results from abnormally shaped mitotic figures after methanol / acetic acid fixation. They consistently present a "parachute-like" bulk chromatin with extended chromatin fibers that tether centromeres at a remote, yet normally shaped spindle apparatus. These abnormal mitoses are completed within the regular time range and the cells proceed into interphase. Typically one multilobulated nucleus is formed, which shows an abnormal distribution of centromeres. Despite these enormous abnormalities these cells survive for several hours before going into apoptosis. The formation of such abnormal mitotic figures hints to a condensation failure and suggests that the impaired condensation, possibly in combination with DNA breaks, leads to chromatin fragility, which is highly susceptible for shattering during fixation. With decondensation in interphase the nuclear framework obviously gets stabilized again, as shattering from interphase cells could never be found. Our observation likely exemplifies the simultaneous contribution and close functional relationship of proteins that alter the helical DNA structure and are involved both in DNA repair and chromatin condensation. Shattering can be the result when those proteins are depleted for repair tasks and thus no more available for proper condensation during the subsequent mitosis.

P46

X CHROMOSOME IN ACTIVATION PATTERNS IN HUMAN PLACENTA

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In mammals, transcriptional silencing of all but one X chromosome present in somatic cells ensures dosage compensation for X-linked genes among females (XX) and males (XY) in a process called X chromosome inactivation (XCI). XCI can be random, where the inactive X in each cell is chosen by chance; or imprinted, where the X chromosome activity depends on its parental origin. In marsupial female embryonic cells and in rodent extra-embryonic tissues, XCI is imprinted and the paternal X is always inactivated. In contrast, in embryonic cells of eutherian mammals, either the paternal or the maternal X chromosome is randomly inactivated. In human extra-embryonic tissues, results concerning XCI pattern are still controversial, and are mostly based on the analysis of only one or two X-linked genes in different cell types. We have used 25 SNPs in expressed regions of 20 genes along the entire X chromosome to investigate the patterns of XCI in human placenta. Using direct sequencing of cDNA, allele-specific expression was analysed in 21 full-term placental fragments. Nine samples presented monoallelic expression of X-linked genes submitted to XCI, indicating a completely skewed pattern of XCI. Three placentae, however, displayed biallelic expression of every gene examined, suggesting random XCI. Another sample showed skewed XCI, where for every gene examined one allele had higher level of expression than the other. Classification of additional eight samples was not clear due to a heterogeneous expression pattern among

different genes. Our results indicate that the pattern of XCI in human placenta is random, and occurs as a mosaic of relatively large patches of cells with either maternal or paternal XCI. The variability of the data on individual samples can explain the lack of agreement among studies described in the past as a result of the small number of genes investigated

P47

BIODIVERSITY OF POINT MUTATION IN BRCA1 AND BRCA2 IN EGYPTIAN`S WOMEN PATIENTS AND THEIR RELATIVES

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An early age of breast cancer onset is clearly an indicator of BRCA1 as well as BRCA2 mutation carrier status since one of the characteristics of hereditary breast cancer is a tendency towards younger age of onset. This study was carried out for 300 patients and their relative, the mean age at diagnosis of breast cancer were 42.4 and 34.3 years respectively. For studying the biodiversity of point mutation between the patients and their relative four exones (8, 9, 11 and 13) were amplified using specific PCR, followed by SSCP analysis and DNA sequencing. Exon 11 is a large exon and comprising 60% of coding sequence that contained either hot spots or founder mutations. For BRCA2, a study has provided evidence that mutation in 3.3 kb nucleotide region of exon 11 which denote the ovarian cancer cluster region (OOCR) are associated with a higher incidence of ovarian cancer relative to breast cancer. The BRCA2 mutation in exon 9 is outside the OCCR. This explains why all the Egyptian breast cancer patients having this mutation in young age, less than forty. In addition, the identified repeated mutation in exon 13 of BRCA1 gene is a non-sense mutation (C4446T) was detected in 20% of the examined families. The last investigation showed that 13.3% of probands and half their asymptomatic relatives had mutation in exon 8. In conclusion, 60 family out of 300 Egyptian families were found to have inherited mutation in both BRCA1 and BRCA2 gene. They are double heterozygotes, as well as it is obvious that BRCA1 and BRCA2 mutations had been found to account for greater proportion of breast cancer patients among the studied families.

P48

THE ROLE OF KLF1 IN THE TRANSCRIPTIONAL CLUSTERING OF ACTIVE ERYTHROID GENES

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Recent evidence supporting the existence of intra- and inter-chromosomal interactions between genomic regions in higher eukaryotes suggests that a functional interplay exists between genome architecture and gene expression. However, the underlying mechanisms determining or driving these interactions are not fully known. We used RNA and DNA FISH (fluorescence in situ hybridisation), 3C (Chromosome Conformation Capture) and ChIP (Chromatin Immunoprecipitation) techniques to investigate the nuclear organisation of actively transcribed genes in transcription factories. Our results show that co-regulated genes preferentially cluster at specialised transcription factories. We found that the erythroid-specific transcription factor Klf1 (Krüppel-like factor 1) is required for associations between Klf1 co-regulated genes in shared factories. We show that a Klf1-ER fusion protein can induce rapid intra- and inter-chromosomal interactions upon nuclear translocation in an erythroblast cell line lacking endogenous Klf1. Importantly, these interactions are prevented by drugs affecting actin polymerisation. Our data show that actin polymerization and Klf1 are required to direct spatial re-location of Klf1-reguated genes to transcription factories, and suggests the possibility that co-regulated active genes cooperate to create specialised transcription factories that are optimised for their efficient high-level transcription.

P49

THE TRANSCRIPTION FACTOR ZBP-89 SUPPRESSES P16 EXPRESSION THROUGH A HISTONE MODIFICATION MECHANISM TO AFFECT CELL SENESCENCE IN CANCER CELLS

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The transcription factor ZBP-89 has been implicated to induce growth arrest and apoptosis. Our experimental

data show that ZBP-89 is able to restrain senescence in NCI-H460 human lung cancer cells, through epigenetically regulating p16INK4a expression. Specifically, our results indicate that knockdown of ZBP-89 by RNA interference (RNAi) stimulated cellular senescence in NCI-H460 cells, as judged by the SA-ß-gal activity assay and SAHF assay. This process was dependent on p16INK4a, as it could be reversed by RNAi-mediated p16INK4a silencing. We also show that histone deacetylases HDAC3 and HDAC4 inhibited the p16INK4a promoter activity in a dose-dependent manner. Furthermore, ChIP experiments indicated that HDAC3 is recruited to the p16INK4a promoter by ZBP-89 and that p16INK4a silencing involves histone deacetylation. Moreover, immunofluorescence and co-immunoprecipitation assays revealed that ZBP-89 and HDAC3 formed a complex. These data suggest that ZBP-89 and HDAC3, but not HDAC4, can work coordinately to restrain cell senescence by through p16INK4a silencing. Such changes in chromatin organization at a key tumor suppressor loci could play a role in tumorigenesis. Keywords: ZBP-89; cell senescence; p16; HDAC3; lung cancer cells.

P50

DETERMINING HOW PARP DYNAMICS INFLUENCES CHROMATIN STRUCTURE AND GENE REGULATION IN DROSOPHILA MELANOGASTER

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Nucleosomes provide a barrier to RNA Pol II transcription. We have recently shown that, for the D. melanogaster hsp70 gene, the loss of histones throughout the gene occurs within the first two minutes of heat shock at a rate faster than transcribing Pol II and extends to previously defined insulator elements. Interestingly, loss of histones is dependent upon the activity of Poly-ADP-Ribose Polymerase (PARP) [Petesch and Lis, Cell 2008]. We are therefore very interested in understanding the mechanism by which PARP mediates these large changes in chromatin structure. PARP can bind to histones at the dyad axis of nucleosomes, excluding histone H1 from binding the same nucleosome [Kim et. al, Cell 2004]. Consequently, understanding the localization and dynamic behavior of PARP's interaction with nucleosomes may provide insight into its influence on chromatin structure upon gene activation. We have established an experimental system uniquely suited to investigating this question: imaging living D. melanogaster salivary glands in real-time via Multiphoton Microscopy (MPM) [Yao et. al, Nature 2006]. The deep-penetration and low background of MPM provides an ideal approach for examining the recruitment and exchange of GFP-tagged PARP at individual gene loci with high temporal and spatial resolution. Preliminary results indicate that upon heat shock PARP turnover upon chromatin switches from highly dynamic to greatly stable. We hypothesize that, since PARP binds chromatin at hsp70 before and after heat shock, the change in dynamics may play a key role in mediating the changes in chromatin structure at hsp70. Examining the dynamics of PARP co-localized with RNA Pol II at a variety of loci may suggest if this is a general feature of active transcription. Additionally, by using PARP inhibitors we can test if the change in the stability of PARP's interaction with chromatin is dependent upon its catalytic activity.

P51

UNUSUAL CLINICAL PHENOTYPE IN A PATIENT WITH DELETION OF THE DOWNSTREAM REGULATORY REGION OF PAX6

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The spatiotemporal expression of a gene depends on its regulatory elements. It is now clear that these can be located distantly from their target gene. One of the best characterized distant gene regulators is the downstream regulatory region (DRR) of PAX6. The region was discovered when a subset of patients with aniridia was found to have chromosomal rearrangements with breakpoints distal from PAX6. Here we describe a patient with a deletion encompassing the DRR of PAX6 who has an unusual phenotype: the patient has Axenfeld Rieger Syndrome with a Rieger anomaly of the eye and abnormal dentition. To our knowledge, Rieger syndrome with extraocular presentation has been reported only once in a patient with complete deletion of the gene. Our data broadens the phenotypes related with PAX6 alterations. We will present clinical and molecular data from the analysis of this patient.

P52
H2B UBIQUITINATION CONTRIBUTES TO BARRIER ACTIVITY AT THE HS4 INSULATOR

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Introduction: The HS4 insulator is a boundary element within the chicken beta-globin locus that separates the beta-globin genes from the upstream condensed chromatin. This insulator possesses both barrier activity and enhancer-blocking activity, where the barrier activity can protect transgenes from position effect silencing. It has been found that HS4 is enriched with histone acetylation, H3K4 dimethylation as well as H4R3 dimethylation regardless of neighbouring gene expression. Such active histone marks are recruited by USF proteins and are required for the barrier activity. We now find that H2B ubiquitination may play a role in barrier activity.
Methods & Materials: We performed native and crosslinking ChIP to detect the presence of ubiquitinated histones, RNF20 and PAF1 at HS4. Cell lines were established to study the effect of stable RNF20 knockdown on H2B ubiquitination and other histone modifications.
Results: Our native ChIP result showed that ubiquitinated histone was present at HS4; however, its level is markedly decreased when the USF binding site was deleted. The ubiquitinated histone is suggested to be ubiquitinated H2B as RNF20 and PAF1, enzymes required for H2B ubiquitination, are shown to interact with HS4. Knockdown of RNF20 led to a global decrease of H3K4 trimethylation and a local loss of H3K4 di- and tri-methylation levels at HS4 specifically.
Conclusions: Ubiquitinated H2B is suggested to be present at HS4 and may function as a master switch of H3K4 di- and tri-methylation there. Therefore, H2B ubiquitination would be crucial for barrier activity because it may work on top of the histone modification cascades. Moreover, the importance of the USF binding site to maintain the ubiquitinated histone level suggests that the ubiquitination activity is recruited by USF proteins.
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P53
X CHROMOSOME INACTIVATION PATTERNS IN HUMAN PLACENTA

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In mammals, transcriptional silencing of all but one X chromosome present in somatic cells ensures dosage compensation for X-linked genes among females (XX) and males (XY) in a process called X chromosome inactivation (XCI). XCI can be random, where the inactive X in each cell is chosen by chance; or imprinted, where the X chromosome activity depends on its parental origin. In marsupial female embryonic cells and in rodent extra-embryonic tissues, XCI is imprinted and the paternal X is always inactivated. In contrast, in embryonic cells of eutherian mammals, either the paternal or the maternal X chromosome is randomly inactivated. In human extra-embryonic tissues, results concerning XCI pattern are still controversial, and are mostly based on the analysis of only one or two X-linked genes in different cell types. We have used 25 SNPs in expressed regions of 20 genes along the entire X chromosome to investigate the patterns of XCI in human placenta. Using direct sequencing of cDNA, allele-specific expression was analysed in 21 full-term placental fragments. Nine samples presented monoallelic expression of X-linked genes submitted to XCI, indicating a completely skewed pattern of XCI. Three placentae, however, displayed biallelic expression of every gene examined, suggesting random XCI. Another sample showed skewed XCI, where for every gene examined one allele had higher level of expression than the other. Classification of additional eight samples was not clear due to a heterogeneous expression pattern among different genes. Our results indicate that the pattern of XCI in human placenta is random, and occurs as a mosaic of relatively large patches of cells with either maternal or paternal XCI. The variability of the data on individual samples can explain the lack of agreement among studies described in the past as a result of the small number of genes investigated.

P54
EPIGENETIC REGULATION OF THE C2H2 ZINC FINGER GENE FAMILY BY THE KAP1 CO-REPRESSER COMPLEX

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Introduction: H3me3K9 is a histone methylation mark associated with transcriptional repression. The KRAB-associated protein-1 (KAP1) co-repressor complex has been implicated in the regulation of this histone mark, based on a series of in vitro and artificial recruitment experiments. In the canonical model, KAP1 is recruited to the genome via interaction with a Kruppel-associated Box domain, which is present in half of all C2H2 zinc finger (ZNF) proteins. KAP1 then acts as a scaffold protein, recruiting Mi-2? (a protein involved in histone deacetylation), SETDB1 (a histone methyltransferase), and HP1 (a protein that binds to the modified histone). Thus, the ZNF-mediated recruitment of KAP1 is thought to initiate the assembly of negative regulatory factors at promoters, to produce distinct, stable, and heritable heterochromatic regions, resulting in the repression of transcription. In this study, we test this model of KAP1 regulation in vivo.
Methods & Materials: To elucidate KAP1 binding pattern in vivo, we performed a whole genome ChIP-Chip assay using an antibody that recognizes the KAP1 co-repressor protein in the Ntera2 human cancer cell line. Further, to study recruitment mechanism, we have cloned specific binding sites into episomes for analysis by episome ChIP.
Results: Our ChIP-chip studies indicate that complexes containing KAP1 bind to over 7,000 targets in the human genome, including a significant number of C2H2 KRAB zinc finger genes. Interestingly, we found that KAP1 binding is enriched within the coding regions of the KRAB ZNFs, at highly repetitive domain structures located at the 3' end of the genes.
Conclusions: We are investigating a novel mechanism for the recruitment of the KAP1 co-repressor complex to the 3' ends of the ZNF genes that is dependent upon chromosomal structure, as opposed to a standard recruitment mechanism mediated by a consensus motif for a site-specific DNA binding factor.

P55
CONSOLIDATION OF THE CANCER EPIGENOME TO REGIONS OF UNIFORM CHROMATIN

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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.

P56
SKEWED X-INACTIVATION AND HYPOMETHYLATION OF IGF2/H19 IMPRINTING CENTER IN SPONTANEOUS ABORTIONS WITH TRISOMY 16 MOSAICISM

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Introduction: Trisomy 16 is the most frequent chromosomal abnormality among human spontaneous abortions. However, the exact developmental mechanisms compromised under this aneuploidy are not clear. We have shown the dose-dependent association of asymmetrical X-chromosome inactivation (XCI) with the level of trisomic cells in human placenta. It provides the first evidence for possible linkage of tentative X-inactivation trans-factor to chromosome 16. Literature

data indicate that CTCF-factor, which expresses from chromosome 16 and has binding sites on chromosome X, potentially can be a desired factor. It is also known that CTCF has binding sites on imprinting centers (IC) and protects maternal or paternal chromosome from inactivation. So, we suggest that embryos with trisomy 16 will have the excess of the factor and therefore hypomethylation of IC.
Methods & Materials: Retrospective interphase fluorescence in situ hybridization with centromere-specific DNA probe D16Z1 was performed on extraembryonic mesoderm (EM) and cytotrophoblast (CT) of 15 first-trimester spontaneous abortions with trisomy 16 after conventional cytogenetic analysis. X-inactivation was assessed by methyl-sensitive PCR of androgen receptor locus. IGF2/H19 IC was analyzed in extraembryonic tissues of embryos with trisomy 16 using methyl-specific PCR.
Results: FISH-analysis of native extraembryonic tissues with trisomy 16 has revealed the additional disomic cell line with frequency 2-86% in CT and 1-95% in EM. Asymmetrical XCI was observed in embryos with more than 80.9% of cells with trisomy 16 in the placental tissues. Hypomethylation of IGF2/H19 was found in most of analyzed cases and was predominantly associated with CT with high level of mosaicism (> 74%).
Conclusions: Dose-dependent association of skewed XCI with trisomy 16 provides the first evidence for possible linkage of tentative trans-factor to chromosome 16. Hypomethylation of IGF2/H19 IC in extraembryonic tissues of embryos with trisomy 16 mosaicism can be the other proof of the existance of such factor and its linkage to chromosome 16.

P57
IDENTIFICATION OF CHROMOSOMAL BREAKPOINTS IN TRANSFORMED BREAST CELL LINES AND VALIDATION OF THE RESULTING CANDIDATE GENES IN LARGE BREAST CANCER COLLECTIONS

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Chromosomal copy number alterations and chromosomal rearrangements are frequent mutations in human cancer. Unlike copy number alterations, little is known about the role and occurrence of chromosomal rearrangements in breast cancer. This may be due to the fact that analysis of chromosomal rearrangements is, in the main, limited to studies on cultured cells. In order to identify gene rearrangements in breast cancer that were generated in conjunction with chromosomal rearrangements, we studied the chromosomal breakpoints of radiation-transformed epithelial breast cell lines (B42-11 and -16) using BAC array-based mapping of breakpoints (array painting). We validated the candidate genes in large breast cancer tumour sets by FISH (n=70) and by analysis of a publicly available breast cancer expression data set (n=328, Loi et al.). FISH analysis on breast IDC (n=70) found 28% (19 out of 68) of cases positive for rearrangement of the gene Has2, 15% (10 out of 67) of Grid1 and 13% (8 out of 62) of ret. Cases positive for a rearrangement of Grid1 were significantly associated with expression of P53 (p=0.003) and Ki67 (p=0.012). Furthermore we showed that Has2 expression is down-regulated in larger tumours (tamoxifen treated) and tumours of the elderly (> 60 years). Grid1 expression was significantly higher in tumours with higher grade. The genes Arf1, Cpm and Tbx3 were up-regulated in high-grade tumours whilst Arf1 was also up-regulated in larger tumours. Our data demonstrate that gene rearrangements identified in a cell culture model are prevalent in breast cancer. Furthermore the expression of some of the genes was regulated in clinico-pathologically defined tumour groups. This supports the biological importance of gene rearrangements and their potential role as prognostic biomarkers in the treatment of breast cancer.

P58
THE INTERACTION NETWORK OF STEM CELL TRANSCRIPTION FACTOR OCT4

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Embryonic stem (ES) cells maintain their identity using transcriptional networks dominated by specific transcription factors, including Oct4 and Sox2. Oct4 and Sox2 also have strong cellular reprogramming power as their ectopic expression, together with 2 other factors, reprograms somatic cells into ES cell-like iPS cells. Physical interactions between transcription factors could play an important role in maintaining the transcriptional network but they are often weak and therefore difficult to detect, especially with the limited amount of stem cell

material available. We have developed technology for efficiently purifying tagged transcription factors and their interaction partners from stem cells and successfully applied this to ES cell transcription factor Oct4. We identified many novel putative Oct4 interactors, which we can verify by immunoprecipitation of endogenous Oct4. We thereby expand the known Oct4 interaction network several fold. We showed that one novel Oct4 interaction partner, the reprogramming factor Esrrb, acts as an Oct4 co-factor in the regulation of the Nanog gene. Technology of this kind will be important if we want to understand the transcriptional circuitry of stem cells and iPS cells, which will be essential for their manipulation for medical purposes.

P59

EPIGENETIC ANALYSES OF A FAMILIAL t(6;14) ASSOCIATED WITH AUTOIMMUNE POLYENDOCRINE SYNDROME

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Epigenetic mechanisms play a major role during the life cycle of mammalians, including man, and the disruption of the principal epigenetic pathways can lead to silencing or inappropriate expression of genes, with associated pathologies. The aim of this study is to investigate the effect of chromosomal translocations on short- and long-range epigenetic modification of gene regulatory regions, including methylation of CpG-islands and histone modifications. We identified a familial t(6;14)(p22.2;q12) translocation where the carriers had different combinations of hypothyroidism, psoriasis and pernicious anemia, iron deficiency anemia, type 2 diabetes, suggestive of Autoimmune Polyendocrine Syndrome type II (APS2). Some of the APS types are associated with a specific HLA haplotypes. We have mapped both breakpoints - the 14q breakpoint was within an intergenic region and the 6p breakpoint was located distal to the HLA locus. The data from Affymetrix gene expression analysis of skin fibroblasts from two of the translocation carriers have shown that among the genes displaying differential expression, two were in the vicinity of the chromosome 6p breakpoint, but a third was located megabases from the breakpoint. To further test the hypothesis that the translocation breakpoint(s) may be associated with long range position effects, we will perform global DNA methylation analysis, gene expression analysis (RNA-Seq) and analyses of histone modifications (ChIP-seq) of cells from these translocation carriers by high throughput sequencing (Illumina Genome Analyzer (Solexa)).

P60

CHARACTERIZATION OF A NOVEL dL(3)mbt COMPLEX AND ITS IMPACT ON GENE REGULATION

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The family of MBT-domain containing proteins in Drosophila melanogaster consists of three members, namely dScm, dSfmbt and dL(3)mbt. MBT domains have not only been shown to read the histone code via specifically binding to mono- and di-methylated lysines within histone tails, but also have been implicated in the formation of higher order chromatin structures (1). dScm as well as dSfmbt, both belonging to the group of polycomb proteins, act in transcriptional repressive complexes. Previous studies found dL(3)mbt to be a substoichiometric subunit of the Myb-MuvB/dREAM complex and in this context to be involved in the repression of a certain subset of dE2F-regulated genes (2). To test wether dL(3)mbt exists in other high molecular weight complexes, we generated dL(3)mbt specific antibodies. Initial gel filtration analysis using nuclear extracts from embryonic Drosophila cell lines revealed, that the bulk of dL(3)mbt, though detected in the high molecular weight range, is separated from RBF2, a Myb-MuvB/dREAM complex specific subunit. Previously, we demonstrated an association between dL(3)mbt and the histone deacetylase dRpd3 (3). In order to identify additional dL(3)mbt interaction partners, we established a S2 cell line, stably expressing Flag-tagged dL(3)mbt. Using anti-Flag affinity purification we identified dLint-1 (L(3)mbt interacting protein), an up to now uncharacterized protein. dLint-1 is highly conserved among several other Drosophila species and possesses a cysteine/histidine rich region in the C-terminus. dLint-1 exhibits the same elution profile in gel filtration analysis as dL(3)mbt, supporting the view that both proteins form a stable complex. Furthermore, we knocked down dL(3)mbt in Kc cells by RNA interference (RNAi) and performed expression profiling, with the objective to identify dL(3)mbt target genes. Interestingly, dL(3)mbt target genes are also co-regulated by dLint-1. Co-

immunoprecipitations support that dL(3)mbt, dLint-1 and dRpd3 reside in the same complex (dLint-complex) and the associated HDAC activity seems to contribute to the repression of dLint-1 complex target genes. Current experiments aim to characterize dLint-1 in more detail and to obtain more insight into the mechanism of gene regulation by the dLint-1 complex.

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P61

GENOMEWIDE ANALYSIS OF dMec COMPLEX BINDING SITES

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Gene accessibility regulates many cellular processes from DNA-damage response to cell proliferation. Among other mechanisms, chromatin remodeling allows the opening and the closure of DNA regions to regulate gene accessibility to the transcription machinery. In Drosophila, several chromatin remodeling complexes, such as the Nucleosome Remodeling and Deacetylation (dNuRD) complex, have been discovered. dNuRD has an ATPase activity via its subunit dMi-2. We have shown that dMi-2 can also interact with dMep-1 to form a distinct complex, dMec. dMec is more abundant than the dNuRD complex and it is present in embryos, larval tissues and adult flies. We have shown that dNuRD and dMec can bind the proneural genes from the achaete-scute locus. However, only dMec appears to be important for the repression of these genes in non-neuronal cells. An interaction between Mi-2 and Mep-1 has also been reported in Caenorhabditis elegans. It has been shown in C. elegans that Mi-2/Mep-1 are involved in development, in germline cell proliferation, in sex-determination and in the maintenance of differentiation. Little is known about the genomewide functions of Mep-1 and Mi-2. Beside the proneural genes, no other target genes have been identified to date. Our goal is to determine the target genes of the Mec complex using the DamID technique. Our preliminary results suggest that Mep-1 has 125 target genes, many of which are involved in development and alternative splicing.

P62

ChIA-PET: REVEALING A NUCLEAR INTERACTOME

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The three dimensional organisation of the genome is seen as a new paradigm in transcriptional regulation, with spatial association of genes, regulatory elements and transcription factors required for efficient transcription. However, such a role cannot be established without a complete description of nuclear architecture. We have set out to describe associations between active genes at transcription factories which contribute to the 3-Dimensional structure of a mammalian genome. By combining Chromosome Conformation Capture (3C), Chromatin Immunoprecipitation and high throughput sequencing, we have developed ChIA-PET (Chromatin Interaction Analysis - Paired End Tags): A methodology for analysing the nuclear interactions of the transcribed genome. Experimental validation (3C and FISH) confirms ChIA-PET is predictive of large and fine-scale organisation, revealing preferential chromosome pairing, multi-megabase intrachromosomal and interchromosomal interactions. These interactions describe a non-random, scale-free organisation of transcribed genomic loci. We have identified sub-population specific interactions which delineate spatial and possibly functional networks of transcribed genes, further strengthening the links between genome organisation and transcriptional state.

P63

PROMOTER HYPERMETHYLATION OF PHACTR3, A POTENTIAL NEW BIOMARKER FOR COLORECTAL CANCER DETECTION

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Introduction: Aberrant promoter hypermethylation of (tumor suppressor) genes occurs in many cancers, including colorectal cancer (CRC). When highly specific and sensitive, this epigenetic alteration could be used as a biomarker for early detection of the disease. The aim of this study was to identify new hypermethylated genes for the early detection of colorectal adenoma and carcinoma, and to test their sensitivity and specificity in tissue and stool samples.
Methods & Materials: Candidate genes were selected based on microarray expression data available from 37 colorectal adenomas and 31 colorectal carcinomas and based on computational analysis of the promoter regions. Genes were selected when their mRNA expression was downregulated in carcinomas compared to adenomas and when the promoter harbored more than four specific binding patterns. Quantitative Methylation Specific PCR (QMSP) was used to measure methylation levels in 94 carcinoma tissue samples and 77 resection ends, and in three independent sets of stool samples comprising 85 CRC patients, 40 patients with colorectal adenomas, and 95 individuals without colon pathology.
Results: PHACTR3 was selected and validated for DNA methylation. In tissue samples, we observed a sensitivity of 62% with a specificity of 100% for detecting CRC. The presence of methylated PHACTR3 detected adenomas with a sensitivity of 17%, 23% and 47%, and CRC with a sensitivity of 58%, 64% and 70%, in stool sample set one, two, and three, respectively. The specificity was correspondingly 89%, 88%, and 92%. When taking a cut-off of 100 methylated copies, a specificity of 100% could be obtained, at cost of the sensitivity.
Conclusions: PHACTR3 methylation detected adenomas and carcinomas in stool with a sensitivity of 27% and 61%, respectively, with a specificity of 100%. Therefore, PHACTR3 could be used as a novel biomarker, in the combination with others, for the early detection of CRC in a stool-based DNA methylation test.

P64
THE NUCLEOSOME BINDING PROTEIN HMGN3 REGULATES GLYT1 EXPRESSION

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HMGN (high-mobility-group nucleosome binding) proteins are small, basic proteins that bind specifically to nucleosomes. They are known to affect gene expression, DNA repair and recombination due to their ability to modulate chromatin structure. However, they bind without specificity for the underlying DNA sequence, and little is known about where they bind in the genome. We have previously shown that over-expression of HMGN3 leads to an increase in Glyt1 expression (Glycine transporter 1 gene). Glyt1 is responsible for transporting glycine at synaptic junctions and is a drug target for schizophrenia. Our aim was to investigate the mechanism by which HMGN3 promotes Glyt1 expression. Glyt1 has alternative promoter usage, which is dependent on cell type. Induction of HMGN3 via doxycycline increased transcription from the most active promoter Glyt1a, while suppressing transcription from the weaker internal promoter, Glyt1b. Using ChIP, we found that HMGN3 is bound predominantly across the transcribed region of the gene, while little is found at the Glyt1a promoter. Induction of HMGN3 expression results in a significant increase in H3K14 acetylation, sharing a similar profile across the gene to that of HMGN3. HMGN family members have been shown to increase acetylation by HATs in vitro; HMGN3 may directly stimulate H3K14ac at the Glyt1 gene. Other histone marks such as H3K4me3, H3K36me2 and H3K27me3 showed no correlation with HMGN3 binding. Our results suggest that HMGN3 may promote transcription elongation rather than initiation. By ChIP, the binding of all the forms of RNA Pol II increases across the Glyt1 gene after HMGN3 induction, with the phosphorylated form showing the most correlation with HMGN3 binding. Our data is consistent with a model whereby HMGN3 increases H3 acetylation across the transcribed region of Glyt1, stimulating the elongation by the RNA Pol II complex. An increased rate of elongation may also be responsible for suppression of the weak Glyt1b promoter.

P65
VISUALIZATION AND QUANTIFICATION OF NASCENT TRANSCRIPT SITES BY ENERGY FILTERING TRANSMISSION ELECTRON MICROSCOPY

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Non-Random and preferential co-association of transcribing genes is becoming an accepted phenomenon, however, the environment in which this occurs is still contested. Fluorescence in situ hybridization (FISH) is the most valuable method for investigating the organisation of specific nascent transcripts, yet cannot

give more than positional information. To extend the resolution and information provided by FISH beyond the limits of light microscopy, we have developed EMISH (electron microscopy in situ hybridization): a protocol for performing RNA FISH that allows for examination of nuclear architecture at ultra-structural resolution. Nascent transcripts were detected in mouse fetal (E 14.5) liver cells using the EMISH protocol. Energy filtering transmission electron microscopy (EFTEM) in combination with correlative microscopy was used to show that nascent transcripts are associated with nitrogen rich domains, containing phosphorus rich granules, in pockets of low chromatin density. Sites of transcription have a nitrogen:phosphorus (N:P) ratio of - 4.63 ± 1.76, which is unique from other nuclear structures. This allows us to make precise measurements between specific nascent transcripts that are hypothesized to co-associate and to characterize the sub-nuclear environments in which they exist.

P66

HIGH THROUGHPUT SEQUENCING TECHNOLOGY APPLIED TO A 3Mb CANDIDATE REGION WITHIN 11q13 IN THE QUEST FOR BREAST CANCER SUSCEPTIBILITY GENES

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Breast cancer (BC) is the most frequent malignant tumour among women with approximately one million new cases per year around the world. About 5% of all BC cases are considered to be due to the segregation of a germinal mutation within a family and only about 25% of these cases can be explained by mutations in known genes (mainly BRCA1 and BRCA2). We have previously performed a linkage analyses in non-BRCA1/2 familial breast cancer (Gonzalez-Neira et al. BMC Genomics) and identified a region within 11q13 as candidate to carry a breast cancer susceptibility gene (Rosa-Rosa et al. Breast Cancer Res Treat, 2009). We have delimitated a final region of 3 Mb within 11q13, which contains more than 70 genes, using the information obtained from three high-heterozygosity STR markers genotyped in three families putative linked to this region. Then, we have applied the new generation of high throughput sequencing technologies (SolexaTM) to perform the mutational screening of the exonic sequence of the 70 genes, in at least three members from each of the three families, and also in four individuals from the general population (used as controls). We are currently analysing the generated data by using different available bioinformatics programmes and developing other necessary bioinformatics tools.

P67

ANALYSIS OF THE HIGHER-ORDER CHROMATIN STRUCTURE OF THE HUMAN 4q35 LOCUS INVOLVED IN THE FACIO-SCAPULO-HUMERAL DYSTROPHY

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The subtelomeric regions are variable regions composed of mosaic repetitive motifs found throughout the genome. In the long arm of chromosome 4 two types of alleles have been described in the population, and it has been reported that FSHD co-segregates preferentially with the 4qA allele containing ?-satellite repeats. In order to test the role of these elements we have cloned a 1.4 kb fragment immediately adjacent to the D4Z4 motif of either the 4qA or the 4qB alleles in the experimental system described above and tested its impact on the expression of a reporter gene alone or in the presence of one or several copies of D4Z4. The fragment corresponding to the 4qA acts as a silencer element that reinforces the TPE by inhibiting the expression of the reporter gene when it is upstream. This silencing effect is inhibited when D4Z4 is placed between this element and the reporter gene which is in agreement with the insulator activity we described for D4Z4. However, the element adjacent to D4Z4 and corresponding to 4qB does not provoke this repression and behaves like an insulator or an enhancer and synergizes with D4Z4 when both are combined. These data strongly suggest a protector effect of this allelic type towards FSHD. We observed that the 4qA allele induces gene silencing whereas the 4qB protects gene expression from position effect variegation. We are currently investigating the cross-talks between these two different subtelomeric regions and different number of D4Z4 repeats. I already obtained some different constructs with a variable number of D4Z4 elements and either the 4qA or 4qB allele but additional clonings are needed in order to increase our collection and mimic the normal and pathological conditions. Then, constructs containing different number of D4Z4 element with either the 4qA or 4qB specific sequence will be transfected into human cells and the expression of the

reporter gene will be followed after an extended time in culture. The chromatin structure of the different constructs will be analyzed by chromatin immunoprecipitation (antibodies specific for different modification of histones, the role of the different epigenetic modification will be evaluated after treatment with Trichostatin A, Mythramycin and Dystamycin) and the respective influence of 4qA or 4qB sequences on the methylation of the D4Z4 array will be investigated (Southern blot and ChIP with antibodies to methyl-cytosine, treatment of the cells with 5-aza-2'-deoxycytidine).

P68

A NEW APPROACH TO IDENTIFY HUMAN PROTEINS THAT BIND METHYLATED DNA

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Epigenetic phenomena are key contributors to the architecture of eukaryotic genomes. These processes act on chromatin, and they are used to render the genome dynamic. Epigenetic cross-talk between DNA methylation, histone modification and non-coding RNA is involved in normal development and in tumorigenesis. Among epigenetic processes, DNA methylation is strongly correlated with transcriptional repression and is linked to cancer. DNA methylation acts via specialized proteins able to recognize methylated DNA and induces hetechromatinization. Therefore the study of these proteins is essential for our understanding of nuclear organization and its role in gene transcription. The techniques currently used to identify these proteins are biochemical and cannot be easily applied to certain interesting cell types, such as Primordial Germ Cells or fertilized embryos. To overcome this limitation, we have developed a new approach to identify methyl binding proteins. The technique is based on T7 phage display: a cDNA is expressed on the phage surface, which is then incubated with a prey, methylated DNA. The methylated DNA is immobilized on a streptavidin-coated plate via biotin interaction. Phages interacting with the prey are selected and amplified in bacteria. Several rounds of selection are performed until the enrichment of specific phages is achieved. Our preliminary experiments with controls are very promising and we believe this approach will be powerful tool to identify new methylated DNA binding protein. Then, phage display screening is engineered to study easily and quickly diverse cell types, as well PGCs as tumor cells. Our next step will be to screen a breast cancer cDNA library in order to characterize proteins that bind methylated DNA during tumorigenesis. Our long term aim is to address the role of epigenetic components, such as DNA methylation, in the interplay between nuclear organization and cancer.

P69

CHROMATIN LOOPS STEMMING FROM R-LOOPS

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Introduction: We have shown (Szekvolgyi et al., PNAS, 104/38:14964-14969, 2007) that the genomic DNA of resting and proliferating mammalian cells and yeast spheroplasts harbor ss breaks, primarily nicks, positioned at loop-size intervals that could be efficiently labelled in situ by DNA polymerase I holoenzyme, but not by Klenow fragment and terminal transferase unless after ribonucleolytic treatments. The RNA molecules involved comprise R-loops detected by an RNA/DNA hybrid specific antibody. The integrity of the genomic DNA was also studied at the breakpoint cluster region (bcr) of the Mixed Lineage Leukemia (MLL) gene that has been previously shown to be nick-prone. *Methods & Materials:* Single-strand breaks within the MLL gene were detected by biotin-dUTP in situ nick end labelling. Immunofluorescence and ChIP experiments were performed using anti-R-loop, anti-biotin and anti-topo II alpha antibodies. QPCR was carried out with primers spanning the breakpoint cluster region of the MLL gene. FISH detection of the MLL bcr was performed with a dual color, break-apart probe. *Results:* Quantitative microscopic (CLSM, LSC) analysis of nicks and R-loops over a population of cell nuclei demonstrates that the R-loops are indeed juxtaposed with the nicks. Data obtained by immuno-FISH and ChIP experiments were in line with this result: the breakpoint cluster region of the MLL gene not only colocalized with nicks detected by biotin-dUTP nick end labelling, but the bcr sequence appeared to be enriched within ChIP samples obtained with either the anti-R-loop or anti-biotin antibodies. On the other hand, topo II alpha, although associated with the MLL bcr as shown by ChIP, exhibits no genomewide colocalization with either the nicks or the R-loops.

Conclusions: The data allow envision a model with =50 kb chromatin loops emanating from their anchorage distinguished by nick-accumulation and the presence of RNA/DNA-hybrids.

P70

SIGNATURE GENES FOR BEHÇET'S DISEASE - BLOOD GENOMIC EXPRESSION PROFILE

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Background: Behçet\'s disease (BD) is a multisystemic immuno-inflammatory disorder characterized by a generalized vasculitis, particularly at the orogenital mucosa and eye. It is a complex disease with genetic and environmental risk factors implicated in its aetiology. The only established genetic risk factor for BD is the HLA-B*51 allele, which accounts for approximately 20% of its overall genetic component. To further understand BD´s genetic architecture and pathogenesis, we performed genomic expression profiling of patients and controls. Genes differentially expressed constitute preferential candidates for subsequent investigations and points towards gene networks and pathways involved in disease etiology.
Methods: We performed gene expression profiling in peripheral blood mononuclear cells (PBMCs) of 15 BD patients and 15 sex- and age-matched controls, using Affymetrix GeneChip Human Genome U133 Plus 2.0 microarrays. Results were analysed with Partek software. Significant functional groups of genes, affected networks and pathways were analysed using different approaches.
Results: We identified 541 differentially expressed genes among cases and controls (1.2 fold-change cutoff and p-value<0.05) with a significant over-representation (2.2×10-4<P-VALUE<4.4×10-4) td process.

P71

FORKHEAD PROTEINS:MASTER REGULATORS OF GENETIC MALE INFERTILITY?

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Introduction: Despite advances in assisted reproductive technologies, infertility in men is poorly understood health problem. Interesting causes of male infertility have been pinpointed. Forkhead box (FOX) proteins are required for male fertility (Blomqvist et al. 2006; Behr et al. 2007). Congenital Bilateral Absence of the Vas Deferens (CBAVD), a form of male infertility, results from Cystic Fibrosis Transmembrane conductance Regulator (CFTR) mutations (Claustres M, 2005). However, the functional significance of a few unclassified CFTR nucleotide variants remains unclear. The aims of this study were to evaluate on the one hand the functional relevance of CFTR nucleotide variants recently identified on putative FOX-binding cis-elements and the other hand the role of FOX proteins in the CFTR transcriptional regulation.
Materials and Methods: Gene reporter assays were carried out to distinguish CBAVD-causing mutations from linked neutral variants. ChIP assays, were used to evaluate whether FOX proteins bind to CFTR untranslated sequences. Transient co-transfection experiments either with FOX expression vectors or specific siRNAs were performed to evaluate the role of FOX in the CFTR transcriptional regulation.
Results: We showed that the mutated alleles identified in both promoter and intronic regions significantly decrease the CFTR transcription in a cell-type-specific manner. We demonstrated the abilities of FOXI1 and FOXA3 to both bind and repress the CFTR transcription in the wild-type context and, to a higher extent, in the mutated context.
Conclusion: Our works identify new CBAVD-causing regulatory mutations and suggest that FOXI1 and FOXA3 might be master regulators of male infertility. However, further experiments should determine whether chromatin remodelling could be responsible for FOX-mediated CFTR transcriptional repression. In addition, it will be interesting to specifically search FOXI1 and FOXA3 mutations in CBAVD patients in whom only one CFTR mutation was found so far. These FOX genes could be new candidates genes in human idiopathic infertility cases.

P72

MAPPING THE ARRANGEMENT OF NICKS MARKING LOOP-SIZE DOMAINS IN YEAST CHROMATIN

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Introduction: Agarose embedded, deproteinized S. cerevisiae and mammalian chromatin exposed to S1 nuclease digestion, or upon denaturation, yield DNA fragments of ~50-150 kbp, due to the presence of preformed single-strand (ss) discontinuities bordering these loop-size chromatin domains (Székvölgyi et al., PNAS 104/38:14964-14969, 2007).
Methods & Materials: Detection of ss breaks in agarose embedded yeast genomic DNA was by S1 nuclease digestion on non-denaturing gels or by urea/agarose gel electrophoresis of denatured samples, using either FIGE or CHEF. In order to compare the distribution of ss discontinuities in the different chromosomes, they were separated by CHEF prior to denaturing analysis in a 2nd dimension. Quantitative image analysis was performed on full-field fluorescent microscopic images, after appropriate averaging and background correction. In Southern hybridization, after S1 treatment or denaturation of the restriction enzyme digested agarose-embedded genomic DNA samples, P32-labeled ds or ss specific probes were used.
Results: We extended the earlier observations by comparing the global distribution of ss breaks in two distinct yeast species, both at the individual cell and chromosome level. Based on the number of chromatin particles yielded by the nuclei upon denaturation, the ss breaks appear to separate ~30 kb regions on either strand. In mapping experiments by indirect end labeling, discrete bands as well as stochastic nicking were detected in S. cerevisiae chromosome I, as well as in XII within the rDNA cluster. In the latter, the site-specific nicks appear to coincide with the transcription termination sites of the 5S and 25S rDNA genes.
Conclusions: These results are in line with a model of chromatin loops organized in conjunction with transcriptional regulation. Stochastic occurrence of the initial nicking event may also contribute to loop-size fragmentation if further lesions within the same loop are prevented by relaxation of the supercoils.

P73
E3-MEDIATED REGULATION OF HEDGEHOG EXPRESSION

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Morphogens represent potent intercellular signalling molecules control both animal developmental and homeostasis. While much is known about the events occurring in a morphogen-stimulated cell, very little is known about the events that govern the expression of morphogens. Recent work in Drosophila identified a unique E3 ubiquitin protein ligase, Hyperplastic Discs (Hyd), which suppresses the expression of the morphogens Hedgehog and Decapentaplegic (BMP-like). Hyd mutant animals exhibit cell autonomous morphogen overexpression and concomitant tissue hyperplasia. A number of tumour suppressor proteins and oncoproteins are either E3s themselves, or are targets for ubiquitylation. Hence ubiquitylation is heavily implicated in many aspects of tumourigenesis. Morphogens also play a role in many cancers and are important for stem cell self-renewal. Correct temporal and spatial control of their expression is therefore essential for determining development, cell fate and cancer. Similarly, Polycomb (PcG) silencer complexes are also involved in governing similar processes. Interestingly, the PRC1 complex includes a number of E3 proteins and, in combination with histone ubiquitylation, strengthens a role of ubiquitylation in controlling gene expression. Expression of hedgehog is under the control of PcG and one particular focus of this work will to examine the relationship between Hyd\'s E3 activity and histone ubiquitylation status at the hedgehog locus. Methods to be used will inclde a combination Drosophila genetics and in vivo biochemistry and proteomics.

P74
MAPPING THE REGULATORY ORGANIZATION OF MOUSE CHROMOSOMES WITH SLEEPING BEAUTY

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Many vertebrate genes are apparently controlled at the transcriptional level by large sets of cis-regulatory elements, often separated from their target by large distances and unrelated genes. These intricate situations most likely played a central role in maintaining chromosomal syntenies during evolution. Furthermore, the widespread structural variations found between individual human genomes are likely to impinge on the functional properties of these chromosomal regulatory

landscapes, contributing to phenotypic diversity and disease susceptibility. However, apart for few peculiar loci, the regulatory organization of the mammalian genome is mostly unknown. To get a better map of the "regulatory genome", we have devised a strategy to assess the regulatory potential present at multiple genomic positions. For this purpose, we took advantage of the Sleeping Beauty (SB) transposon system to generate mouse lines carrying single copy-insertions of a reporter construct. Our transposon comprises a LacZ reporter gene driven by the human beta-globin minimal promoter, which acts as a sensor for regulatory inputs. The transposition of this SBlac transgene is induced in the germline of transgenic males carrying in addition the SB transposase driven by the protamine 1 gene regulatory region. Transposition occurs at a very high efficiency, both in trans to other chromosomes and - in about half of the cases - in cis, reflecting the known "local hopping" properties of the SB system. These properties offer the possibilities to obtain insertions distributed on most chromosomes, as well with a high density on few regions of interests. We have currently recovered more than one hundred insertions, both in gene-dense and gene-poor regions. In many of these, we found LacZ expression, often overlapping with expression domains of adjacent genes. We will present our analysis of these lines, and discuss the properties of these emerging genomic regulatory maps.

P75

ESTIMATION OF SEQUENCE ERRORS AND CAPACITY OF GENOMIC ANNOTATION IN TRANSCRIPTOMIC AND DNA-PROTEIN INTERACTION ASSAYS BASED ON NEXT GENERATION SEQUENCERS

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The transcriptome or the interactome at unprecedented depth. These techniques yield short sequence reads that are then mapped on a genome sequence to predict putatively transcribed or protein-interacting regions. We argue that factors such as false locations, sequence errors, and read length impact on the mapping prediction capacity of these short reads. Here we suggest a computational approach to measure those factors and analyse their influence on both transcriptomic and epigenomic assays. This investigation provides new clues on both methodological and biological issues. First, we estimate that 4.6% of reads are affected by SNPs. Second, we show that the nucleotide error probability is low, and it significantly increases with the position in the sequence. Third, by choosing a read length above 19 bp, we practically eliminates the risk of finding irrelevant positions. However, the number of uniquely mapped reads decreases with sequences above 20 bp. Following our procedure, we obtain 0.6% of false positives among genomic locations. Therefore, even rare signatures, if they are mapped on the genome, should identify biologically relevant regions. This indicates that digital transcriptomics may help to characterise the wealth of yet undiscovered, low abundance transcripts. Next-generation sequencing technologies, able to yield millions of sequences in a single run, allow to interrogate the transcriptome or to assay protein-DNA interactions (by Chromatin Immuno-Precipitation and sequencing (also called ChIP-seq)) at a genome-wide scale. These assays yield short sequences (<40 bp), also called tags, that need to be mapped to the genome sequence. To each tag is associated the number of times the same sequence has been experimentally detected: its occurrence number. For transcriptomic assays, for instance, a tag with a high occurrence number likely is the biologically valid signature of an abundant transcript, while a tag with a low occurrence number may either result from a sequencing error or identify a rare RNA. The mapping is a compulsory step to first predict, and then annotate regions of interest on the genome. Usually, only genomic locations that are unambiguously mapped by a tag are further analysed. Those high-throughput assays are intended to predict a maximum number of genomic locations of interest. Obviously, this induces a balance between the number of mapped tags and the number of tags that map a unique genomic location, and this balance is controlled by the tag length. The sequencing technique generally dictates the tag length. Nevertheless, once a certain length is sequenced (e.g., 36 bp with a Solexa/Illumina 1G machine) it is still possible to map only sub-parts (a prefix, a suffix, a substring) of the tags to the genome, thereby artificially reducing the tag length and modifying the balance. Presently, we lack a statistical method to evaluate the influence of the tag length on the capacity of prediction for different assays and sequencing techniques, as well as the importance of sequence errors. Our contribution is threefold. Based on word statistics, we design a program that computes the theoretical probability of mapping a genomic location by chance for a given tag length. Using an efficient algorithm to map short tags on complete genome sequence, we investigate how the prediction capacity varies with tag length. Finally, we propose a method to

estimate the probability of a tag to be altered by a sequencing error. We apply it to derive a probability of having an erroneous nucleotide at a given position in the tag for the Sanger and Solexa sequencing techniques, and for both transcriptomic and ChIP-seq experiments. We investigate on real data sets how the number of uniquely predicted genomic regions varies with tag length and background distribution. This enables a technical assessment of such assays and the indirect measurement of the impact of some biological phenomena (e.g., the number of reads affected by SNPs). Our analysis delivers the first estimates of sequence error rate for transcriptomic and DNA-protein interaction assays based high-throughput sequencing.

P76
HEN1-DIRECTED LABELING OF microRNA

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MicroRNAs are a class of small RNAs (18-25 nt) that suppress the translation of target mRNA and regulate gene expression, playing an important role in a great variety of biological pathways in many species. MicroRNAs contribute to heterochromatization and chromatin compaction, repress activity of mobile elements and thereby maintain genome organization and stability. MicroRNAs even modulate methylation changes to the epigenome controlling DNA methyltransferases (for example miR-29 family directly targets both DNMT3A and -3B). In view of numerous functions the connection of microRNAs with a wide range of human diseases comes as no surprise. Differences in microRNA expression patterns are significant in cancer, diabetes, heart malfunctions, neurodegenerative diseases etc. For this reason detection techniques for high-throughput microRNA profiling need to be developed. We employ enzymatic modification reactions, catalyzed by microRNA methyltransferase HEN1 for specific microRNA labeling. HEN1 transfers methyl groups from cofactor S-adenosyl -L-methionine (AdoMet) onto the 2'OH group of 3'-terminal nucleotide of microRNA duplexes in Arabidopsis. As mammalian microRNAs are not naturally methylated, HEN1-based strategy is particularly attractive for microRNA labeling in vitro. We have used synthetic AdoMet analogs with saturated and unsaturated extended side chains for our experiments. Method of periodate treatment and subsequent ß-elimination was optimized and subjected for detection of modified microRNAs. Mass-spectrometry analysis confirmed the transfer of that extended side chains with reactive functional group to miR173*/miR173 duplex. These functional groups react with NHS-ethers and thus are fruitful for microRNA labeling.

P77
AN EXHAUSTIVE METHOD FOR CpG ISLAND ANNOTATION

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Mammalian genomes are depleted of CpG dinucleotides, with the exception of small CpG-rich regions that are called CpG islands. It has been shown that CpG islands colocalize with transcriptionally active euchromatin and overlap with three quarters of human gene promoters. Alteration of the methylation status at CpG islands sites is an epigenetic regulation mechanism and plays a major role in disease, development and cell differentiation. In spite of their widely accepted importance, the scientific community has not yet agreed on a generally accepted definition of CpG islands. Instead, several sequence based definitions with quite different thresholds are in use. We observed that the widely applied sliding window approach for CpG islands identification possesses an intrinsic bias that leads to incomplete annotations. In consequence, annotations diverge even for a fixed set of parameters when different search programs are applied. We present the CgiHunter software tool that implements a provable exhaustive annotation algorithm for CpG island annotation. While this tool alone cannot solve the ongoing debate on the exact definition of CpG islands, it guarantees to generate unbiased genome-wide annotations for a chosen set of criteria. Further, it provides a method to incorporate experimentally derived methylation data into the annotation process and therefore enables a better discrimination between epigenetically active CpG islands and inactive CpG-enriched regions.

P78
NoRC REGULATES HIGHER ORDER rDNA STRUCTURE

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The Nucleolar Remodeling Complex (NoRC) is a multifunctional chromatin dependent regulator of rRNA genes (rDNA), which plays a role in nucleosome positioning, transcriptional repression, epigenetic silencing and replication timing. Here we show the functional role of the multiple DNA binding domains in the large, regulatory subunit Tip5. Tip5 harbors 4 AT-hook domains and a newly described extended AT-hook domain. DNA binding experiments exhibit the binding properties of the individual AT-hook domains and the cooperative effects of Snf2h and Tip5 in DNA binding. The AT-hooks of Tip5 anchor the complex and the associated rDNA to the nuclear matrix in vivo. Functional studies underpin the role of the strong DNA binding activity of Tip5 in transcriptional repression within chromatin and in the spatial organization of rDNA in the nucleolus.

P79

A NUCLEOSOMAL SWITCH REGULATES THE ACTIVITY OF rRNA GENES

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Eukaryotic genomes are packaged into nucleosomes that occlude the DNA from interacting with most DNA binding proteins. Nucleosome positioning and chromatin organisation is critical for gene regulation. We have investigated the mechanism that establishes specific nucleosome positions at the promoter of active and silent rRNA genes (rDNA). Reconstitution of nucleosomes on the rRNA gene leads to transcriptional repression as a result of sequence-dependent nucleosome positioning at the rDNA promoter. The repressed structure is the default chromatin organisation of the rRNA genes. Transcription activation requires a chromatin remodelling dependent switch in chromatin organisation that repositions the promoter-bound nucleosome. Inhibition of ATP-dependent chromatin remodelling by phosphokanamycin inhibits activation of RNA polymerase I (Pol I) transcription on reconstituted chromatin. Once initiated, Pol I elongates through chromatin without displacing nucleosomes. The results reveal the functional importance of ATP-dependent nucleosome positioning in establishing the epigenetically active or silent state of rRNA genes.

P80

GENETIC PROFILING OF HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA

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Persistent infection with high-risk human papillomavirus (hrHPV) plays an essential role in cervical carcinogenesis. Presently, cervical cancer screening is performed by the Pap test. Addition of hrHPV testing leads to earlier detection of high-grade premalignant lesions and has a negative predictive value approaching 100% [Bulkmans et al., 2007]. However, this also results in detection of women with transient infections that will not develop lesions. Therefore, novel biomarkers, based on host-cell alterations driving malignant transformation, are needed to reliably identify hrHPV-positive women with increased risk of cervical cancer. To identify these alterations we focus on high-grade lesions, which may arise 2-3 years following hrHPV infection. Only a minority will progress to cervical carcinoma, a process that takes 10-30 years, suggesting that high-grade lesions represent a heterogeneous disease of which only advanced stages are likely to have invasive potential. Recent microarray comparative genomic hybridization (maCGH) analysis of 46 high-grade lesions revealed two distinct subsets; profiles of one which closely resembled cervical carcinomas [Wilting et al., 2008]. To determine how various chromosomal profiles correlate to duration of existence of lesions and progression risk, maCGH was performed on 'early' and 'advanced' high-grade lesions, a distinction made on basis of the duration of hrHPV presence. Unsupervised hierarchical clustering analysis revealed two clusters. One cluster contained 57% of the advanced and 30% of the early lesions, showing frequent (>25% of the cases) gains at chromosomes 1,3&8 and losses at 2,7,11,12,16&17. These alterations did not or, to lesser extent, occur in the other cluster, containing most of the early lesions. For a complete picture chromosomal profiles of low-grade lesions and high-grade lesions adjacent to carcinomas, the latter representing advanced disease, will also be included in the analysis. Future

biomarkers based on alterations specific for advanced high-grade lesions will aid in the triage of hrHPV-positive women.

P81
DECIPHERING THE CIS-REGULATORY ARCHITECTURE OF Tlx1-Fgf8 INTERVAL

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In higher organisms gene expression is guided by cis-regulatory elements (CREs) that can be scattered several hundred kilobases from the target gene(s) and embedded inside other functionally unrelated genes in the region. The corresponding large chromosomal domains are usually extensively conserved across different animals, and several genetic diseases are associated with the disruption of the normal structure of some of them. This strongly suggests that the architecture of a locus can modulate the activity and target specificity of the associated CRE and therefore be crucial for gene expression. Accordingly, morphological evolution which occurs largely through expression changes of functionally conserved proteins can be driven by mutations in defined CREs, but also by modifications of the higher-order organization of a locus. We are studying the cis-regulatory architecture of the TLX1-FGF8 interval, which maps to human chromosome 10q24. The gene order over the region is highly conserved in tetrapods and some smaller syntenic blocks are found within all vertebrates and beyond. Furthermore, severe limb deformations are observed when this region is structurally altered in humans or mice. In teleosts, we noticed that despite substantial changes of gene content and order, the disposition of non-genic elements was much more conserved, defining two separate blocks: Fbxw4-Fgf8 and Btrc-Lbx1. To analyze the role of these blocs, we have engineered mouse lines with overlapping chromosomal rearrangements in this interval. We have tested the enhancer activity of several of these evolutionary conserved elements in transgenic mouse embryos and found many of them active in expression domains overlapping wih Fgf8 and possibly other genes in the region. Altogether, these elements define a pattern broader than the one of the endogenous genes, underscoring the importance of other elements to restrain and direct regulatory activities of the CREs to the proper target genes and tissues.

P82
DOSAGE OF CHROMATIN REMODELLING ENZYMES IS CRITICAL FOR HUMAN HEART DEVELOPMENT

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Introduction: Recently, several well-defined human developmental disorders have been shown to result from a deregulation of chromatin remodelling, mainly through loss-of-function mutations or deletions of genes that underlie chromatin regulation. Many of these disorders are associated with congenital heart defects (CHDs). *Materials and methods:* We screened 150 patients with an idiopathic syndromic CHD by array CGH at a 1Mb or a 10kb resolution (on BAC/PAC- or Agilent 244K oligo-microarray). Detected imbalances were confirmed using an independent method (FISH and/or rtqPCR). *Results:* In 27/150 patients (18%) we detected a causal cryptic chromosome aberration. In 12/27 imbalances, a gene known to cause CHDs is affected. 5 of these imbalances affect genes that encode enzymes known to be involved in chromatin remodelling: *EHMT1* was deleted in two patients, in one patient *NSD1* was deleted. *CREBBP* was duplicated in one patient. In a final patient, we detected an intragenic *ATRX* duplication that causes an apparent loss-of-protein as demonstrated by Western Blotting.
Conclusions: A genome-wide screen for genetic causes of syndromic heart defects in 150 patients led to the detection of causal micro-imbalances in 27 patients, affecting a known CHD-causing gene in 13 patients. Interestingly, 5 of these are involved in chromatin remodelling, indicating the frequent involvement of disturbances of epigenetic remodelling in CHDs. For some factors, our findings expand the dosage spectrum that is associated with pathology: first, *CREBBP* appears dosage-sensitive not only for deletions, but also for duplications. Such a duplication was recently described in additional patients and we thus show that *CREBBP* duplications represent a novel genomic disorder, linked to a disturbed epigenetic remodelling. Second, we demonstrate a loss of the ATRX protein, which is– in contrast to reports – apparently compatible with life. These findings lead us to propose a novel, epigenetic strategy for etiological diagnosis of CHDs, which we will present.

P83

ROLE OF NUCLEAR PORE COMPONENTS IN GENE REGULATION

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No abstract avauilable

P84

DARWIN AMONG THE CELLS: CHROMATIN STRUCTURE, MUTATION AND CANCER

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Introduction: It has long been suspected that the structural and functional organisation of the genome may impose constraints on its evolutionary history and influence predisposition to disease. At the same time, the distribution of genes differentially expressed in cancer has been shown to be nonrandom.
*Methods & Materials:*Here we undertake a computational study of gene expression, mutation and higher order chromatin structure in the human genome.
*Results:*We demonstrate significant correlations between chromatin structure and gene dysregulation in many cancers. We also provide evidence for the mutations and adaptive processes responsible for this dysregulation.
Conclusions: Apparently random patterns of mutation and gene expression change in cancers closely follow the underlying chromatin landscape. These phenomena appear to be universal across many diverse cancers.

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EPIGENETIC INTERACTION BETWEEN Mecp2 AND ORGANIC POLLUTANTS IN MURINE NEURODEVELOPMENT

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Background: Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked gene MECP2 that encodes methyl CpG binding protein 2. MeCP2 is a known epigenetic modulator of gene expression required for postnatal neuronal maturation. In previous studies, Mecp2-deficient mice showed delayed neuronal maturation exhibited by alterations to the level and localization of histone H3K9 acetylation, an epigenetic marker of gene activity. Also, 80% of autism brain samples showed decreased MeCP2 expression and exhibited similar alterations to H3K9 acetylation patterns in cortical neurons. RTT is one of several neurodevelopmental disorders with a known genetic cause of epigenetic abnormalities, highlighting the importance of epigenetic mechanisms in postnatal brain development. However, neurodevelopmental disorders which have unknown genetic etiologies such as autism and mental retardation are likely to be caused by a combination of genetic and environmental factors. The recent increase in the incidence of autism cases suggests that environmental factors may play a role. The widespread use of persistant organic polybrominated diphenyl ethers (PBDEs) as commercial flame retardants over the past decade has raised concern about human exposure to this new pollutant and potential effects on the developing brain.
Objectives: This study aims to test the hypothesis that perinatal exposure to brominated diphenyl ether 47 (BDE-47) affects the development of social and cognitive behavior through epigenetic changes in neurons during development in a mouse model genetically susceptible to an autistic phenotype.
Methods: Heterozygous Mecp2308/+ dams were exposed orally via cornflake to either vehicle control (corn-oil), 0.03 mg BDE-47/kg/day, or 0.1 mg BDE-47/kg/day for a 10-week perinatal period (4 weeks prior to mating, 3 weeks in utero, 3 weeks lactation). Dams were bred to wildtype males in order to yield four possible genotypes per treatment group - Mecp2+/+, Mecp2308/+, Mecp2+/y, Mecp2308/y. The pups undergo behavioral testing throughout development until approximately pnd70 when they are sacrificed and their tissues are collected. A tissue microarray was constructed consisting of triplicate 6um cores from each of the various genotypes and treatment groups. Immunofluorescence staining was performed and laser scanning cytometry (LSC) was used to quantify changes in histone acetylation and MeCP2.
Results: The 0.1 mg BDE-47/kg/day exposure negatively impacted fertility and litter survival specifically in Mecp2-mutant but not wild-type C57Bl6/J mice, suggesting an increased genetic susceptibility of Mecp2-mutant mice to BDE-47 in reproductive success.

Preliminary behavioral studies on pups from 0.03 mg BDE-47/kg/day exposed Mecp2308/+ dams indicate significant defects in at least one measurement of social behavior (ultrasonic vocalizations) but no changes in growth, reflex, and motor skills. Preliminary immunofluorescence and LSC data have shown changes in the levels of MeCP2 and histone acetylation in cerebral cortical neuronal nuclei in BDE-47 exposed mice influenced by Mecp2 genotype.
Conclusions: Perinatal exposure to low doses of the organic pollutant BDE-47 combined with genetic susceptibility alters epigenetic patterns in maturing neurons and ultrasonic vocalizations in mice.

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NUCLEAR REPOSITIONING AND INTER-CHROMOSOMAL GENE ACTIVATION BY AN ECTOPIC BETA-GLOBIN LCR

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The architecture of DNA in the cell nucleus is an emerging key contributor to genome function. Based on microscopy studies, genes have been found to locate at different positions in the nucleus depending on their expression status. Such relocation is often mediated by regulatory DNA elements like enhancers that control the transcriptional output of these genes. Here, we have investigated in detail the nuclear environments that genes leave and enter when activated by a tissue-specific enhancer. For this, we generated transgenic mice carrying the prototype of a strong, tissue-specific transcription regulatory DNA element, the beta-globin locus control region (LCR), targeted into a gene-dense region of mouse chromosome 8. Previously we have shown that the ectopic LCR enhances the expression of genes neighbouring in cis up to 150 kilobases away from the integration site. Here, we have applied 4C technology and demonstrate that with or without the LCR essentially the same genomic regions are contacted, both in cis and in trans (on other chromosomes). Few or no new contacts are made by the LCR, but the interaction frequency with some loci on other chromosomes increases, in agreement with the observation that it locates its integration site more often outside the chromosome territory. We find no indication that the LCR searches for erythroid-specfic genes or other preferred genomic partners. However, microarray gene expression analysis reveals one gene that is upregulated in trans. This is the beta-globin bh1 gene, located on chromosome 7. Extensive expression analysis by RT-qPCR confirms this observation. Both with and without the LCR interchromosomal contacts between the integration site on chromosome 8 and the beta globin locus on chromosome 7 are formed, but are rare (allelic interaction frequency is 2.5%). Since bh1, a natural target gene of the beta-globin LCR, is the only gene in the genome that is upregulated in trans in our transgenic animals, the data show that enhancer-promoter compatibility, perhaps even more than interaction frequency, determines whether a gene responds to an enhancer. The data confirm, and predict, that interchromosomal gene regulation in mammals will be rare.

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REGULATION OF HOX GENE EXPRESSION BY THE CHROMATIN ASSOCIATED PROTEIN LEDGF/p75

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Lens epithelium derived growth factor (LEDGF)/p75 functions is a chromatin associated protein that functions as a tether during lentiviral intergration. Its N-terminal PWWP domain can bind to cellular chromatin, while the integrase domain from viruses such as HIV bind to an integrase binding domain on LEDGF/p75 in its C-terminus. We are interested in the cellular function of LEDGF/p75 and have generated a mouse line with mutant LEDGF/p75 from a gene-trap line that we identified in a screen for nuclear and chromosomal proteins. The LEDGF/p75 homozygous mutant mice showed homeotic transformations suggesting that LEDGF/p75 may play a role in Hox gene regulation. We are investigating which genes are deregulated in LEDGF/p75 mutant animals by microarray expression analysis from both whole embryos or mouse embryonic fibroblast lines derived from wild type and LEDGF/p75 mutant mice. We are also specifically looking for deregulation of Hox genes by qPCR and whether mutant LEDGF results in an unusual nuclear organisation of the Hox clusters or affects chromatin modifications in the Hox loci.

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ROLE OF ONCOGENES IN CHROMOSOME REGION 17p11.2-p12 IN OSTEOSARCOMA TUMORIGENESIS

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Osteosarcoma is the most common type of malignant solid tumor occuring in children. Tumors arise mostly from osteoblastic cells and often develop in the metaphysical region of the long bones. Osteosarcomas are characterized by frequent chromosomal aberrations involving gains, deletions and amplifications. The chromosomal region 17p11.2-p12 has been shown to be aberrantly organized in about 25% of high grade osteosarcoma cases. The first part of our project is the fine mapping of the amplified segments of this region to a resolution of 75-100 base pairs. Our interest focuses on the involvement of Low Copy Repeats (LCRs) as structural elements mediating the amplifications. To identify common regions of amplifications, possibly containing the causative oncogenes, a cohort of approximately 80 patients will be screened. In parallel studies, microarray analyses will be performed to determine the expression status of genes in the 17p11.2-p12 region. The second part of our project focuses on previously described candidate genes. In preliminary studies, we found several genes to be amplified and overexpressed in this region. They will be tested for their oncogenic potential by overexpressing these genes in normal human osteoblasts and determine whether transfected cells gain a proliferative advantage compared to untransfected cells. Concurrently, osteosarcoma cells with an overexpressing candidate gene will be cultured and the candidate gene silenced by using RNAi technology to check for anti-proliferative effects.