contrast to WT, T-bet/- showed an attenuated vascular dysfunction, ROS production and expression of NADPH oxidase subunits in response to AT II. Furthermore, mRNA expression and protein tyrosine nitration in the aorta was markedly reduced in T-bet/- mice which showed a significant decrease of macrophages in the vascular wall caused by a reduced presence of pro-inflammatory cells after AT II treatment compared to WT. In a rescue experiment we transferred WT CD4+ T cells in T-bet deficient mice which resulted in increased vascular oxidative stress and dysfunction. We conclude, that T-bet mediates, at least in part, ATII induced vascular damage and might represent a novel target to treat vascular dysfunction and inflammation in arterial hypertension.

4248-Pos Board B198
Do Multiple PHDS Matter? An Analysis of the Dual PHD Domains of Rco1
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Post-translational modifications (PTMs) on histone tails are important for many DNA-templated processes, including transcription elongation by RNA polymerase II (RNAPII). Histone PTMs directly alter the accessibility of chromatin and serve as binding sites for effector proteins that mediate numerous downstream biological processes. During transcription elongation in budding yeast (Saccharomyces cerevisiae), the C-terminal domain of RNAPII is hyperphosphorylated at serines 2 and 5 (Ser2 and Ser5) of the YSPTSPS consensus repeat. This has been shown to be coupled to ultrathin nuclear lamina (HMT) recruitment to phosphorylated Ser2 and Ser5, promoting the catalysis of lysine 36 (H3K36) methylation on the nucleosomal H3 tail in the bodies of genes. H3K36 methylation is recognized by the Rpd3S histone deacetylase (HDAC) complex, consisting of Rpd3, Sin3, Ume1, Rco1, and Eaf3. Deacetylation of histones behind elongating RNAPII has been shown to suppress cryptic initiation of transcription within the bodies of genes by decreasing chromatin accessibility. While it has been shown that recognition of H3K36 methylation by Rpd3S requires the chromodomain of Eaf3 and the first PHD domain (PHD1) of Rco1, little is known regarding the importance of other potential histone-interacting domains within the complex. We are specifically interested in the second PHD domain (PHD2) of Rco1 due to its sequence homology to PHD1. We hypothesize that the second PHD domain of Rco1 contributes to the recognition of the Rpd3S complex on nucleosomes. Using a combination of yeast genetics and in vitro biochemical and biophysical assays, we are gaining a better understanding of the functional role of Rco1 PHD2. This study will lend insight into the importance of recognition of histone modifications by multi-domain protein complexes, and will allow us to better understand the function of the Rpd3S complex.

4249-Pos Board B199
Robust and Sensitive Control of Ammonium Sequestration in Escherichia coli
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The sequestration of essential nutrients is vital for organisms. Some nutrients, in particular gas molecules such as CO2 and NH3, are readily diffusible across the cell membrane. The large membrane permeability of these molecules is both a blessing and a curse: It obviates the need of transporters when the ambient nutrient level is high; however, when the ambient level is low, maintaining a high intracellular nutrient level against passive back diffusion is both challenging and costly. Here we study the delicate management of ammonia (NH3) sequestration by exponentially growing E. coli cells, using microfluidic chambers which provide steady, low ammonium concentrations not attainable previously in batch or continuous culture. Ammonium is the preferred nitrogen source for many microorganisms including E. coli, and the membrane protein AmbB is crucial for maintaining rapid cell growth at low ambient ammonium levels. Using a combination of time-lapse microscopy, gene expression and flux analysis, we show that as the ambient ammonium concentration is reduced, E. coli cells first maximize their ability to assimilate ammonia diffusing into the cytoplasm before abruptly activating AmbB for ammonium transport. The onset of ammonium transport varies under different growth conditions, always just barely maintaining the internal ammonium concentration such that cells can grow as if ammonia is replete. Quantitative data analysis along with mathematical modeling reveal an integral feedback strategy by which two sensors of the cellular nitrogen status are used to provide seamless coordination of the two lines of defense against ammonium shortage. Implementation of this tight control on ammonium sequestration ensures that the expensive cost of upholding the internal ammonia concentration against back diffusion be kept at a minimum, a survival strategy possibly employed by a variety of organisms in dealing with essential but rapidly diffusible substances.

4240-Pos Board B200
Designed Red Fluorescent Calcium Sensor with Ultrafast Kinetic Properties
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Transient change of cytosolic calcium level leads to physiological actions, which are modulated by the intracellular calcium store, as well as membrane calcium channels and vesicles/endosomes. To probe calcium responses in such high calcium environments simultaneously with the calcium transients in intracellular plasma, there is a pressing need to develop a calcium sensor featured with 1) fast kinetic properties, 2) a different spectral window from other calcium indicators, and 3) pH-independent fluorescence change. In this study, we first report our development of fast red calcium sensors using our protein design instead of using endogenous calcium binding proteins or natural calcium binding motifs. Calcium binding results in fluorescence signal increase with a Kd in the submilimolar level. The red calcium sensors with fluorescence emission in the near infrared region allow us to monitor calcium signaling together with GFP-based calcium indicators. The pH stability of the red sensors was dramatically enhanced, with pKd below 5, compared to the GFP-derived calcium sensors. High resolution NMR has been applied to probe the mechanism of calcium-induced conformational change of designed calcium binding proteins. Our results show the developed calcium sensors are able to monitor calcium dynamics at several cellular environments responding to perturbations of extracellular calcium signaling.

Chromatin

4241-Pos Board B201
Spatio-Temporal Organization of Transcription Compartments Within Living Cells
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Recent studies have shown that transcriptional activity in the nucleus is organized in compartmentalized foci, where genes either loop out or are co-clustered to form active chromatin hubs. In addition, our studies are beginning to reveal that gene-active chromosomes share physical proximity within the 3D architecture of the cell nucleus. However, the spatio-temporal organization of TFs and its functional implications are unclear. Using high-resolution live-cell fluorescence imaging and spectroscopy, we analyze the dynamic organization of Transcription Compartments (TCs). For this we labeled TCs using fluorescent UTPs which co-localize with active RNA Pol-II antibody in a transcription dependent manner. Interestingly time-lapse imaging of these compartments exhibited a dynamic behavior with runs, pauses and steps. This dynamic organization of TCs was dependent on ATP, lamin B1, histone acetylation levels, cytoplasmic to nuclear anchorage and transcriptional activity. Importantly during runs, TCs are mobile within the inter-chromosome territories. The spatio-temporal organization of TCs that we observe may provide possible mechanisms to alter gene expression programs upon integration of physico-chemical signals to the nucleus.

4242-Pos Board B202
Geometric Regulation of Chromatin Remodeling is Dependent on Acto-Mysin Contractility
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Cells and tissues respond to geometric constraints by altering their gene expression programs. Modulation of gene expression requires post-translational modifications, such as histone acetylation, to alter chromatin compaction states and hence accessibility to gene regulatory sites for transcriptional machinery. But the mechanisms underlying geometrical constraints impinging on chromatin remodeling is largely unknown. In this work we show that cellular geometric constraints impinge on both the spatial organization of actin cytoskeleton and nuclear morphology (shape and size). These changes are found to be dependent on acto-mysin contractility as revealed by the phosphorylation of myosin light chain.
chain kinase (MLCK). Further geometric constraints regulate the nuclear to cytoplasmic ratio of HDAC3, a histone-deacetylase enzyme. Taken together, our work suggests that cellular geometric cues regulate chromatin remodeling processes via modulating acto-myosin contractility and nuclear-cytosplasmic shuttling of histone deacetylase enzymes.

2433-Pos Board B203
Impact of Nuclear Morphology on Gene Expression during Cellular Differentiation and Development
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Morphogenetic movements elicit differential gene expression programs during developmental processes, but the spatio-temporal evolution of nuclear morphology and its impact on genome function for lineage specificity is still unclear. Our results suggest that there exist an inter-play between nuclear morphology, inter-cellular connections and gene expression during the early development of Drosophila embryo. Quantitative morphometric analysis during development revealed the coupling between acto-myosin assembly and the emergence of nuclear shape. Physical perturbation to groups of cells in the developing embryo, using magnetic traps, alters nuclear morphology and induces defects in morphogenetic movement. As a consequence, these defects result in an altered segmental gene expression pattern. Our results highlight the importance of the emergence of prestressed nuclear morphology to genome regulatory processes.

2434-Pos Board B204
The Effect of Nucleosome Stability on Gene Expression Level
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Nucleosomes, which are the basic packaging units of chromatin, are stably positioned in promoters upstream of most stress-induced genes. These promoter nucleosomes are generally thought to repress gene expression due to exclusion; they prevent transcription factors from accessing their binding sites on the DNA. Some promoter nucleosomes, however, do not directly occlude transcription factor binding sites, and therefore their role in gene expression remains unknown. To understand the non-exclusive role of nucleosomes in gene expression, we designed a model promoter in budding yeast where a nucleosome intervenes between a transcription factor binding site in nucleosome depleted region and the transcription start site. To vary nucleosome stability, we constructed promoter variants with several different GC contents in the nucleosomal DNA sequence. We then measured the downstream gene expression level from these promoter variants at different induction levels using a fluorescent protein reporter. Our preliminary data show that relatively high nucleosome stability does not always correlate with relatively low gene expression level. This result suggests that nucleosomes might contribute to gene expression in a cooperative manner either by bringing otherwise distant DNA sites close together or by anchoring the SWI/SNF chromatin remodeling complex for efficient removal of adjacent nucleosomes.

2435-Pos Board B205
Structural Transitions in Higher Order Chromatin Assembly and Nuclear Plasticity during Stem Cell Differentiation
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Undifferentiated cells integrate physico-chemical cues from the local micro-environment to elicit lineage specific gene expression programs. However the underlying mechanisms of this physical plasticity and how it impinges on gene expression is still unclear. In this study, using high resolution live-cell fluorescence polarization imaging, we analyze the spatio-temporal aspects of nuclear organization and chromatin structure in mouse embryonic stem (ES) cells and contrast them to a primary embryonic fibroblasts (PMEF). Higher-order chromatin compaction states exhibit unique features in ES cells, marked by homogeneous chromatin compaction but heterogeneity at the population level, but PMEFs evidence an inverse correlation. In addition, the nuclear lamina and actin cytoskeleton is highly flexible in ES cells, but are frozen in PMEFs. This transition in nuclear architecture resembles that of fluid-like to solid-like transitions. Further the temporal evolution of nuclear plasticity is studied by differentiating ES cells on gelatin coated dishes. Taken together these results suggest that ES cells exhibit a broad epigenetic free energy landscape transitioning into a frozen configuration in higher-order chromatin assembly as lineage specific gene expression programs emerge.

2436-Pos Board B206
Single-Molecule Studies of the Bacterial Segregosome Component Spo0J
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The ParAB system is a broadly conserved module involved in bacterial plasmid partitioning and chromosome segregation. The core DNA binding component of the system, ParB, binds to consensus parS sites in plasmid or chromosomal DNA but also interacts with adjacent DNA non-sequence-specifically. We have used single molecule techniques, including a new “DNA motion capture” assay, to characterize the interactions of the Bacillus subtilis ParB homolog Spo0J with double-stranded DNA. In contrast to previous models of ParB polymerization on DNA to form a filament, our results suggest that Spo0J forms higher-order complexes by trapping DNA loops.

2437-Pos Board B207
3D Organization of the Interphase Nucleus using Soft X Ray Tomography
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The nucleus, although it had been studied for many decades, remains the body of many mysteries. In eukaryotic cells, DNA is found in two coexistent readable forms, an open, gene-rich region (euchromatin) that allows transcription of DNA into RNA, and a compacted region (heterochromatin) that contains silenced genes. In response to numerous extracellular stimuli, the cell must express specific proteins, which means that the DNA coding for these proteins must be in a transcriptionally active form. Consequently the balance between euchromatin and heterochromatin must be regulated during cell differentiation. Understanding this regulation requires fine-tuned biochemical and biophysical analyses and cutting-edge imaging techniques. Thanks to the soft X-ray microscope of the National Center for X-ray Tomography (Uchida et al., 2010; Larabell et al. 2010), high-resolution images of the 3D organization of the nucleus in the native state are now achievable. Cryo-immobilization of the cells (fast freezing) for x-ray imaging avoids chemical fixation artifacts associated with TEM. Imaging with X-rays in the water window energy range allows a natural contrast between water and biomolecules. X-ray imaging avoids perturbations caused by chemicals and osmotic changes associated with dehydration required for TEM, which cause deformation of nuclear structures (Finan and Guilack, 2010). As a result, x-ray tomography yields the first 3D views of nuclear organization in intact cells with a resolution of 50 nm. Our study indicates that heterochromatin forms a continuous network in 3D space, with no evidence of the chromatin patches described in TEM. Likewise, euchromatin regions are continuous. In addition distinct 3D chromatin patterns are linked to the differentiation/maturate state of the cell. Studying the 3D pattern of the nucleus using soft x-ray tomography is shedding new light on our understanding of cell differentiation.

2438-Pos Board B208
Ultrastructural Probes of Clusters of Open Regulatory Elements (Core) Within Chromatin
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Introduction:
Mammalian cells during interphase display clustering of DNase I-sensitive sites within the 10 nm micro-fibers of active euchromatin (Song L, et al, Genome Research, August 19, 2011). Over 870,000 distinct CORE sites were identified as poised or active in gene transcription across an analysis of seven human cell lines, (ibid).

Methods:
We have developed a high-resolution electron microscopic technique for detecting DNase I-sensitive sites within intact single human cells. All such sites are confined to the euchromatin portion of the cell nucleus, and can be analyzed for location, number, shape, and size within each single cell analyzed within native biopsied tissue. Such ultrastructural probes can detect sites greater than 10 nm in diameter, and offer a global 3D view in 10 nm thick serial sections of the probed tissue after instant fixation during biopsy.