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Saturday April 4

LONG RANGE CONTROL OF GENE EXPRESSION

Chair: Wendy Bickmore

IL.16 08.30 – 09.15

CHROMATIN MODIFICATIONS DIFFERENTIATE IMPRINTED GENES AND TISSUE-SPECIFIC SILENCED GENES

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We have used ChIP-Chip and ChIP-Seq to analyze chromatin modification in MEFs on mouse Chr.17 that contains the imprinted Igf2r gene cluster and applied an algorithm named BLOCs (Broad Local enrichments), that allows the identification of spreading histone modifications. Our results show that silent tissue-specific genes and non-transcribed intergenic regions are contained in large K3K27me3 BLOCs, indicating a negative correlation between H3K27me3 and transcription for these genes (Pauler et al., 2008). However silent alleles of the imprinted Igf2r and Air genes, lack this modification and instead contain focal peaks of heterochromatin only at their promoters that contains all known marks of classic heterochromatin i.e., H3K9me3, H3K20me3 and HP1 (Regha et al., 2007). Thus imprinted gene silencing may not model epigenetic silencing of tissue-specifically expressed genes. Our recent analysis of the onset of imprinted expression in development provides a molecular explantation for these differences. We show, using an ES imprinting model that contrary to expectation, that imprinted Igf2r expression arises not by silencing the paternal Igf2r promoter, but by preventing its upregulation (Latos et al., Development in press).

References:

[1] Pauler FM, Sloane MA, Huang R, Regha K, Koerner M, Tamir I, Sommer A, Aszodi A, Jenuwein T, Barlow DP. Genome Res. 2008 Dec 1. [Epub ahead of print]
[2] Regha K, Sloane MA, Huang R, Pauler FM, Warczok KE, Melikant B, Radolf M, Martens JH, Schotta G, Jenuwein T, Barlow DP. Mol Cell. 2007 Aug 3;27(3):353-66.
[3] Latos PA, Stricker SH, Steenpass L, Pauler FM, Huang R, Senergin BH, Regha K, Koerner MV, Warczok KE, Unger C, Barlow DP. Development 2009 in press.

O14 09.15 – 09.30

GENOMIC DNA HYPOMETHYLATION BY HDAC INHIBITION INVOLVES DNMT1 RELEASE FROM NUCLEAR COMPONENTS

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Introduction: Histone deacetylase inhibitors are promising anti-tumor drugs acting through reactivation of epigenetically silenced tumor suppressor genes. Several HDAC inhibitors are currently in clinical trials both for haematological and solid tissue malignancies. A synergistic effect by HDAC inhibitors (HDACi) and DNA methylation inhibitors on cancer cells suggests the possibility of an interaction mechanism between these two classes of drugs.
Methods & Materials: Here, we have analyzed the effect of HDACi TrichostatinA (TSA) treatment on DNA methylation in Hep3B cells. We have used several molecular techniques such as LUMA, Bisulfite-sequencing and confocal microscopy to analyze the effect of TSA on DNA methylation machinery.
Results: Our data indicate that TSA treatment reduces the level of global DNA methylation, with some gene specificity. Analysis of fluorescent-tagged DNA methyltransferases (DNMTs) by flourescence correlation spectroscopy (FCS) revealed that TSA treatment has a direct effect on their nuclear mobility. The movement of DNMT3a and DNMT3b was attenuated after TSA treatment while DNMT1 moved faster. These data suggest that TSA treatment changes the interaction between DNMTs and nuclear components in a DNMT-specific manner. In addition, TSA treatment reduced the expression of DNMT3a and DNMT1 at the protein level.
Conclusions: These data may shed light over the synergistic effect of HDAC inhibitors and DNA methylation inhibitors previously reported in several

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studies and may also help to define improved policies for cancer treatment.

O15 09.30 – 09.45

HISTONES DEACETYLASES AS NOVEL FACTORS OF HEAT SHOCK RESPONSE

Sabrina Fritah-Hnida; Edwige Col; Cyril Boyault; Susanne Chiocca; Saadi Khochbin; Caroline Jolly; Claire Vourc'h.
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Heat shock induces major changes in gene expression. Besides the up-regulation of specific genes, among which hsp genes, heat shock also induces a general shut-down of gene expression underlined by a global chromatin remodelling. While the mechanisms involved in the heat-induced activation of hsp genes have been investigated in details, the molecular events associated with the global down-regulation of the genome are poorly understood. By using a combination of in situ and molecular approaches, we have shown that heat shock induces a massive and reversible deacetylation of core histones affecting specific epigenetic marks of histones H3 and H4. We have characterized the molecular mechanisms underlying the heat-induced deacetylation, and have identified HDAC1 (Histone Deacetylase 1) and HDAC2 as the key actors of this event. Interestingly, we found that HDAC1 and HDAC2 are complexed with HSF1 (Heat shock factor 1), in a stress dependent manner. Using HSF1-/- Mefs, we showed that HSF1 is involved in the control of H4 acetylation level before and during stress. Finally, the impact of HDAC1 and HDAC2 on capsase 3 mediated apoptosis after heat shock has been investigated. Taken together this study identifies HDAC1 and HDAC2 as novel important factors of heat shock response and reveals a new role for HSF1 in the control of histone acetylation..

IL.17 09.45 – 10.30

GENE REGULATORY NETWORKS DURING DEVELOPMENT: DISSECTING THE LOGIC

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One of the central challenges in biology is to understand how the genome is utilized to orchestrate the development of complex tissues and organisms. While genetic studies have identified a number of essential transcription factors required for cell fate specification, little is known about the molecular mechanisms by which these regulators function. Few of their direct target genes or effector molecules are known. Moreover, the architecture of the underlying transcriptional network in which they operate remains elusive. Our work attempts to bridge this gap, by integrating genetic, genomic and computational approaches to understand the transcriptional network that drives the selection of cell fates within the mesoderm. By combining ChIP-on-chip through a time-course of Drosophila development we are systematically identifying cis-regulatory module occupancy during developmental progression. These data are enriched by expression profiling of mutant embryos for each transcription factor. The topology of the network was unexpected, showing extensive combinatorial regulation and temporal enhancer occupancy. Current work is focused on understanding how these diverse combinatorial binding 'codes' give rise to specific patterns of enhancer expression.

References:

[1] Bonn S, Furlong EE. cis-Regulatory networks during development: a view of Drosophila. Curr Opin Genet Dev. 2008 Oct 16.

[2] Jakobsen JS, Braun M, Astorga J, Gustafson EH, Sandmann T, Karzynski M, Carlsson P, and Furlong EE (2007). Temporal ChIP-on-chip reveals Biniou as a universal regulator of the visceral muscle transcriptional network. Genes & Development. 2007 Oct 3; 21: 2448-2460.

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[4] Sandmann T, Jensen LJ, Jakobsen JS, Karzynski MM, Eichenlaub MP, Bork P, Furlong EE (2006). A temporal map of transcription factor activity: Mef2 directly regulates target genes at all stages of muscle development. Dev Cell, June; 10(6): 797-807.

10.30 – 11.00 TEA AND COFFEE

IL.18 11.00 – 11.45

THREE-DIMENSIONAL FOLDING OF

CHROMOSOMAL DOMAINS IN RELATION TO GENE EXPRESSION

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We have proposed that the genome can be viewed as a three-dimensional network that is driven by physical and functional associations between genomic loci (Dekker, Nature Methods 2006; Science 2008). Comprehensive analysis of this genomic network through large-scale chromatin interaction mapping will provide important information about the spatial organization of chromosomal domains, the relationships between genomic elements, and will reveal the logic of long-range gene regulation.
Recently we developed the Chromosome Conformation Capture - Carbon Copy (5C) technology that combines 3C with deep-sequencing for high-throughput mapping of interactions between genomic elements (Dostie et al. Genome Res. 2006). Using 5C we have generated comprehensive interaction maps of Mb-sized domains in the human genome. We will present results that indicate widespread long-range interactions among genes, enhancers and insulator/boundary elements, providing new insights into the nature and potential functional role of these associations.
The unique power of 5C as compared to other high-throughput 3C based methodologies is that exceptionally dense interactions matrices can be generated for large chromosomal domains. The comprehensive nature of these interaction maps allows the determination of the overall spatial conformation of chromatin at unprecedented resolution. We will present a novel approach to convert 5C interaction maps into 3D models of higher order chromatin organization.

O16 11.45 – 12.00

IDENTIFICATION OF NUCLEOLAR ASSOCIATED CHROMATIN LOCI REVEALS CLUES FOR NUCLEAR ARCHITECTURE

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Introduction: The nuclear position of chromosomes in distinct territories is non-random, and it is likely that this organisation of chromosomes affects gene expression. However, relatively little is known about the role of nuclear bodies in the organization of chromosomal regions. The most prominent nuclear body is the nucleolus, whose major function is the biogenesis of ribosome subunits. The nucleolus has been linked also to multiple forms of human disease, where altered nucleolar function and/or structure is evident, such as several types of cancer. Nucleoli are assembled around chromosomal loci containing ribosomal DNA (rDNA) genes. In addition to rDNA, other chromatin sequences also surround the nucleolar surface and possibly even loop into the nucleolus. However, at present little is known about the role or composition of these nucleolar-associated chromatin regions. Our aim is to identify and characterize non-rDNA chromatin sequences that associate with nucleoli, and to study the role they may play in nucleolar and nuclear structure and functions.
Methods & Materials: We used DNA samples derived from highly purified nucleoli and from total nuclear DNA in two parallel approaches. First, the DNA samples were labelled and used as probes for FISH analyses on Comparative Genome Hybridization (CGH) slides, containing human lymphocyte metaphase spreads. Hybridization of the rDNA clusters confirmed the specificity of the enriched nucleolar DNA. Second, we used the same human DNA samples derived from purified nucleoli and total genomic DNA to identify specific nucleolar-associated chromatin loci by Illumina-Solexa deep sequencing; a powerful technique that sequences millions of short 35 bp DNA fragments in parallel.
Results and Conclusions: Our results show specific non-rDNA clusters to be enriched in DNA isolated from purified nucleoli. Combined bioinformatics and statistical analyses of the resulting sequence data so far have identified candidate regions of the human genome that may preferentially associate with nucleoli. We are currently analysing these by FISH to confirm independently their association with the nucleolus, to identify in what fraction of cells each region is associated with nucleoli and to study the functional importance of these associations. Importantly, in addition to these highly interestingly individual loci, our deep-sequencing approach allows us to study the nuclear, and particularly the nucleolar architecture in general. Our preliminary

results show specific regions of human chromosomes to be associated with nucleoli, most likely reflecting the position of those chromosomes within the nucleus, and thereby possibly affecting gene expression.

IL.19 12.00 – 12.45

MOLECULAR MECHANISMS REGULATING THE EPIGENETIC STATE OF RRNA GENES

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Epigenetic control mechanisms silence half of the ribosomal RNA genes (rDNA) in eukaryotes. Silencing is brought about by NoRC, a SNF2h-containing remodeling complex, that recruits chromatin modifying activities and establishes heterochromatic features at the rDNA promoter, including specific histone modifications, de novo DNA methylation and recruitment of HP1. Association with nucleolar chromatin and transcriptional repression requires the interaction of TIP5, the large subunit of NoRC, with RNA that originates from the intergenic spacer and overlaps the rDNA promoter. These intergenic transcripts are processed into 150-300 nt RNAs (pRNA) and are stabilized by binding to NoRC. Antisense-mediated depletion of pRNA leads to displacement of NoRC from nucleoli and decreases rDNA methylation, whereas overexpression of pRNA leads to heterochromatin formation, de novo DNA methylation and transcriptional repression. Data will be presented showing that pRNA recruits DNMTs to the rDNA promoter, suggesting a NoRC-independent role in rDNA silencing. Moreover, we show that Gadd45a (growth arrest and DNA damage inducible protein 45 alpha) counteracts NoRC-dependent rDNA silencing. Demethylation of rDNA is initiated by recruitment of Gadd45a to the rDNA promoter by TAF12, a TBP-associated factor that is contained in Pol I and Pol II-specific TBP-TAF complexes. Once targeted to rDNA, Gadd45a triggers demethylation of promoter-proximal DNA by recruiting the nucleotide excision repair (NER) machinery to remove methylated cytosines. Knockdown of Gadd45a, XPA, XPG, XPF, TAF12 or treatment with drugs that inhibit NER causes hypermethylation of rDNA, establishes heterochromatic histone marks and impairs transcription. The results demonstrate that methylation and rDNA silencing is a dynamic and reversible process and suggest a mechanism that recruits the DNA repair machinery to the promoter of active rRNA genes, keeping them in a hypomethylated state.

References:

[1] Mayer et al (2006). Intergenic transcripts regulate the epigenetic state of rRNA genes. Mol Cell. 22, 351-361.
[2] Grummt I. (2007). Different epigenetic layers engage in complex crosstalk to define the epigenetic state of mammalian rRNA genes. Hum Mol Genet. 16, R21-27.
[3] McStay B, Grummt I. (2008).The epigenetics of rRNA genes: from molecular to chromosome biology. Annu Rev Cell Dev Biol. 24, 131-157.

12.45 – 13.45 LUNCH

Chair: Job Dekker

IL.20 13.45 – 14.30

THE RELATIONSHIP BETWEEN THE NUCLEAR PERIPHERY AND GENE EXPRESSION IN HUMAN CELLS

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In mammals, regions with low transcriptional activity (gene-density) are located close to the nuclear periphery. Inactive transgenes and some endogenous inactive genes also locate close to the nuclear periphery and their movement away from there correlates with transcriptional activation. However, a crucial issue is whether this level of nuclear organisation directly affects gene function, rather than merely reflecting it. To provide direct experimental evidence that proximity to the nuclear periphery can facilitate transcriptional repression in mammalian cells we relocated lacO-tagged human chromosomes to the nuclear periphery via interaction with lac repressor that is fused to proteins of the inner nuclear membrane. We show that this can reversibly down-regulate the expression of some endogenous human genes located near the lacO sites. However, other genes appear unaffected and we show that location at the nuclear periphery is not incompatible with active transcription. We will discuss the mechanism of this transcriptional suppression. Evidence to suggest that this mechanism may influence gene expression in cases of human genome rearrangements will be presented.

O17 14.30 – 14.45

VISUALISATION OF HETEROCHROMATIN-MEDIATED SILENCING: TARGETING OF HP1 AND SUV39H1 TO CHROMOSOMAL REGIONS IN VIVO IN MAMMALS

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Over 95% of the murine and human genomes are comprised of non-coding DNA elements. It is becoming apparent that such non-coding DNA plays a role in gene regulation and can regulate the formation and extent of chromatin domains. Specific repetitive elements are thought to perform this function, for example by acting as chromatin insulators, encoding regulatory RNAs, or providing nucleation sites for heterochromatin. Therefore, the expression of a gene is dependent on the local sequence environment in which the gene is embedded. Heterochromatin in particular, is highly enriched in repetitive DNA sequences, and has the characteristic ability to spread from localised nucleation sites, thereby regulating gene expression in a region-specific manner. Typically, this leads to epigenetic repression of target genes. Interestingly, heterochromatin is also known to be required for activation of several genes, suggesting that the sensitivity of genes to heterochromatic silencing is dependent on the sequence composition of its chromosomal location. We have developed a novel genome browser with the unique ability to simultaneous visualise transcriptional microarray and chromatin immunoprecipitation data along linear chromosomes, allowing the identification of co-regulated genes in multiple mutants where chromatin modifiers have been disrupted. In addition, the browser displays the location of non-coding DNA elements, enabling us to analyse the composition of chromosomal domains with respect to DNA sequence motifs. We will present how we have used these tools to identify endogenous genes and chromosomal regions that respond in different ways to changes in the dosage of Suv39h1 and HP1 in ex vivo murine T cells, and how differences in sensitivity to these factors correlates with the presence of distinct DNA motifs. Supported by the Medical Research Council.

O18 14.45 – 15.00

FUNCTIONAL INTERACTIONS OF CONSERVED NON-CODING (CNC) SEQUENCES WITH OTHER CNC USING CIRCULAR CHROMOSOME CONFORMATION CAPTURE (4C)

Daniel Robyr; Marc Friedli; Corinne Gehrig; Mélanie Arcangeli; Marilyn Marin; Alexandra Quazzola; Sonia Verp; Didier Trono; Stylianos E. Antonarakis.
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The comparison of human chromosome 21 (Hsa21) sequences with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of a least 100 nucleotides of ungapped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs). Very little is known about the function of most CNCs. We speculated that a functional CNC might interact with its genomic target (i.e. an enhancer would bind to it's cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by chromosome conformation capture (4C) whose identity is determined by subsequent high-throughput sequencing. We have identified genomic loci that interact physically with a selection of 10 CNCs from human chromosome 21. We have uncovered evidences that CNCs are capable not only to interact with loci on other chromosomes but that these are enriched for CNCs as well. Interestingly, we have identified two evolutionary conserved DNA elements (separated by 89kb) that have the ability to interact with the oligodendrocyte gene OLIG2 and that drive the expression of a reporter gene in the mouse embryo in regions corresponding to its native expression. This study also demonstrates how powerful chromosome conformation capture is when applied to the identification of functional DNA elements targets.

O19 15.00 – 15.15

DYNAMIC ORGANIZATION OF NUCLEAR LAMINA ASSOCIATED CHROMATIN DURING DIFFERENTIATION

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Introduction: A recent high-resolution genome-wide mapping study in our group has systematically identified genomic regions that interact with the nuclear lamina in human fibroblasts (Guelen et al 2008, Nature 453:948-951). These so called Lamina Associated Domains (LADs) were found to be large contiguous regions (100 kb- 10 Mb) with low gene density and low gene expression, often flanked by one or more of three features, CTCF binding, CpG islands, and promoters oriented outwards from the LAD. In an effort to further elucidate the rules that underlie the formation of LADs, we study the dynamics of LAD organization during differentiation.
Methods & Materials: We constructed full-genome lamina-association maps in Embryonic Stemcells (ESs), Neuronal Precursor Cells (NPCs) derived from ESs, and Mouse Embryonic Fibroblasts (MEFs), using DamID of LaminB1.
Results & Conclusions: We found that all 3 cell-types contain LADs and that these LADs are very similar in size distribution and general features. Comparison of LADs in the 3 cell-types revealed that the majority of LADs is constant between the cell-types, however there is also a significant subset of LADs unique to each cell type. Although the lamina is generally a repressive environment, we find a subset of genes that are actively transcribed while associated with the lamina. In these genes only the promoter dissociated from the Lamina. However, for the majority of genes located in LADS, increase in transcription is strongly correlated with loss of Lamina association. Genes that lose Lamina association are enriched for cell-type specific GO categories (e.g. neuronal differentiation for NPCs). Furthermore, the loss of association is mostly limited to the transcription unit and does not affect the flanking intergenic regions. This suggests that, rather than large reorganization of LAD structure, differentiation and associated transcriptional changes affect LAD and chromosome organization mostly in a topical manner.

O20 15.15 – 15.30

DYNAMIC, TRANSCRIPTION-INDEPENDENT HISTONE MODIFICATIONS AT HOXB PROMOTERS IN EMBRYONIC STEM CELLS

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Introduction: Histone modifications have been closely associated with changing levels of gene expression, but their role in determining, or possibly predicting, patterns of expression is uncertain. Here we explore the link between histone modifications and Hoxb gene expression in mouse embryonic stem (ES) cells.
Methods: Native chromatin immunoprecipitation using the antibodies to H3K9ac, H3K4me3 and H3K27me3 was performed on chromatin from untreated ES cells and cells exposed for varying times to the deacetylase inhibitor and teratogen sodium valproate.
Results: Levels of the "active" modifications H3K9ac and H3K4me3 at Hoxb promoters varied widely from gene to gene, but were closely correlated. Hoxb9 showed much the highest level of active modifications and Hoxb3 the lowest, but otherwise there was no overall correlation between modification levels and position in the cluster. The repressive modification H3K27me3 was found at equivalent levels across the cluster. Treatment with valproate induced a significant increase in the levels of H3K9ac and H3K4me3 at all Hoxb promoters, but not other genes, whilst H3K27me3 was unaffected. All Hoxb genes were silent in undifferentiated ES cells, but expression was activated at defined times of differentiation in the expected 3' to 5' sequence, Hoxb1 after two days and Hoxb9 beyond eight days. The valproate-induced increase in active modifications did not induce Hoxb expression from the cluster in undifferentiated cells, nor did we see any major shift in the timing of Hoxb expression in cells transiently exposed to valproate (ie. hyperacetylated) during the first eight hours of differentiation.
Conclusions: Despite the absence of detectable transcription, the active modifications H3K9ac and H3K4me3 vary widely across the Hoxb cluster and are closely linked. Artificially induced hyperacetylation in undifferentiated cells is not sufficient to trigger Hoxb expression, or to disrupt the temporal sequence of Hoxb expression on differentiation.

15.30 – 16.00 TEA AND COFFEE

IL.21 16.00 – 16.45

GENOME STRUCTURAL VARIATION

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In my talk I will touch on some of the latest developments in the identification and analysis of copy number variants both in normal populations and in disease. As array-CGH resolution has developed we have been able to identify smaller copy number changes in individuals. Using a set of 20 NimbleGen arrays each containing 2.1 million probes and tiling the human genome with long oligonucleotides we have been able to identify CNVs as small as 500 bp. We have used this array set to screen 40 normal individuals and have made 51,981 CNV calls with on average 1,300 CNVs per comparison (18-30 Mb) and corresponding to 9,299 discrete CNV loci which cover 4.8% of the human genome (135.6 Mb) thus greatly extending the number of known CNVs. More importantly, the high resolution of this array set has allowed us to more accurately define CNV breakpoints and go on to design specific arrays for CNV genotyping which will allow the application of CNV in association studies. We have been able to extend the number of CNV genotyping assays from a few hundred to approximately 5,000 and these are now being applied in large association studies such as the Wellcome Trust Case Control Consortium studies CCC1+ (19,000 samples) and CCC2 (~100,000 samples). For patients with developmental disorders, these genotyping assays have the potential to allow us to determine the role ofcommon, apparently benign CNVs in modifying disease severity and onset.

In the last part of my talk, I will describe how new generation sequencing is already being used to identify structural arrangements and mutations in normal individuals and patients and discuss the potential of this technology to replace DNA arrays for identification of copy number changes.

O21 16.45 – 17.00

ORGANIZATION AND EVOLUTION OF CENTROMERIC REGIONS IN THE WHITE-CHEEKED GIBBON

Angelo Cellamare; Francesca Antonacci; Marika Malig; Evan E. Eichler; Mario Ventura.
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Alpha-satellite is a family of tandemly repeated DNA found at the centromeric regions of all human and primate chromosomes. Its evolutionary history is incomplete due to the difficulty in sequence assembly and rapid evolution when compared to most genomic sequences. As revealed by patterns of monomer organization, there are two major types of alpha-satellite DNA in the human genome, designated higher-order and monomeric. Higher-order-satellite DNA is made up of monomers arranged in multimeric repeat units that are highly similar from repeat unit to repeat unit. In contrast, monomeric a-satellite lacks detectable higher-order periodicity, and its constituent monomers are far less homogeneous than are higher-order repeat units. Using several approaches, we have characterized a-satellite centromeric sequences from the white-cheeked gibbon Nomascus Leucogenys (NLE). Our sequence analyses demonstrate that gibbon a-satellite sequences are formed by a monomeric unit of ~171 bp and they lack high-order structure and subfamily organization found in humans. These data suggest, according to the evolutionary distances between gibbon species and human, the hypothesis that higher order a-satellite DNA emerged more recently respect to the monomeric structure during the evolution. FISH characterization showed for the first time, in addition to the centromeric localization, telomeric and chromosomal interstitial a-satellite localizations. Three colors FISH experiments using intertitial NLE a-satellite clones, human and gibbon BAC clones spanning NLE evolutionary breakpoint on NLE chromosomes 3,5,9,14 showed a colocalization of these three probes. (http://www.biologia.uniba.it/gibbon)
These results strongly support the hypothesis that the interstitial a-satellite regions owing to their highly repetitive nature might have represented preferential sites of chromosome breakages thus contributing to the occurrence of rearrangements within the gibbon karyotype.

O22 17.00 – 17.15

CHROMOSOME INSTABILITY IS COMMON IN HUMAN CLEAVAGE STAGE EMBRYOS

Thierry Voet[1 *]; Evelyne1 Vanneste[2 *]; Cédric Le Caignec[1, †]; Michèle Ampe[3]; Peter Konings[4]; Cindy Melotte[1]; Sophie Debrock[2]; Mustapha Amyere[5]; Miikka Vikkula[5]; Frans Schuit[6]; Jean-Pierre Fryns[1]; Geert Verbeke[3]; Thomas D'Hooghe[2]; Yves Moreau[4]; Joris R. Vermeesch[1].
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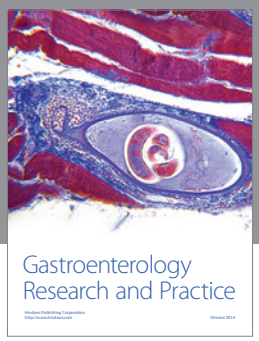
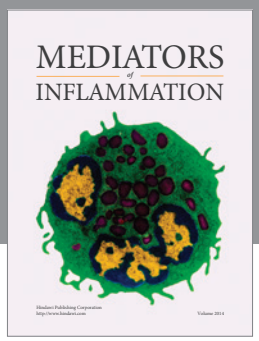
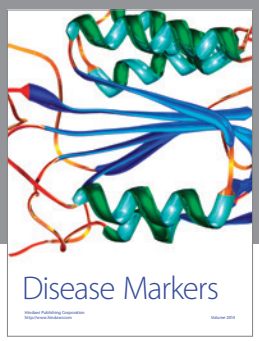
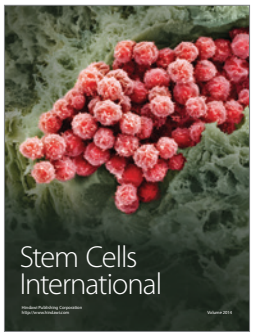
No abstract available.

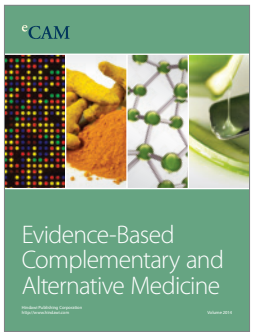
17.15	**POSTER PRIZE**
1830 – 19.00	**COACH TO DINNER**
19.00 – 20.00	**RECEPTION**
20.00 – on	**CONFERENCE DINNER**

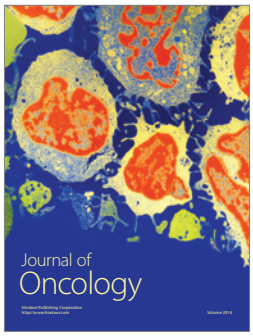


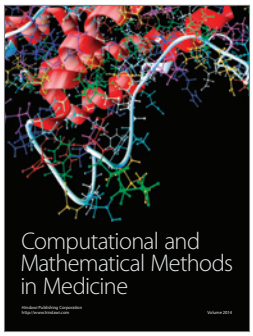


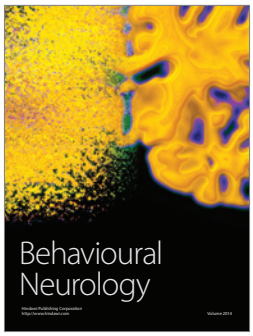


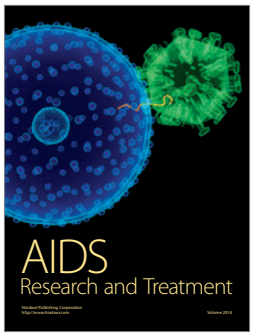


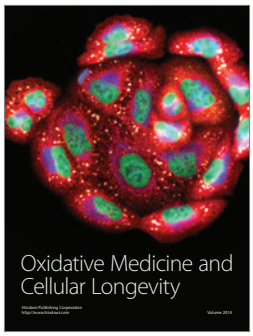












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