14th Dutch Chromatin Meeting - Leiden

Programme & abstracts

Location: Lecture hall C1, Gorlaeus Laboratoria, Einsteinweg 55, 2333CC, Leiden

9h00	**Registration, coffee & tea**
9h30	Opening
9h35	Keynote lecture 1: Marcelo Nollmann (CNRS, Montpellier, France): Synergies between chromosome dynamics and DNA transactions
10h25	Selected talk 1: Eva Brinkman (NKI, Amsterdam): Fidelity and kinetics of DNA repair after Cas9-induced double strand breaks
10h45	**Coffee & tea**
11h05	Selected talk 2: Blaise Weber (University of Amsterdam) Identification and characterization of distant enhancers in *Zea mays*
11h25	Selected talk 3: Cristina Ribeiro da Silva (Erasmus MC, Rotterdam) SWI/SNF ATPases BRM and BRG1 promote genome stability by stabilizing transcription factor TFIIH
11h45	Selected talk 4: Nicolaas Hermans (Leiden University) Single Molecule Spectroscopy on Native Chromatin of Yeast 18S rDNA
12h05	**Lunch & posters**
14h00	Invited lecture: Daan Noordermeer (I2BC, Gif-sur-Yvette, France): Paternally imprinted gene loci are dynamically organized into allele-specific sub-TADs
14h50	Selected talk 5 Nelleke Spruijt (UMC Utrecht/Radboud University Nijmegen) ZMYND8 co-localizes with NuRD on target genes and regulates PAR-dependent recruitment of GATAD2A/NuRD to sites of DNA damage
15h10	Selected talk 6: Ramon van der Valk (Leiden University): Environmental factors *directly* modulate bacterial genome organization and function
15h30	**Coffee & tea**
16h00	Selected talk 7: Simona Antonova (UMC Utrecht): Subtle Differences Distinguish Core Complex Assembly of the Human TFIID and SAGA Transcription Regulators

16h20 Selected talk 8: Aniek van der Vaart (Utrecht University): A dosage-dependent tumor suppression function of SWI/SNF chromatin remodeling complexes in the C. elegans model

16h40 Keynote lecture 2: Gaelle Legube (CBI, Toulouse, France): Chromatin and chromosome dynamics at DNA double-strand breaks

17h30 Closing remarks & drinks

Selected talks

1. Fidelity and kinetics of DNA repair after Cas9 - induced double-strand breaks

Eva K. Brinkman[1], Tao Chen[1], Marcel de Haas[1], Linda Holland[1], Waseem Akhtar[2] and Bas van Steensel[1]

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How quickly is a double-strand break (DSB) in the human genome repaired, and how does the interplay between various repair pathways affect the kinetics of repair? So far the kinetics of re-joining of broken DNA ends has primarily been studied in bulk, after exposure of cells to very high doses of damaging agents. Repair rates have also been indirectly inferred from studying the formation and clearance of "repair protein foci" by microscopy. Yet, the kinetics of the actual re-joining of two DNA ends after induction of a DSB at a single defined genomic location are not known. In addition, we lack accurate estimates of the fidelity of such a discrete repair event.

We developed a strategy to directly measure the kinetics and fidelity of NHEJ in single loci in human cells. We use a cell line with a tightly inducible Cas9 that enables the timed generation of a DSB at a locus of choice. We then use a combination of (i) our recently developed TIDE method, (ii) a novel assay to detect DSBs at single loci, and (iii) high throughput sequencing to quantitate intact, broken and repaired DNA over time. The resulting data are fed into a mathematical model that estimates key parameters of repair kinetics and fidelity.

Application of the above-mentioned strategy revealed that the half-life of DSB repair is ~8 hours, indicating that repair after a Cas9 cut is rather slow. Moreover, perfect repair of the DNA seems to be almost absent, meaning that the repair is exclusively error-prone resulting in small indels at the site of the break. Taking advantage of unique mutation signatures we found that two repair pathways (classical non-homologous end-joining and alternative end-joining) are both active at the same locus but with very different kinetics. Moreover, we show that the balance between the two pathways can be altered by additional IR damage elsewhere in the genome.

Our strategy to determine the kinetics and fidelity of DSB repair at single loci – and to infer contributions from different repair pathways – should be generally applicable. To dissect the effect of chromatin context on the Cas9 cutting and repair rate we are currently targeting barcoded transposons that are randomly inserted in the genome with Cas9. The cell pool used enables us to parallel track the kinetics of cutting and repair for thousands of individual sites in time. Preliminary results show that local sequence and chromatin state influences cutting and repair rate. This strategy offers new possibilities to study the effects of chromatin context on DSB repair kinetics, fidelity and pathway choice. We expect that these quantitative analyses will contribute to a better understanding of DSB repair and the impact of chromatin on this process.

2. Identification and characterization of distant enhancers in *Zea mays*

Blaise Weber[1], Rurika Oka[1], Johan Zicola[2], Jan-Jaap Wesselink[3], Huub Hoefsloot[1], Franziska Turck[2] and Maike Stam[1]

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Correct temporal and spatial regulation of gene expression is crucial for the successful development of an organism. Regulation of gene expression is in part accomplished through the coordinated action of *cis*-regulatory elements such as enhancers. Whereas regulatory sequences are still poorly characterized in plants, they have been extensively characterized in mammals. Active enhancers are for example found to be associated with specific features such as particular histones marks, chromatin accessibility, low DNA methylation, presence of enhancer-specific transcripts (eRNAs) and the ability to physically contact their target via the formation of chromatin loops.
Aiming at a better identification and characterization of plant enhancers we are generating a cartography of those elements in the crop plant *Zea mays*. Putative regulatory sequences are being identified using published methylC-seq data sets and newly generated DNAseI-seq, ChIP-seq, RNA-seq and CAGE-seq data sets. Our data indicate the existence of about 3,000 putative distal enhancers, which also include known and experimentally validated enhancers in maize. The enhancer candidates are characterized by increased chromatin accessibility, low DNA-methylation levels and H3K9ac enrichment. Selected candidate sequences are currently being validated using *in planta* reporter systems. Furthermore, the RNA-seq data are used to predict the target genes of the candidate enhancers, and this prediction is currently being tested by using 4C (Circular Chromosome Conformation Capture).

3. SWI/SNF ATPases BRM and BRG1 promote genome stability by stabilizing transcription factor TFIIH

Cristina Ribeiro-Silva, Özge Z. Aydin, Wim Vermeulen and Hannes Lans

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DNA damage interferes with transcription and replication and causes genomic instability, which is a hallmark of cancer. The DNA damage response (DDR) effectively preserves DNA integrity through a network of multiple DNA repair and associated signaling pathways. Modification of chromatin structure are not only necessary to accommodate DNA associated processes such as transcription but also to enable repair of damaged DNA. Mutations in chromatin remodeling enzymes, in particular in subunits of SWI/SNF ATP-dependent chromatin remodeling complexes, are observed with high frequency in many different types of cancer. It is, however, unclear whether altered chromatin remodeling compromises DNA repair which consequently contributes to genomic instability in tumorigenesis.

Using genetic screening in *C. elegans*, we found that SWI/SNF subunits proteins protect organisms against UV irradiation. To better understand the relevance of SWI/SNF in genome stability, we studied its involvement in mammalian Nucleotide Excision Repair (NER). NER is a versatile DNA damage response pathway that removes a wide variety of DNA lesions, including those induced by UV-light and many carcinogens. Our results show that both SWI/SNF catalytic ATPase subunits, BRM and BRG1, are critical for stability and efficient damage recruitment of general transcription factor IIH (TFIIH). TFIIH functions in transcription initiation and verifies the presence of damage during NER, which is necessary for the loading of downstream endonucleases that remove DNA lesions. BRM and BRG1 promote TFIIH complex stability by stimulating transcription of one of its core subunits, GTF2H1. As a consequence, loss of BRM or BRG1 leads to lowered transcription levels and defective NER.

Our data suggest that involvement of chromatin remodelers in maintaining genome stability goes beyond providing access to DNA of damage detection proteins. Furthermore, the dual function of TFIIH in both transcription and NER may explain compromised genome integrity when SWI/SNF is mutated in cancer cells.

4. Single molecule experiments on native chromatin of yeast 18S rDNA

N. Hermans, J. Huisman, T. Brouwer, G.P.H. van Heusden, J. Griesenbeck and J. van Noort

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The assembly of DNA into chromatin is involved in the regulation of gene expression. To fully understand this process there is a need for detailed structural knowledge of natively assembled chromatin, preferably at the single-molecule level to avoid ensemble and temporal averaging. This requires isolation of specific chromosomal loci for *in vitro* analysis. Current purification methods often involve chemical cross-linking to preserve the chromatin composition, which limits their application in structural studies. Here we present a method for the purification of native nucleoprotein fragments based on their DNA sequence without cross-linking, and the subsequent analysis in single-molecule force spectroscopy experiments. We used this method to study native chromatin fragments of the 18S rDNA from the yeast *Saccharomyces cerevisiae*. The natively assembled fibers reproduce some of the features measured on chromatin fragments reconstituted with purified histone proteins. However, the isolated fibers display compositional heterogeneity and the number of nucleosomes per fiber is often larger than expected based on sequence alone. Overall, we show single-molecule experiments on specific chromatin loci are both feasible and informative, especially when integrated with biochemical analysis and modelling.

5. ZMYND8 co-localizes with NuRD on target genes and regulates PAR-dependent recruitment of GATAD2A/NuRD to sites of DNA damage

Cornelia G. Spruijt[1,2], Martijn S. Luijsterburg[3,$], Roberta Menafra[2,4], Rik G.H. Lindeboom[2], Pascal W.T.C. Jansen[2], Raghu Ram Edupuganti[2], Marijke P. Baltissen[2], Wouter W. Wiegant[3], Moritz C. Voelker-Albert[1,5], Filomena Matarese[2], Anneloes Mensinga[1], Ina Poser[6], Harmjan R. Vos[1], Hendrik G. Stunnenberg[2], Haico van Attikum[3] and Michiel Vermeulen[2]

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The multi-protein NuRD complex has roles in transcription regulation and the DNA damage response. We show that ZMYND8 bridges NuRD to a number of putative DNA-binding zinc finger proteins. Furthermore, the MYND domain of ZMYND8 directly interacts with PPPLΦ motifs in the NuRD subunit GATAD2A. Since GATAD2A and GATAD2B define mutually exclusive NuRD subcomplexes, ZMYND8 only associates with GATAD2A-containing NuRD complexes. ZMYND8 and NuRD share a large number of genome-wide binding sites, mostly active promoters and enhancers. However, depletion of ZMYND8 does not affect NuRD occupancy genome-wide and only slightly affects expression of NuRD/ZMYND8 target genes. In contrast, the MYND domain in ZMYND8 facilitates the rapid, poly(ADP-ribose)-dependent recruitment of GATAD2A/NuRD to sites of DNA damage to promote repair by homologous recombination. Altogether, we show that a sub-stoichiometric interaction with a specific NuRD subunit paralogue provides unique functionality to distinct NuRD subcomplexes.

6. Environmental factors *directly* modulate bacterial genome organization and function

Ramon A. van der Valk[1], Jocelyne Vreede[2], Liang Qin[1], Geri F. Moolenaar[1], Andreas Hofmann[3], Nora Goosen[1] and Remus T. Dame*[1,4]

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Bacteria frequently need to adapt to altered environmental conditions. Adaptation requires changes in gene expression, often mediated by global regulators of transcription. The nucleoid-associated protein H-NS is a key global regulator in Gram-negative bacteria, and is believed to be a crucial player in bacterial chromatin organization, due to DNA bridging. H-NS activity in vivo is modulated by physico-chemical factors (osmolarity, pH, temperature) and interaction partners. Mechanistically it is unclear how functional modulation of H-NS by such factors is achieved. Here, we show that a diverse spectrum of H-NS modulators alter the ability of H-NS to bridge DNA. Changes in monovalent and divalent ion concentrations drive an abrupt switch between a bridging and non-bridging DNA binding mode. Similarly, synergistic and antagonistic co-regulators modulate the DNA bridging efficiency. Structural studies suggest a conserved mechanism: H-NS switches between a "closed" and an "open", bridging competent, conformation driven by environmental cues and interaction partners.

7. Subtle differences distinguish core complex assembly of the human TFIID and SAGA transcription regulators

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The basal transcription factor TFIID engages DNA and modified chromatin at RNA-polymerase-II-dependent promoters to form the transcription pre-initiation complex. These crucial functions are reflected in the structural complexity, shared subunits and large size of the TFIID complex. Insight into structure and *in vivo* assembly of TFIID has been limited. Current models suggest formation of a core complex, comprised of symmetrically arranged TAF4, -5, -6, -9 and -12 subunits, but precise molecular details are lacking. Here, we show the existence of a core complex in cells prior to nucleation of full TFIID. We provide an atomic model for the TAF5/6/9 subcomplex supported by *in vivo* analysis of TAF5 and TAF9 mutations and by cryo-electron microscopy. Our data reveal both a similar core for SAGA histone acetylase coactivator as well as distinct structural requirements for TFIID and SAGA assembly of the shared TAF9 subunit, highlighting similarities in *in vivo* assembly pathways of large heterogeneous protein complexes.

8. A dosage-dependent tumor suppression function of SWI/SNF chromatin remodeling complexes in the *C. elegans* model

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SWI/SNF chromatin remodeling complexes are important regulators of transcription that alter the position of nucleosomes and are frequent targets of loss of function mutations in human cancer. Our group showed previously that loss of SWI/SNF subunits in *C. elegans* results in mild overproliferation of muscle precursor cells, while combined loss of SWI/SNF and additional G1/S cell cycle regulators leads to a dramatic tumorous phenotype (Cell, 2105). To study the SWI/SNF tumor suppressor function in more detail, we used RNAi, created an ATPase-dead *swsn-4* Brm/Brg1 missense mutant, and generated conditional knock-out alleles of different SWI/SNF subunits by CRISPR/Cas9-assisted recombineering. The RNAi experiments showed that the SWI/SNF-A (BAF) complex is specifically required for cell cycle exit during terminal differentiation. The *swsn-4* ATPase-dead and knock-out mutants showed highly similar phenotypes, indicating that the ATPase activity is essential for SWI/SNF complex function in controlling cell cycle exit. CRE recombinase expression in the mesoblast lineage resulted in complete null alleles of either the ATPase subunit *swsn-4* or the BAF-specific subunit *swsn-8* (human BAF250/ARID1). Interestingly, mesoblast-specific SWI/SNF knockout and knockdown by RNAi resulted in opposite phenotypes. Our data imply a dosage-dependent SWI/SNF function, which may help explain the nature of SWI/SNF mutations in human cancers.

Posters

Speeding up neuronal maturation in microRNA-based trans-differentiation

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The overexpression of microRNAs 9 and 124 in combination with specific transcription factors is able to transdifferentiate adult human fibroblasts directly into neurons. However, it takes 6-12 weeks before these fibroblast-derived neurons are mature in terms of synaptic activity. In contrast, overexpression of Ngn2 in induced pluripotent stem cells (iPSC) induces differentiation into mature neurons within 2-3 weeks. Here, we compared microRNA-based transdifferentiation with the fast iPSC differentiation to identify transcription factors (TFs) than can improve the maturation efficiency in microRNA-based transdifferentiation. To this end, we assayed genome-wide chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) three weeks after induction of transgenes in the following cell-types: iPSCs, iPSC-derived neurons through overexpression of Ngn2, adult human fibroblasts, and adult human fibroblast overexpressing either Ngn2, miR-9-124 or both Ngn2 and miR-9-124. We used known TF DNA binding motifs to predict genome-wide TF binding in accessible regions. Multiple linear regression analysis shows that motifs associated to two specific TF families are highly enriched in chromatin regions that are more accessible in iPSC-derived neurons compared to fibroblast-derived neurons, indicating that TFs belonging to these families might explain the observed difference in maturation efficiency. We are currently validating these predictions *in vitro*.

The spatial structure of euchromatin in *A. thaliana*

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In the interphase nucleus of *Arabidopsis thaliana* constitutively silenced heterochromatin appears as highly condensed chromocenters. In contrast, most genes are located in the less compacted euchromatin. Depending on their activity genes are assumed to be more or less decondensed. We are interested if the linear genome takes up a conformation that reflects this as a spatial segregation of active and silenced genes. By staining active and silencing histone marks, RNA polymerase II and DNA in isolated nuclei we are able to observe the spatial arrangement of chromatin by microscopy. We see slight differences in the intensity of DNA stainings within euchromatin. These correlate with stainings for active histone marks. Elongating RNA polymerase II, however, localizes to sub-micrometer domains depleted of chromatin. To elucidate the functional relevance of this finding we are study the spatial positioning of the flowering repressor gene FLOWERING LOCUS C (FLC). FLC can be permanently silenced by a several weeks-long cold treatment. We observed the FLC locus to be located out of its immediate genomic neighbourhood frequently in both the active and the silenced state. Looping may provide a way for loci to reposition to specific compartments. Preliminary data points to colocalization of active FLC with other active genes.

Designer nucleosomes for next generation assay and tool development

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Assays with epigenetic enzymes such as histone methyltransferases or histone acetylases and related screenings for inhibitors of the respective enzymes are usually run with native or recombinant nucleosomes, single histone proteins, or histone derived peptides.

As some epigenetic enzymes require highly specific substrates with defined histone modifications, we developed a new generation of epigenetic substrates: Designer Nucleosomes (dNuc**).** dNUCs are semi-synthetic nucleosomes incorporating specific histone post-translational modifications (PTMs). These reagents represent a powerful new technology – critical in understanding chromatin biology and for the development of novel drug targets and precision therapeutics. We have established an EpiStandard nucleosome assembly process that minimizes the presence of contaminating histone proteins and free DNA from final preparations. Importantly, all PTMs are seamlessly incorporated into histones to best recapitulate *in vivo* chromatin conformations. All of the dNuc-based substrates are free of unnatural substrate mimics (such as methyl-lysine analogs), which have shown to alter interactions with enzymes, effector binding proteins, and antibodies. The first set of dNucs focused on PTMs with strong associations with diseases such as various types of cancers, including H3K4me3, H3K4me1, H3K9me3, H3K27me3, H3K36me3, H4K12ac, and H4K5,K8,K12,K16ac (H4-tetra). Further dNUCs will be developed to meet the growing needs of the epigenetic community and will be released in the near future.

DNMT3B mutations in ICF1 and FSHD2

Marlinde L. van den Boogaard[1], Richard J.L.F. Lemmers[1], Judit Balog[1], Peter. E. Thijssen[1], Mariëlle Wohlgemuth[2], Mari Auranen[3], Safa Baris[4], Ercan Nain[4], Ayça A. Kıykım[4], Lucia Spossito[5], Caner Aytekin[6], Patrick J. van der Vliet[1], Kirsten R. Straasheijm[1], Rob F.P. van den Akker[1], Marjolein Kriek[1,7], Marlies E.Y. Laurense – Bik[7], Monique M. van Ostaijen - ten Dam[8], Kerstin B.M. Hansson[7], Sari Kiuru-Enari[3], Maarten J.D. van Tol[8], Rabi Tawil[9], Stephen J. Tapscott[10], Baziel G.M. van Engelen[2], Lucia Daxinger[1] and Silvère M. van der Maarel[1]

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DNA methyltransferase 3B (DNMT3B) is considered a de novo methyltransferase, which has been shown to CpG methylate a variety of genomic regions, including repeats. In 1999, recessive mutations in DNMT3B were identified as the cause of Immunodeficiency, centromeric instability and facial anomalies syndrome type 1 (ICF1). ICF is a rare condition, mainly characterized by hypo- or agammaglobulinemia in the presence of B cells, developmental delay and facial anomalies. As a consequence of the DNMT3B deficiency, ICF1 cases present with hypomethylation of centromeric repeats and other repeats such as the subtelomeric D4Z4 repeat.

More recently, dominant mutations in DNMT3B were found in two families with fascioscapulohumeral muscular dystrophy type 2 (FSHD2). FSHD2 is characterized by hypomethylation of the D4Z4 repeat, which is associated with transcriptional derepression of DUX4, a copy of which is located within each unit of the D4Z4 repeat. DUX4 encodes a germ line transcription factor, which is normally repressed in somatic tissue. Its expression is harmful to skeletal muscle cells.

A comprehensive clinical and epigenetic comparison of mono- and biallelic DNMT3B mutation carriers in ICF1 families and FSHD2 families identified some epigenetic similarities. However, muscular dystrophy has not been reported in ICF1 patients or their parents and no immunodeficiency was detected in FSHD2 families. This indicates that recessive or dominant mutations in the chromatin modifier DNMT3B can cause very discordant phenotypes.

High throughput assessment of context-dependent effects of chromatin proteins

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Chromatin proteins control gene activity in a concerted manner. We developed a high-throughput assay to study the effects of the local chromatin environment on the regulatory activity of a protein of interest. The assay combines a previously reported multiplexing strategy based on barcoded randomly integrated reporters with Gal4-mediated tethering. We applied the assay to Drosophila Heterochromatin protein 1a (HP1a), which is mostly known as a repressive protein but has also been linked to transcriptional activation.

Recruitment to over one thousand genomic locations revealed that HP1a is a potent repressor able to silence even highly expressing loci. However, the local chromatin context can modulate HP1a function. In pericentromeric regions, HP1a-induced repression was enhanced by two-fold. In regions marked by a H3K36me3-rich chromatin signature, HP1a-dependent silencing was significantly decreased. We found no evidence for an activating function of HP1a in our experimental system. Furthermore, we did not observe stable transmission of repression over mitotic divisions after loss of targeted HP1a.

The multiplexed tethered reporter assay can be applied to a large number of chromatin proteins and will be a useful tool to dissect combinatorial regulatory interactions in chromatin.

High-throughput and multiplexed (phospho-)protein detection via a novel immuno-sequencing method

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Large compound screens are one of the first steps to assess the mechanism of action (MMOA) in the early stages of drug development. The techniques currently used in these screens allow the evaluation of only one or very few phenotypes, which could result in high number of false positives. To increase the number of different molecular phenotypes that are measured, we aim to develop a method that allows highly multiplexed and high-throughput assessment of molecular phenotypes. Our novel 'immuno-Sequencing' (iSeq) technique combines (I) specific protein detection via antibody-DNA conjugates, with (II) sequencing the (antibody-specific) DNA barcodes. The DNA sequencing read-out of the antibodies allows 1) amplification of signal, 2) increased multiplexing and 3) high sample-throughput within one experiment. First, we produce covalent and cleavable antibody-DNA conjugates using click-chemistry. Second, we perform multiplex immunostaining with >60 antibody-DNA conjugates and developed a PCR based sequencing sample-preparation workflow to label hundreds of samples simultaneously. Finally, a iSeq dedicated computational pipeline is used for data (pre-)processing and analysis. By combining different types of antibodies, we anticipate iSeq being broadly applicable for high-throughput studies of proteins and signalling events in academia and industry.

TAF1 involvement in neurodegenerative conditions

Simona Capponi, Roy Baas, Richard van Schaik, Robert van Es, Harmjan R. Vos and H. Th. Marc Timmers

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Regulation of transcription is a critical process for cell proliferation, differentiation, and development. The basal transcription factor TFIID recognizes the core promoter region of target genes and nucleates the pre-initiation complex assembly to commence transcription. TFIID is composed of TBP (TATA-binding protein) and thirteen TBP-associated factors (TAFs). The TAF1 protein is the largest subunit of TFIID. It interacts with TBP and TAF7 and it binds acetylated histones via its double bromo-domain.
Despite its basal function, TAF1 has been associated with neurodegenerative conditions. The reduced expression of its neuron-specific isoform (NTAF1) has been associated with X-linked dystonia-parkinsonism. In addition, eleven TAF1 mutations have been described in Intellectual Disability (ID).

In our study, we evaluated the assembly of the TFIID complex in disease-associated conditions. We generated inducible HeLa cell lines expressing GFP-tagged NTAF1 or different ID-associated mutations. After induction, nuclear extracts were affinity purified and analysed with MS. While NTAF1 displayed a similar MS pattern compared to the canonical protein, one of the ID-associated mutations (I505N) showed a massive disruption of the TFIID complex, with only the TAF1-TAF7 sub-complex being still preserved. Further studies aiming at understanding the transcriptional program sustained by NTAF1 or ID-associated mutations are currently ongoing.

Identification and analysis of primate specific regulatory networks

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Whole genome sequencing of different primates has identified millions of genomic changes that could account for the phenotypic differences between these species. Most of these changes reside in the non-coding part of the DNA, potentially affecting non-coding gene regulatory elements such as enhancers and promoters.

To get a better understanding of the important regulatory changes that occurred in the primate lineage, we have annotated active regulatory elements on the common marmoset genome using ChIP-sequencing on different tissues. The common marmoset is a small new world monkey that diverged from the human lineage 45My ago. Comparison of the marmoset regulatory networks with those in similar human tissues enables us to assess regulatory changes that first appeared within the primate branch and then became fixed.

The identification of these primate specific regulatory networks may shed light on how the physiological features that set primates apart from other mammals have evolved.

Interaction of a histone chaperone involved in DNA repair with a core histone complex

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Histone chaperones catalyze the assembly and disassembly of nucleosomes, the basic unit of chromatin. As such, histone chaperones play a crucial role in the replication, transcription, and repair of our genome where access to the DNA is required. DNA repair factor APLF has been identified to have histone chaperone activity. In particular, APLF's C-terminal acidic domain is vital for its DNA repair and histone chaperone activities. We have investigated the interaction of this domain with the histone H2A-H2B complex using microscale thermophoresis, isothermal titration calorimetry, and high resolution NMR spectroscopy. Our results show that the C-terminal acidic domain of APLF binds in a complex binding mode with high affinity and specifically to the histone H2A-H2B dimer. A model of the APLF chaperone mechanism is presented.

The role of nucleoid-associated proteins in the compaction of the bacterial chromosome

Frédéric G.E. Crémazy[1], Fatema-Zahra Rashid[1], Andreas Hofmann[2], James Haycocks[3], Dieter W. Heermann[2], David Grainger[3] and Remus T. Dame[1]

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Fully unfolded, the circular, 4.64 Mb-long chromosome of Escherichia coli K-12 has a diameter of 0.5 mm — 500 times larger than the diameter of a typical E. coli cell. Therefore, the bacterial genome needs to be compactly folded to be packaged inside the bacterial cell; and at the same time, remain accessible to proteins involved in DNA transactions.

Members of the nucleoid associated protein (NAP) family are architectural proteins that consistently bind along the bacterial chromosome and collectively control its conformation and its level of compaction. They maintain genome integrity, modulate DNA accessibility, and regulate DNA transactions by influencing the local topology of DNA. Additionally, NAPs promote long-distance interactions between DNA fragments through the formation of large loops. Moreover, by adapting their molecular functions, NAPs can be direct effectors of environment stimuli or changes in growth regime. Consequently, they mediate drastic changes in the transcriptome of the cell through remodeling of the chromosomal structure.

We are investigating the contribution of environmentally driven changes in chromosome structure on the activity of the associated regulons using High-resolution chromosome conformation capture (Hi-C) and Targeted genomic labeling (FROS).

Development of microfluidic chip for automated Chromatin Immunoprecipitation and library construction

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Chromatin Immunoprecipitation sequencing (ChIP-Seq) is the key experimental approach to locate histone modifications and transcription factors in the genome, yet for robust and automated detection this assay requires large numbers of cells. We are developing a miniaturizated ChIP-seq workflow that will enable automated ChIP-seq at great throughput and sensitivity at reduced labour and reagent costs. Multiple prototype polydimethyl-siloxane microfluidic chips have been produced and iteratively optimized. The microfluidic chip contains reactors that facilitate immunoprecipitation of chromatin and library construction in a parallelized fashion. Our automated workflow performs highly reproducible chromatin immunoprecipitations on thousands of cells for multiple histone modifications. We also show DNA purification and library construction starting from lower picogram range of DNA as input.

SWI/SNF chromatin remodelling in Nucleotide Excision Repair

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Nucleotide Excision Repair (NER) is a highly versatile DNA repair pathway, capable of removing a wide range of helix-destabilizing DNA lesions, including those induced by solar UV-light (CPDs and 6-4PP photolesions), cigarette smoke and several commonly used chemotherapeutics. Despite our detailed knowledge on the core NER machinery, it is still largely unclear how NER operates within the complex chromatin environment as present in mammalian cells and how the dynamic remodelling of chromatin takes place during NER to regulate its efficiency. ATP-dependent chromatin remodelling factors are thought to regulate chromatin accessibility through movement of nucleosomes along the DNA or by transfer of nucleosomes from and to DNA. In mammals, different ATP-dependent chromatin remodelling complexes have been identified of which the SWI/SNF family is among the best characterized. Previously, we identified different SWI/SNF chromatin remodelling subunits that play a role in the UV-induced DNA damage response by showing that mutation of BRM/BRG1, SNF5, BAF155, PBRM1 and BAF250 sensitizes *C. elegans* to UV irradiation. Preliminary experiments with the respective mammalian orthologs of these remodelling proteins suggest that different SWI/SNF chromatin remodelling subunits differently affect mammalian global genome NER and transcription coupled NER. However, the precise molecular mechanism of SWI/SNF involvement in the DNA damage response remains undefined. To better understand how these important remodelling complexes act during NER, we started a systematic in-depth analysis of the function of SWI/SNF-dependent chromatin remodelling subunits during NER.

Gene regulation and genome compaction by archaeal histones

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To organize their genomes, archaea express histone proteins that are homologous to their eukaryotic counterparts H3 and H4. Eukaryotic histones are believed to originate from archaeal histones; this is underlined by the notion that histones from the recently discovered phylum Lokiarchaeota seem to be intermediates between archaea and eukaryotes, as are the organisms themselves. Unlike eukaryotic histones, which predominantly wrap DNA around an octameric histone core, archaeal histones seem to be able to bind to DNA as dimer, tetramer or larger multimer. Some archaea express multiple histones, which allows formation of heterocomplexes that may add a level of complexity to archaeal genome organization. Here, we examined the histones HMfA and HMfB from *Methanothermus fervidus*. Our data indicate that tetramers are formed on high-affinity sites on the genome and that higher concentrations allow for highly cooperative aspecific genome compaction. We speculate that aspecific binding is important for genome organization, whereas specific binding may have a more regulatory function. HMfA and HMfB have equal affinities for an aspecific substrate, but the affinities of HMfA and HMfB for a high-affinity site are very different, opening up the possibility of growth phase specific regulation via these proteins.

Molecular mechanisms that distinguish TFIID housekeeping from regulatable SAGA promoters

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An important distinction is frequently made between constitutively expressed housekeeping genes versus regulated genes. Although generally characterized by different DNA elements, chromatin architecture and cofactors, it is not known to what degree promoter classes strictly follow regulatability rules and which molecular mechanisms dictate such differences. We show that SAGA-dominated/TATA-box promoters are more responsive to changes in the amount of activator, even compared to TFIID/TATA-like promoters that depend on the same activator. Regulatability is therefore an inherent property of promoter class. Further analyses show that SAGA/TATA-box promoters are more dynamic because TBP recruitment through SAGA is susceptible to removal by Mot1. In addition, the nucleosome configuration upon activator depletion shifts on SAGA/TATA-box promoters and seems less amenable to preinitiation complex formation. The results explain the fundamental difference between housekeeping and regulatable genes, revealing an additional facet of combinatorial control: an activator can elicit a different response dependent on core promoter class.

Unravelling the role of the H4-tail and the linker histone H1 in chromatin (un-)folding

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Gene expression in eukaryotes is regulated by DNA condensation into chromatin fibers. 147 bp of DNA wrapped around a histone octamer form a nucleosome, which can further interact with its neighbors into a higher-order chromatin structure. Its geometry is highly debated though, as it depends on a variety of factors including: nucleosomal repeat length, the linker histone content and post-translational modifications.
Here we used multplexed magnetic tweezers to measure the unfolding of individual chromatin fibers. With the recombinant histones H4 and H2A containing cystein mutations we introduced a covalent bond between adjacent nucleosomes and stabilized the fibers against unfolding. Under „cross-linking" conditions we observed the same folding of the fibers as compared to wild-type, but increased rupture forces upon stretching. This shows that H4-tail and H2A interaction drives chromatin fiber compaction.

Linker histone H1 has also been implied to strengthen chromatin folding. We observed an increased stability and hysteresis in force-extension curves of the H1 – containing fibers. We quantified this non-equilibrium behavior using the novel statistical mechanics model that includes transition rates between the unfolding states. This detailed structural interpretation elucidates the physics of chromatin folding and will help to understand the mechanisms of gene regulation.

A molecular switch for polyploidization through heterochromatin disruption in Arabidopsis embryonic cells

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Polyploidy is a common phenomenon in all eukaryotic organisms that is typically achieved by non-separation of duplicated chromosomes during mitosis or meiosis. The AT-hook containing, nuclear localized protein (AHL) genes encode DNA binding proteins with diverse roles in plant development. AHL15 overexpression in Arabidopsis results in efficient induction of somatic embryogenesis (SE) on seedling cotyledons , a considerable portion of which appears to be polyploid. Here we show, using the centromere-specific histone H3 (CENH3)-GFP or the Histone 2B (H2B)-GFP reporters, that AHL15 overexpression-induced polyploidy specifically arises from endomitosis events during the induction of somatic embryos , and not during mitotic divisions of leaf and root cells (Fig.3 C-F). In search for factors that lead to this polyploidization, we observed a remarkable disruption of the heterochromatin structure in embryonic cells compared to non-embryonic cells. By using the H2B-GFP reporter we could show chromosome mis-segregation and binucleated cells in embryonic cells. Heterochromatin disruption is well known to lead to chromosome mis-segregation in animal cells, and we hypothesize that this is the common mechanism through which AHL15 can simultaneously alter the developmental program of cells, while disrupting the segregation of their chromosomes during mitosis. Currently we are investigating if chromatin modification is altered by AHL15 overexpression.

Genomes in general follow a loop aggregate/rosette architecture – the underlying reasoning of nature

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Recently we determined the 3D architecture and dynamics of the human and mouse genomes by a systems genomics combination of a novel superior selective high-throughput high-resolution chromosomal interaction capture (T2C), a novel FCS microscopy technique, polymer modelling, and architectural and DNA sequence scaling analysis. Here we show by a reanalysis of published data that the architecture – a quasi-fibre, folding into stable loops forming stable aggregates/rosettes is not only present in this data and thus contrasts the suggestions put forward in recent publications, but also that this architecture is a general property from bacteria to mammalian genomes. A synoptic comparison not only reveals that this multi-loop aggregate/rosette (MLA/R) architecture is independent of genetic loci, species, cell type and states, but also shows minor though characteristic functional differences which are only variations of a theme. Again we find in the reanalysis the same fine-structured multi-scaling behaviour in the architecture and the DNA sequence, thus both are tightly evolutionarily entangled. This universal architectural behaviour has its reason in the co-evolutionary connection to its function – the storage, translation, and finally replication of genetic information: stability, accessibility, and reproduction ability of genomes. In principle genome architecture, dynamics, and function show a strong interdependence of genotype and phenotype, in which both are an inseparable system from the single basepair to the entire genome/cell nucleus. Hence, we not only determined the three-dimensional organization and dynamics but also show that this is a general phenomenon, explaining why genomes are organized in such a consistent systems manner. Thus, after ~170 years of intense research on this topic we finally get a broad understanding of genome architecture and function, which is of fundamental importance for diagnosis, treatment, and genome manipulation in the future.

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Multi-contact 4C reveals multi-way three-dimensional chromatin conformation

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Spatial chromatin organization is increasingly recognized as a regulator of nuclear processes such as gene activity. Most methods developed to unravel three-dimensional (3D) chromatin conformation analyze pair-wise chromatin contacts, but are incapable of identifying simultaneous associations among multiple loci. Yet, exactly this type of 'beyond two-way' contact information will provide the insights required to start composing a more complete picture of 3D genome organization at the level of the individual allele.

We describe a novel chromatin conformation capture technology that allows direct identification of multi-way chromatin contacts. Multi-Contact 4C (MC-4C) applies the Pacific Biosciences third-generation long-read single molecule real time (SMRT) sequencing technology to an intermediate product of the conventional chromatin conformation capture (3C) protocol. For a given allele of interest, MC-4C can easily identify 4 to 8 spatial neighbors, based on proximity-ligation events. With many thousands of identified multi-way (>3) contacts per targeted locus, MC-4C presents a unique and promising new technology to distinguish co-operative from mutually exclusive 3D chromatin structures.

Promoter selection determinants in the biogenesis of coding and non-coding RNAs

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Transcription regulation involves the interplay between regulatory DNA sequences, such as enhancers and the core promoter, chromatin structure and regulatory protein complexes. The core promoter defines the transcription start site and is the focal point for transcription regulatory signals.

The TFIID complex is the first basal transcription factor to bind the core promoter and facilitates recruitment of RNA polymerase II and other basal transcription factors. The crucial subunit of the TFIID complex is the TATA-binding protein (TBP), which has a high affinity for promoter sequences such as TATA boxes. Furthermore, the TFIID complex is able to mediate regulatory signals and recognize histone modifications via several other subunits, such as TAF1 and TAF3.

In this project, the contributions of the core promoter sequence as well as histone modifications to TFIID binding, PIC assembly and mRNA synthesis will be assessed on a genome-wide scale. To this end, 34 HeLa cell lines with GFP-tagged TBP, TAF1 or TAF3 mutant proteins, defective in DNA and/or chromatin interactions, have been generated. The functional effect of all mutations will be assessed via quantitative mass spectrometry, live cell imaging and genomic tools such as ChIP-seq, GRO-seq and START-seq. Progress will be presented and discussed.

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Accurate repair of DNA damage is critical for the maintenance of genomic integrity. DNA double-strand breaks (DSBs) are particularly toxic lesions because failure to resolve them can lead to cell cycle arrest, apoptosis, and disease through genetic alterations. Despite extensive research into the molecular pathways of DSB sensing and repair, little is known about management of breaks within different genomic contexts, because there is currently no technique available to accurately locate DSBs in the genome.

DSBs are assumed to be stochastic events that occur differently in each cell. This project aims to extract genomic locations of DSBs from single cells, with better temporal resolution to study break locations after repair has been completed. The resulting damage landscapes will enable systematic study of the response of individual cells to DSBs in different genomic regions. By relating cellular fate to the acquired damage profile and repair response, we expect to get a more detailed understanding of cellular decision-making.

Tools: two complementary methods are used to map DSB repair locations, based on the DamID technique. 1) Single-cell DamID is a genomics approach that allows genome-wide profiling of DSB repair components. 2) The m6A-Tracer is a microscopy tool to visualize dynamics of DSB foci.

The role of histone H1 and its chaperone SET in the DNA damage response

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The DNA damage response involves many signaling cascades and repair pathways to sense and repair DNA lesions. Different types of DNA damage are efficiently repaired by dedicated repair systems each recognizing a specific subset of DNA lesions. Defects in these repair pathways can lead to premature aging and cancer, underscoring the biological relevance of genome surveillance processes. During the last decade it became evident that chromatin plays an important role during the different steps in DNA repair. The chromatin structure can act as an obstacle for repair proteins to bind and is expected to be remodeled to generate a more open conformation to allow efficient repair. However chromatin is not only a barrier for repair, it is also a perfect substrate for many damage signaling events. Thus far most research on the interplay of chromatin with DNA repair is focused on core histones and the role of linker histone H1 has remained largely unclear. This is why we have set out to investigate the function of histone H1 and its chaperone SET in the DNA damage response.

Interestingly, we found that down regulation of SET results in increased resistance to a wide spectrum of structurally different DNA lesions, including those induced by UV, IR and mitomycin C, each of which are repaired by different repair mechanisms. Therefore it is highly unlikely that SET is involved in stimulating the different DNA repair pathways. In line with this, we do not observe differences in the repair efficiency of UV-induced lesion, indicating that SET is a general modulator of the DNA damage response, explaining its role in cell survival upon exposure to genotoxins. One of the main DNA damage signaling pathways, induced after many different types of DNA damage, involves phosphorylation of histone H2AX. Previously it has been shown that SET down regulation results in an enhanced yH2AX signaling after double strand break induction. However, clonogenic survival experiments in H2AX knock down cells still show increased resistance to UV after SET knock down, showing that the enhanced signaling is not the prime cause for the better survival. Live cell imaging studies in combination with immunofluorescence experiment tethering SET to a Lac operon show that SET is responsible for the eviction of histone H1 from the chromatin, probably affecting chromatin compaction and gene specific transcription levels. We found that simultaneous down regulation of SET and histone H1 decreases the effect of SET knock down on the survival, suggesting that the histone H1 chaperone function of SET is the main reason for the higher DNA damage resistance in SET depleted cells.

SMCHD1 regulates a limited set of gene clusters on autosomal chromosomes

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Facioscapulohumeral dystrophy (FSHD) is caused by a contraction of the D4Z4 macrosatellite repeat on chromosome 4 (FSHD1), or by mutations in the *SMCHD1* or *DNMT3B* gene (FSHD2). Both situations result in the incomplete epigenetic repression of the D4Z4 encoded retrogene *DUX4* in somatic cells, leading to the aberrant expression of DUX4 in skeletal muscle. SMCHD1 regulates chromatin repression at different loci, having a role in CpG methylation establishment and/or maintenance. To investigate the global effects of *SMCHD1* mutations on DNA methylation, we combined 450k methylation analysis on mononuclear monocytes from female heterozygous *SMCHD1* mutation carriers and unaffected controls with reduced representation bisulfite sequencing (RRBS) on FSHD2 and control myoblast cell lines. We identified several clustered autosomal loci with hypomethylation: the protocadherin (*PCDH*) cluster on chromosome 5, the tRNA and 5S rRNA clusters on chromosome 1, the *HOXB* and *HOXD* clusters on chromosomes 17 and 2 respectively, and the D4Z4 repeats on chromosomes 4 and 10. Furthermore, an increase in RNA expression was seen in FSHD2 myoblasts for some of the *PCDHβ* cluster isoforms, tRNA isoforms, and a *HOXB* isoform in comparison to controls, as well as *DUX4*. SMCHD1 was bound at DNAseI hypersensitivity sites known to regulate the *PCDHβ* cluster and at the chromosome 1 tRNA cluster, with decreased binding in *SMCHD1* mutation carriers at the *PCDHβ* cluster sites. These results suggest that SMCHD1 acts as a repressor on a limited set of autosomal gene clusters, with *SMCHD1* mutations resulting in hypomethylation of these clusters, and increased expression of some gene cluster isoforms.

Characterizing the epigenome of multiple states of pluripotency using integrative proteomics

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Maintaining pluripotency in mouse embryonic stem cells (ESCs) requires serum and Leukemia inhibitory factor ('serum'), resulting in a metastable state, or a defined medium containing two kinase inhibitors ('2i'), representing the ground state. These functionally pluripotent states show striking differences in their epigenome, such as the near absence of DNA methylation in 2i. We hypothesized epigenetic differences significantly affect chromatin composition. To investigate this, we performed comprehensive chromatin profiling using mass spectrometry. By profiling the proteins on the chromatin and all modifications on the histones, we provide an integrated overview of the epigenome in 2i and serum cells. We identified remarkable differences between 2i and serum in abundance of major epigenetic complexes and their histone modifications, including Polycomb Repressive Complex 2 (PRC2) and the corresponding H3K27me3. We extended our analysis to knockout ESCs for DNA methylation and PRC2 and describe how their interplay affects chromatin composition. Notably, we found that 2i ESCs upregulate DNA methylation upon PRC2 removal, to our knowledge the first example of DNA methylation compensating epigenetic changes. Our integrative chromatomics approach provides a detailed map of the pluripotent epigenome and delivers novel insights into the function of major epigenetic players such as DNA methylation and PRC2.

Single-cell 5hmC sequencing reveals chromosome-wide cell-to-cell variability and enables lineage reconstruction

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The epigenetic DNA modification 5-hydroxymethylcytosine (5hmC) has crucial roles in development and gene regulation. Quantifying the abundance of this epigenetic mark at the single-cell level could enable us to understand its roles. We present a single-cell, genome-wide and strand-specific 5hmC sequencing technology, based on 5hmC glucosylation and glucosylation-dependent digestion of DNA, that reveals pronounced cell-to-cell variability in the abundance of 5hmC on the two DNA strands of a given chromosome. We develop a mathematical model that reproduces the strand bias and use this model to make two predictions. First, the variation in strand bias should decrease when 5hmC turnover increases. Second, the strand bias of two sister cells should be strongly anti-correlated. We validate these predictions experimentally, and use our model to reconstruct lineages of two- and four-cell mouse embryos, showing that single-cell 5hmC sequencing can be used as a lineage reconstruction tool.

ImmunoCRIT to predict resistance in ER$^+$ breast cancer to endocrine therapies using DNA methylation based quantitative tool

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A shift from antitumor immunity towards tumor-immune tolerance plays a major role in progression and aggressiveness of most of the cancers. This balance between immune surveillance and immune tolerance could be expressed as a score called as an ImmunoCRIT (Immuno - Cellular Ratio of Immune Tolerance). It is represented by the ratio of suppressive and effector immune cells tumorous tissues and possibly in blood. Resistance to endocrine therapy in ER$^+$ breast cancer leads to aggressive tumorigenesis.

In the current research, we exploit DNA methylation based analysis to develop an assay for immune suppressor cell type called as MDSCs (myeloid derived suppressor cells). A robust, specific and a highly quantitative qPCR based assay has been developed for this immune cell sub type. Embedded in a panel of already existing immune cell type specific qPCR assay, we aim at testing ImmunoCRIT for its value in the prediction of the risk of resistance in ER+ breast cancers to endocrine therapies

Trained innate immunity can reverse the epigenetic state of LPS induced immunological tolerance in macrophages

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Innate immune memory is the phenomenon whereby innate immune cells such as monocytes or macrophages undergo functional reprogramming after exposure to microbial components (such as immune-tolerance induced by LPS, or trained immunity induced by b-glucan). The inability of tolerant macrophages to respond and eliminate secondary infections is a major cause of sepsis-associated death, while induction of innate trained immunity can have beneficial health effects. We apply an integrated epigenomic approach to characterize the molecular events involved in LPS-induced tolerance in a time dependent manner. Mechanistically, LPS treated monocytes fail to accumulate active histone marks at promoter and enhancers of genes in the lipid metabolism and phagocytic pathways. Transcriptional inactivity in response to a second LPS exposure in tolerized macrophages is accompanied with failure to deposit active histone marks at promoters of tolerized genes. The innate immune trainer, b-Glucan, partially reverses the LPS-induced tolerance *in vitro*. Importantly, *ex vivo* b-Glucan treatment of monocytes from volunteers with experimental endotoxemia (*in vivo* LPS exposure) re-instates their capacity for cytokine production. Tolerance is reversed at the level of histone modification and transcriptional reactivation of otherwise unresponsive genes (Novakovic, et al. Cell *In press*). Further studies on sepsis patients and in vivo models of trained immunity have the potential to translate these findings to the clinic.

Computational genome-wide prediction of transcriptional enhancers in the maize genome

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Biological processes are tightly regulated and one of the major levels of regulation occurs at the gene transcription level. The fine-tuning of gene expression during e.g. development, differentiation and adaptation to environmental changes is accomplished in multiple ways, including gene regulation by transcriptional enhancers. Unlike promoters, which are adjacent to the coding regions they regulate, enhancers can be distantly located. This characteristic of enhancers makes them challenging to locate. It has been shown in mammals that several epigenetic marks and other chromatin features, such as low DNA methylation, high DNA accessibility and histone acetylation, can be used to identify and distinguish enhancers from the rest of the genome.

The aim of our study is to locate distant enhancers in a large plant genome by integrating multiple genome-wide data sets, including DNA methylation, DNase I sensitivity and H3K9 acetylation enrichment. We hereby assumed that plant enhancers have similar characteristics than mammalian enhancers. We compared two different tissues to identify tissue-specific enhancers and their target genes. The crop plant *Zea mays* has been selected because we assumed that its large intergenic regions would accommodate distant enhancers. By taking overlapping regions of the chromatin features, we predicted approximately 3,000 enhancers, which also include known and experimentally validated enhancers in maize. Animal enhancers are indicated to be bi-directionally transcribed and this is associated with two H3K27ac peaks. Our CAGE data suggests uni-directional transcription of our enhancer candidates, and this is supported by a single H3K9ac peak at the candidates A number of the newly identified putative enhancers are being tested for physical interactions with target genes by chromosomal conformation capture technology and for their enhancer function by transient or transgenic expression.

Allele-specific long distance regulation of Il-32 confers susceptibility to HIV-1 infection

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GWAS studies indicate that the non-coding variant rs4349147 is associated with HIV-1 acquisition and viral set point in sero-discordant couples. We found that this variant is located in a DNAseI hypersensitive region in CD4+ Th0 cells suggesting that rs4349147 has regulatory potential. Indeed, CRISPR-CAS9-mediated deletion of the DNAseI hypersensitive region containing rs4349147 in Jurkat cells resulted in severely reduced expression of the IL32 gene, approximately 10 Kb away. 3C experiments identified the presence of a chromatin loop between rs4349147 and the IL32 promoter, demonstrating its function as a long distance enhancer of the IL-32 gene. Using CRSPR-CAS9 gene editing, we generated single rs4349147 alleles, either A or G in Jurkat cell lines, which are heterozygous AG. We found that the activity of the IL32 enhancer element is strongly allele dependent; compared to the G-allele, the rs4349147-A allele displayed diminished IL32 expression. In addition, RNA sequencing analysis indicated a switch in the expression of Il-32 isoforms, concomitant with a reduction in the expression of factors involved in lymphocyte activation in rs4349147-A allele cells. Furthermore, Cytokine arrays showed a reduction in pro-inflammatory cytokines in rs4349147-A allele cell supernatants. Finally, rs4349147-G cells were more prone to infection with HIV virus as compared to rs4349147-A cells. Our data suggests that the rs4349147-G allele results in a pro-inflammatory environment that is more conducive to HIV infection.

Discovery of factors MEDIATing transcription

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The Mediator complex, a 1MDa complex containing 30 subunits, connects transcription factors and RNA polymerase II and thereby facilitates transcriptional regulation. Here we test the role of Mediator complex as a recruitment platform for proteins involved in transcriptional regulation. We purified Mediator complex from neural stem cells and identify its interaction partners by mass spectrometry. We find proteins known to reside at enhancers or promoters but we also discover proteins that were not previously linked to Mediator. These novel mediator-interactors colocalize with Mediator complex on the genome and displacement of Mediator complex affects their genome recruitment. Our data suggest that Mediator complex has a more widespread role as a recruitment platform for enhancer-binding proteins and identifies novel proteins for study in transcriptional regulation.

The role of Ezh2 in intestinal function and tissue maintenance during zebrafish development

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Polycomb group (PcG) proteins are essential regulators of development and epigenetic gene silencing. The PcG protein Ezh2 (Enhancer of zeste homolog 2) is a key component of the Polycomb Repressive Complex 2 and is responsible for repressing gene expression by placing the H3K27me3 epigenetic mark on the genome through its SET methyltransferase domain. Ezh2 is highly conserved in vertebrates, and *ezh2* null mice die around gastrulation. We used zebrafish to study the role of *ezh2* during development. 3 days post-fertilization (dpf), wild type *ezh2* expression is restricted to the brain, gut, branchial arches, and eyes. We identified a nonsense mutation in *ezh2*, upstream of the SET domain. Zygotic *ezh2* mutants (*Zezh2-/-*) survive gastrulation and organogenesis, and die around 11 dpf. *Zezh2-/-* larvae are smaller and leaner than wild type larvae and show severe gastrointestinal defects. At 11 dpf, enterocyte marker *fabp2* expression is non-detectable in *Zezh2-/-* larvae, indicating aberrant tissue maintenance. In histological analyses, disorganized intestinal epithelium is observed in *Zezh2-/-* larvae around the time of death. Interestingly, *Zezh2-/-* larvae ingest and excrete fluorescently labelled food comparable to wild type. Our current focus is to perform RNA- and ChIP-sequencing on dissected intestines to elucidate the alterations in the *Zezh2-/-* mutant transcriptome, and identify intestine-specific Ezh2 target genes occupied by H3K27me3 in wild type genomes. Furthermore, we will evaluate absorptive, proliferative, and apoptotic qualities of *Zezh2-/-* intestines by stainings on larval sections. Our findings suggest that the loss of Ezh2 in zebrafish causes defects in intestinal tissue maintenance.

Normal formation of zebrafish body plan in the absence of ezh2

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Polycomb group (PcG) proteins are transcriptional repressors of numerous genes, many of which regulate cell cycle progression or developmental processes. We used the zebrafish to study Enhancer of zeste homolog 2 (Ezh2), the PcG protein responsible for placing the transcriptional repressive H3K27me3 mark. We identified a nonsense mutant of *ezh2* and generated maternal zygotic (MZ) *ezh2* mutant embryos. In contrast to knockout mice for PcG proteins, *MZezh2* mutant embryos gastrulate seemingly normal, but die around 2 days post fertilization displaying pleiotropic phenotypes. Expression analyses indicated that Ezh2 is important to regulate the maternal load of mRNA in the zygote. At 24 hours post-fertilization (hpf), only a subset of genes important for early development are miss-expressed, suggesting a minor regulatory role for Ezh2 during early zygotic gene expression. Analyses of tissues arising later in development, such as heart, liver, and pancreas, indicated that Ezh2 is required for maintenance of differentiated cell fates. ChIP-sequencing performed at 24 hpf revealed that loss of function of *ezh2* is not associated with a global change in the deposition of the activating mark H3K4me3. We are currently investigating the effect of the loss of *ezh2* on enhancer activity and recruitment of the PcG complex PRC1.

The cohesin release factor WAPL restricts chromatin loop extension

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The spatial organization of chromosomes influences many nuclear processes including gene expression. The ring-shaped cohesin complex shapes the 3D genome by looping together convergent CTCF sites along chromosomes. We show here with high-resolution Hi-C analysis that chromatin loop size can be increased, and that cohesin's DNA release factor WAPL restricts the degree of this extension. WAPL also prevents looping between incorrectly oriented CTCF sites. Through haploid genetics we find that WAPL deficiency bypasses the need for cohesin's DNA loader SCC4 and we reveal that SCC4 promotes the extension of chromatin loops. We provide functional evidence in support of the model that chromatin loops are processively enlarged by the extrusion of DNA from cohesin rings. We conclude that the balanced activity of SCC4 and WAPL enables cohesin to correctly structure chromosomes to ensure proper transcriptional control.

RNA interference screens identify the methyltransferase EHMT1 as a regulator of the DNA double-strand break response

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DNA-double strand breaks (DSB) are among the most dangerous DNA lesions. Accurate and efficient repair of these lesions is critical to maintain genome stability. To this end, cells activate a DSB signalling pathway that leads to several DNA damage-associated histone modifications that make the chromatin amenable for the recruitment of DNA repair proteins.

However, the interplay between these chromatin modifications and the DSB repair machinery remains poorly understood. We therefore aimed at identifying novel chromatin modifying enzymes (CME) involved in the DSB response. A siRNA screen was performed in which we systematically determined the effect of loss of more than 300 CMEs on the accumulation of γH2AX or 53BP1 at ionizing radiation induced DSBs. Among others, we found the histone methyltransferase EHMT1 to negatively regulate 53BP1 assembly at these lesions, while γH2AX was not affected. We further show that EHMT1 is rapidly recruited to DSBs, where it promotes DSB repair via both non-homologous end- joining (NHEJ) and homologous recombination (HR). EHMT1 targets H3K9 and other proteins for methylation and we are currently investigating how these modifications affect the signalling and repair of DSBs.

Super-resolution microscopy to investigate double stranded break repair during meiosis

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During meiotic prophase I homologous chromosomes pair and undergo chromosomal crossover yielding genetically divers haploid gametes. Programmed DNA double stranded breaks (DSBs) are essential for the process of chromosomal pairing and their repair lies at the heart chromosomal crossover. Crossover can take place by alignment of homologous chromosomes on a protein scaffold called the synaptonemal complex (SC). Synapsis of the chromosomes is mediated by the hundreds of induced DSBs. During meiosis these DSBs are repaired by homologous recombination (HR). HR is an error free repair mechanism using DNA from either the sister or one of the homologous chromosomes as a DNA template. During synapsis HR takes place and is driven by two proteins the recombinases RAD51 and the meiosis specific DMC1. Both RAD51 and DMC1 can form filaments on the resected DSBs and search for a homologous DNA template by strand invasion. In contrast with HR in mitosis for which only RAD51 is required in meiosis both RAD51 and DMC1 are essential. RAD51 and DMC1 accumulate on DSB repair foci and with conventional microscopy techniques show overlapping signals. By using the super-resolution microscopy techniques SIM (structured illumination microscopy) and dSTORM (direct stochastic optical reconstruction microscopy) we were able to show that RAD51 and DMC1 do not co-localize in repair foci but occupy distinct areas within the focus.

The use of highly validated rabbit monoclonal antibodies to analyze epigenetic marks and mechanisms in disease

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Significant advances have been made in our understanding of the basic epigenetic mechanisms regulating gene expression and genomic stability, and the impact of epigenetic deregulation on cancer, inflammation, metabolism, and neurological diseases. Much of our knowledge of these mechanisms comes from the utilization of antibodies to probe the protein levels and localization of transcription factors, chromatin regulators and histone modifications in different cell and tissue types, and across the genomes of a multitude of organisms. While antibodies have been a key reagent driving advancements in epigenetic research, there are increasing numbers of publications raising concerns about the quality of the antibodies being used in biomedical research. A very concerning large number of antibodies, both commercially available and those developed by individual laboratories, have not been completely validated, some showing a lack of specificity and sensitivity even in western blot or dot blot assays. Many additional antibodies show specificity in these assays, but fail to work in more demanding assays such as immunofluorescence (IF), flow cytometry (Flow), immunohistochemistry (IHC) and chromatin IP (ChIP). Even high quality, well-validated polyclonal antibodies have issues with reproducibility, as antibody attributes often change from lot to lot. Recent advancements in antibody technologies, specifically the development of rabbit monoclonal technologies presents solutions to many of these problems. We will demonstrate how the utilization of rabbit monoclonal technology combined with thorough antibody validation can lead to generation of high quality rabbit monoclonal antibodies that show exquisite specificity, sensitivity, and reproducibility across multiple applications, including IF, IHC, flow, ChIP and ChIP-Seq.

Transcriptional regulation of human epidermal stem cell differentiation

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Gene expression is regulated on multiple levels which provides the organism flexibility to adapt to environmental cues. This may be of particular importance in the skin, where epidermal stem cells are responsible for keeping the balance between differentiation and self-renewal. In this project we aim to elucidate the role of individual transcription factors in these processes, to analyse the possible functional interaction networks between transcription- and chromatin- factors and to characterise their individual involvement in such networks.

For this, we have silenced 145 transcription factors individually in five different conditions to assess their role in the regulation of human epidermal stem cell differentiation. The overall screen data indicates that there are condition specific effects suggesting that transcription factors regulate different genes during differentiation. Additionally, we have found several factors that have a confirmed role in epidermal stem cell differentiation among the significant hits (e.g. AHR, TP63, IRF6, ETS1). Finally, we have identified some novel transcription factors potentially regulating differentiation and are currently working on their characterization.

In addition to investigating the role of individual transcription factors in differentiation we are also interested in how these genes work together. We have predicted functional interactions between transcription factors and chromatin factors by intersecting the screen data set with a previously generated data set on chromatin factors using a modular Bayesian statistical framework. We are currently investigating several of the identified factors and their predicted interactions for their underlying mechanisms through ChIP-sequencing, RNA sequencing and proteomic approaches.

Annotation of human-specific evolutionary changes at *cis*-regulatory elements in the brain

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Human evolution is characterized by the gain of unique traits such as language, complex social behavior and conceptual learning. These may be the result of an enlarged cerebral cortex, increased connectivity between brain areas and/or specialized neural gene expression programs, but how these properties have evolved is largely unclear. Over the past decades geneticists have mostly focused on protein-coding sequences to try to explain the unique features of the human brain. However, most evolutionary changes occur at noncoding genomic regions. To explore the potential role of noncoding *cis*-regulatory elements in brain evolution, we annotated enhancers in the human, chimpanzee and rhesus macaque brain using ChIP-sequencing. While the majority of enhancers is active in all three primates, we observe considerable quantitative differences between species, as well as emergence of a handful of human-specific elements. In addition, enhancer repurposing was found to occur between functionally distinct regions of the human and rhesus macaque brain; and regulatory compensation, mediated through the gain of enhancers when others were lost, was shown to keep the expression of key genes stable. Our data refine previous predictions about human-specific regulatory changes and pave the way to further uncover elements that were important for evolution of the human brain.

CTCF binding polarity determines chromatin looping

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Chromatin loops organize DNA both structurally and functionally. CCCTC-binding factor (CTCF) is a protein involved in holding chromatin loops together, most frequently at its convergently-oriented binding sites. We used CRISPR/Cas9 genome editing in murine embryonic stem cells to either a) delete, or b) invert the orientation of CTCF binding sites at three loci. In all instances of CTCF core motif deletion, chromatin immunoprecipitation (ChIP) showed that both CTCF and cohesin recruitment were lost, while 4C-seq revealed that chromatin loops with distal, convergent, CTCF sites were disrupted or destabilized. Insertion of oppositely-oriented CTCF recognition sequences restored CTCF and cohesin recruitment, but did not re-establish chromatin loops. By qPCR, we found that loop disruption can, but does not always, lead to local gene dysregulation. We hereby conclude that the binding polarity of CTCF plays a functional role in chromatin organization. In continuing research, we aim to further investigate CTCF's role in gene insulation.

GENOVA: a user-friendly Hi-C exploration package

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The increase in interest for Hi-C methods in the chromatin community has led to a need for more user-friendly analysis methods. The few currently available software packages for Hi-C do not allow a researcher to quickly summarise and visualise their data. An easy to use software package, which can generate a large range of publication-quality plots, would allow researchers to swiftly go from raw Hi-C data to interpretable results.

In the GENome Organisation Visualisation and Analysis (GENOVA) software package, we aim to provide the community with an easy to use environment. The package features both error-detection and visualisation methods for Hi-C matrices. To summarise global chromosome interaction statistics, users can generate relative contact probability plots. Here, the chance of interaction between two loci is plotted as a function of their distance. Another example is PE-SCAn, which allows the user to plot the pairwise interaction frequency of a set of genomic features (e.g. ChIP binding sites).

GENOVA is freely available and enables the analysis of Hi-C interaction matrices on commodity hardware with a basic installation of the R-environment.

Gene regulation of the cohesin-loading factor NIPBL by long-range interactions

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The cohesin complex is crucial for genome stability and shapes the three-dimensional organization of the chromatin fiber by promoting long-range interactions, including promoter-enhancer interactions, together with the chromatin insulator CTCF. Mutations in cohesin complex components or cohesin regulators are found in various types of cancer and human developmental syndromes, termed "Cohesinopathies". However, the mechanism how cohesin mutations lead to disease is still unclear.

The *NIPBL* gene is mutated in more than 60% of the cases of Cornelia de Lange syndrome (CdLS), a developmental disorder characterized by craniofacial anomalies, upper limb malformations and multiple other system abnormalities. Interestingly, cells from patients but also animal models with a heterozygous loss of one *NIPBL* allele are able to maintain 70% wt *NIPBL* transcript levels. This triggered our interest to investigate the transcriptional regulation of the *NIPBL* gene, also with the aim to identify regulatory elements relevant for the diagnostics of CdLS. Interestingly, this gene is also regulated by long-range interactions involving CTCF binding sites. We have identified a conserved distal enhancer for *NIPBL* that is recruited to the NIPBL promoter via interactions between specific CTCF sites. This enhancer regulates the expression of NIPBL but also of the long noncoding RNA *NIPBL-AS1* located upstream and transcribed antisense to *NIPBL*. The *NIPBL-AS1* transcript itself has no role for the activity of the *NIPBL* gene, but we found that the actual transcription of the lncRNA is important to maintain NIPBL transcript levels, providing an interesting insight into the fine-tuning of NIPBL expression.

A PALB2-interacting domain in RNF168 couples homologous recombination to DNA break-induced chromatin ubiquitylation

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DNA double-strand breaks (DSB) elicit a histone ubiquitylation cascade that controls DNA repair pathway choice. This cascade involves the ubiquitylation of histone H2A by the RNF168 ligase and the subsequent recruitment of 53BP1 and RIF1. RIF1 suppresses homologous recombination (HR) by posing a barrier to DNA end resection in G1 phase. This barrier is relieved in S/G2 cells through the action of BRCA1, which favors HR-dependent DSB repair through the assembly of PALB2, BRCA2 and the recombinase RAD51. With the inhibitory impact of RIF1 relieved, it remains unclear if RNF168-induced ubiquitylation influences HR during S/G2 when this repair pathway reaches maximal activation. Here, we uncover a role for RNF168 in coupling the HR machinery to H2A ubiquitylation in S/G2 cells. We show that PALB2 acts as an indirect reader of histone ubiquitylation by physically associating with ubiquitin-bound RNF168. This interaction is mediated by the newly identified PALB2-interacting domain (PID) in RNF168 and the WD40 domain in PALB2, and drives efficient HR by promoting the assembly of DNA repair complexes at DSBs. Our findings uncover that RNF168 couples PALB2-dependent HR to H2A ubiquitylation, which illuminates how RNF168 drives chromatin remodeling to promote DSB repair and preserve genome integrity.

Epigenomic regulation in pediatric Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is a heterogeneous disease where hematopoiesis is disturbed by impaired differentiation and increased proliferation of progenitor cells. Pediatric AML has a high incidence of relapse (~40%) and overall survival rates between 60-70%. There is increasing evidence that epigenomic deregulation is involved in cancer, including adult AML. For example, histone methyltransferase MLL is frequently translocated in pediatric AML, what results in an altered epigenome. Importantly, epigenetic signatures at distal enhancers are highly cell state specific. This study analyses the chromatin landscape of pediatric AML patients at diagnosis and relapse to identify an epigenetic program associated with AML cellular behavior and disease evolution. ChIP-sequencing of the active histone mark H3K27ac shows genome-wide high similarity of diagnosis and relapse samples within patients. In addition, patients that harbor the same molecular aberration (such as a Flt3-ITD or MLL-translocation) cluster together based on H3K27ac enrichment. Two interesting regions that are highly enriched in Flt3-ITD patients cover the HOXB cluster and the homeobox transcription factor NKX2-3. This suggests a regulatory role of homeobox proteins in these relapsed Flt3-ITD patients. To identify whether such regions are specific for a relapsing AML, the epigenome of non-relapsed AML patients will be analyzed.

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For genetically heterogeneous diseases a better understanding of how the underlying gene defects are functionally interconnected will be important for dissecting disease etiology. The Immunodeficiency, Centromeric instability, Facial anomalies (ICF) syndrome is a chromatin disorder characterized by mutations in DNMT3B, ZBTB24, CDCA7 or HELLS. Here, we generated a Zbtb24 BTB domain deletion mouse and found that loss of functional Zbtb24 leads to early embryonic lethality. Transcriptome analysis identified Cdca7 as the top down-regulated gene in Zbtb24 homozygous mutant mESCs, which can be restored by ectopic ZBTB24 expression. We further demonstrate enrichment of ZBTB24 at the CDCA7 promoter suggesting that ZBTB24 can function as a transcription factor directly controlling Cdca7 expression. Finally, we show that this regulation is conserved between species and that CDCA7 levels are reduced in patients carrying ZBTB24 nonsense mutations. Together, our findings demonstrate convergence of the two ICF genes ZBTB24 and CDCA7 at the level of transcription.

The 3-dimensional organization of the bacterial genome and its interplay with gene expression

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Fully unfolded, the circular, 4.64 Mb-long chromosome of *Escherichia coli* K-12 has a diameter of 0.5 mm — 500 times larger than the diameter of a typical *E. coli* cell. Therefore, the bacterial genome needs to be compactly folded to be packaged inside the bacterial cell and at the same time, remain accessible to proteins involved in DNA transactions. The compaction of the bacterial chromosome is driven by Macromolecular crowding in the bacterial cytoplasm and the activity of Nucleoid Associated Proteins (NAPs). NAPs are architectural proteins that bind consistently along the bacterial chromosome and influence its topology by bending, wrapping, and bridging chromosomal segments. They also function as transcription factors and regulate gene expression by directly sensing and responding to environmental cues such as changes in salt concentration or temperature. The dual role of NAPs suggests that the proteins may play a role in regulating gene expression in bacteria by coupling transcriptional activity to the 3-dimensional organization of the chromosome. We use High-Resolution Chromosome Conformation Capture to study the organization of the bacterial genome and to understand the interplay between chromatin structure and gene expression.

Molecular mechanism of nucleosome remodeling by ISWI

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ISWI is a subfamily of ATP-dependent chromatin remodelers that are involved in nucleosome sliding and spacing. To date, no crystal structure is available of the full length ISWI and the molecular mechanism of nucleosome remodeling by ISWI is still under debate. Therefore, we want to study the structure of ISWI and its dynamic interaction with the nucleosome by NMR. The N-terminal region (NTR) of ISWI contains an AutoN motif, thought to be involved in autoinhibition by competing for binding to ISWI's catalytic ATPase domain with the histone H4 tail. So far, preliminary NMR and CD data show that the NTR is mainly disordered, although it may also have a small alpha-helical portion. In further NMR studies, we will investigate how the NTR interacts with the ATPase domain and if this interaction can indeed be attenuated by the presence of a histone H4 tail peptide. In the future, we will also study the interactions of the ATPase domain with the nucleosome, to determine the binding site and the role of the nucleosomal DNA in this binding. This will give us more insight into the molecular mechanism of ISWI remodeling.