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.

Chromatin

2431-Pos Board B201

Spatio-Temporal Organization of Chromatin 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 spatiotemporal 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.

2432-Pos Board B202

Geometric Regulation of Chromatin Remodeling is Dependent on Acto-Mycin Contractility

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Cells and tissues respond to geometric constraints by altering their gene expression programs. Regulation 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-mycin contractility as revealed by the phosphorylation of myosin light 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.

2430-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 submillimolar 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 pKΔ 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.