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.

2428-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. The histone methylation by the SET domain superfamily member Set2 is recruited 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 Rpd3 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 both 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.

2429-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 for ammonia 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.

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 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 $k_d$ 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 $pK_a$ 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
2431-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.

2432-Pos Board B202
Geometric Regulation of Chromatin Remodeling is Dependent on Actomyosin 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-myosin contractility as revealed by the phosphorylation of myosin light chain.