

large  $[Ca^{2+}]_i$  transients just below the cuticular plate, putatively colocalizing with the striated organelle. Depolarization with application of KCl revealed  $[Ca^{2+}]_i$  transients in microdomains at the base of hair cells, likely reflecting voltage gated calcium channels modulating synaptic release. Preliminary results using adult vestibular organs of Gad2-Cre::GCaMP5G-tdTomato transgenic mice reveal highly localized time-resolved  $[Ca^{2+}]_i$  modulation at the tips of stereocilia, in microdomains of hair cells, and at synaptic terminals.

#### 1964-Pos Board B284

##### Targeted Optogenetic Activation of Calcium Transients in Developing Skeletal Muscle Cells

Stephane Sebille<sup>1</sup>, Oualid Ayad<sup>1</sup>, Christian Cognard<sup>1</sup>, Patrick Bois<sup>1</sup>, Aurelien Chatelier<sup>2</sup>.

<sup>1</sup>Université de Poitiers, Signalisation et Transports Ioniques Membranaires, poitiers, France, <sup>2</sup>Université de Poitiers/CNRS, Signalisation et Transports Ioniques Membranaires, poitiers, France.

Activation of calcium transients through the electrical stimulation of myotubes is a pre-requisite for advanced differentiation in developing skeletal muscle. This stimulation occurs early in the muscle development with the appearance of the neuromuscular junction (NMJ) that allows to depolarize cell membrane and to induce post-synaptic potentials. Consequent generated action potentials activate excitation-calcium release coupling mechanism also known to participate in the calcium-dependent differentiation pathways for the maturation of striated muscle cells. To date, depolarization-evoked intracellular calcium increases are mainly investigated *in vitro* using electrical field stimulation or high potassium solution perfusion. However, these methods are not representative of a spatiotemporal NMJ stimulation. Moreover, calcium signaling kinetics and local calcium increases depend on the stimulation approaches. Here we have devised a non-invasive experimental approach to enable genetically targeted photostimulation of developing skeletal muscle cells with fine temporal and spatial resolution. We applied optical stimulation to C2C12 myotubes, genetically engineered to express ChR2-GFP to investigate depolarization-dependent calcium increases initiated from a very small and localized area of light stimulation. We found that the optical stimulation of membrane area smaller than  $1 \mu m^2$  allowed the depolarization of the whole cell membrane and activated depolarization-induced calcium increases in ChR2-expressing myotubes. Optical stimulation conditions (area, surface and light power), associated with the use of pharmacological blockers, were investigated to understand the required conditions for obtaining voltage-dependent calcium transients. Moreover, the analysis of localized calcium increases demonstrates very different kinetics and amplitude of increases in different subcellular compartments. These results suggest that local optical stimulation, that mimics motor neurons inputs, allows to investigate finely the consequent calcium increases and provides new results about calcium homeostasis in subcellular compartments.

#### 1965-Pos Board B285

##### Plasma Membrane Orai and Septin Organization during Calcium Signalling

Zachary Katz<sup>1</sup>, Chan Zhang<sup>2</sup>, Ariel Quintana<sup>2</sup>, Björn Lillemeier<sup>1</sup>, Patrick Hogan<sup>2</sup>.

<sup>1</sup>Salk Institute for Biological Studies, La Jolla, CA, USA, <sup>2</sup>La Jolla Institute, La Jolla, CA, USA.

The STIM-Orai-mediated calcium release-activated calcium channel, or CRAC channel, is a key source of calcium influx that maintains cellular calcium balance and mediates calcium signaling. STIM in the ER membrane senses depletion of ER calcium stores and moves to ER-plasma membrane junctions, then recruits plasma membrane Orai channels to the junctions and directly gates the channels. We have shown previously that septins are crucial for efficient STIM1-Orai1 cluster formation following store depletion. Septins are known to specify diffusive barriers in the plasma membrane and to serve as scaffolds to recruit signalling proteins, but their detailed role in calcium signaling remains to be characterized. Here we utilize live-cell super-resolution microscopy and single-molecule tracking to map Orai1 relative to STIM1 and membrane-localized septins. These observations at high spatial and temporal resolution enable precise analyses of Orai1 movements before and after store depletion, and of the relation of septins to the membrane nano-domains where STIM and Orai interact.

#### 1966-Pos Board B286

##### Lead Tightly Associates to Neuronal Calcium Sensor (NCS) Protein Dream and Promotes Structural Changes Analogous to Calcium Bound Dream

Samiol Azam, Jaroslava Miksovska.

Chemistry and Biochemistry, Florida Int University, Miami, FL, USA. Downstream regulatory element antagonist modulator (DREAM), is a 29 kDa  $Ca^{2+}$  binding protein that controls several neurological processes including gene expression, apoptosis, and modulation of  $K_v4$  voltage channels. Here

we demonstrate that  $Pb^{2+}$  binds to EF-hands in DREAM with an equilibrium affinity higher than that determined for  $Ca^{2+}$ .  $Pb^{2+}$  association triggers changes in the secondary and tertiary structure of the protein that are analogous to those observed in  $Ca^{2+}$  bound DREAM based on the Trp 169 emission data and CD spectra. Namely,  $Pb^{2+}$  binding to DREAM leads to a decrease of Trp169 emission intensity and decrease in the CD signal at 220 nm. The hydrophobic cavity in the C-terminal domain of DREAM is solvent exposed in the presence of  $Pb^{2+}$  as determined using a hydrophobic probe 1, 8-ANS. The  $K_d$  values for 1, 8-ANS binding to  $Ca^{2+}$  and  $Pb^{2+}$  bound DREAM were found to be similar;  $73 \pm 10 \mu M$  and  $103 \pm 10 \mu M$ , respectively.  $Pb^{2+}$  binding to DREAM also modulates DREAM interactions with intracellular partners. For example, titrations of presenilin-1 with DREAM in the presence of  $Ca^{2+}$  or  $Pb^{2+}$  showed that  $Pb^{2+}$  bound DREAM has a similar affinity for presenilin-1 ( $K_d = 2.44 \pm 0.19 \mu M$ ) as  $Ca^{2+}$  bound DREAM ( $K_d = 6.23 \pm 1.60 \mu M$ ). The impact of  $Pb^{2+}$  association on DREAM conformational dynamics was proved in time-resolved fluorescence studies and the thermodynamic parameters for  $Pb^{2+}$  association to EF-hands were obtained using isothermal titration calorimetry (ITC). Those results indicate that DREAM and likely other neuronal calcium sensors bind  $Pb^{2+}$  with a high affinity and  $Pb^{2+}$  association to neuronal calcium sensors may contribute to lead-induced neurotoxicity.

#### 1967-Pos Board B287

##### Rewiring Calcium Signaling for Genome Editing and Transcriptional Reprogramming

Nhung T. Nguyen<sup>1</sup>, Lian He<sup>1</sup>, Yi Liang<sup>1</sup>, Yun Huang<sup>2</sup>, Yubin Zhou<sup>1,3</sup>.

<sup>1</sup>Center for Translational Cancer Research, Institute of Bioscience and Technology, Texas A&M University, Houston, TX, USA, <sup>2</sup>Center for Epigenetics and Disease Prevention, Institute of Bioscience and Technology, Texas A&M University, Houston, TX, USA, <sup>3</sup>Department of Medical Physiology, College of Medicine, Texas A&M University, Temple, TX, USA.

Tools capable of modulating gene transcription in living organisms is very useful for interrogating the gene regulatory network and controlling biological processes. Recently, the CRISPR/Cas9 of *Streptococcus pyogenes* was re-engineered to be catalytically inactive, which functions as a general platform for RNA-dependent DNA binding while leaving the genome intact. This target-gene recognition complex upon fusing to either repressive or activating effectors is able to interfere with transcriptional initiation, transcriptional elongation, RNA polymerase recruitment and transcription factor binding. However, without temporal and/or spatial control, the application of these reprogramming tools will likely cause off-target effects and lack strict reversibility. To overcome this limitation, we report herein the development of a chemical- or light-inducible transcriptional reprogramming device that can control gene expression and cell fate at high spatiotemporal precision. We demonstrated the use of a photo-switchable calcium channel to remotely control calcium and NFAT-dependent transcriptional outputs. By fusing engineered NFAT fragments with Cas9 or dCas9 and transcriptional coactivators, we can harness the power of light to achieve photo-inducible gene editing and transcriptional reprogramming in mammalian cells. This system can also be used to document calcium-dependent activity in mammalian cells after exposure to ligands or chemicals that would elicit calcium response inside cells. [This work is supported by the Welch Foundation and CPRIT].

#### 1968-Pos Board B288

##### Calmodulin Interacts and Regulates Enzyme Activity of the Mammalian Sperm Phospholipase C

Michail Nomikos<sup>1</sup>, Angelos Thanassoulas<sup>2</sup>, Brian L. Calver<sup>3</sup>, Maria Theodoridou<sup>3</sup>, Luke Buntwal<sup>3</sup>, Zili Sideratou<sup>2</sup>, George Nounesis<sup>2</sup>, F. Anthony Lai<sup>3</sup>.

<sup>1</sup>College of Medicine, Qatar University, Doha, Qatar, <sup>2</sup>“Demokritos”, National Centre for Scientific Research, Aghia Paraskevi, Greece, <sup>3</sup>College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom.

Sperm-specific Phospholipase C zeta (PLC $\zeta$ ) is widely considered to be the sole, physiological stimulus responsible for the generation of  $Ca^{2+}$  oscillations that induce egg activation and early embryo development during mammalian fertilization. PLC $\zeta$ , which is delivered from the fertilizing sperm into the egg cytoplasm, catalyzes the hydrolysis of its membrane-bound phospholipid substrate phosphatidylinositol 4,5-bisphosphate [PI(4,5)P<sub>2</sub>], triggering the cytoplasmic  $Ca^{2+}$  oscillations through the inositol 1,4,5-trisphosphate (InsP<sub>3</sub>) signaling pathway. Despite the recent advances the detailed regulatory mechanism of PLC $\zeta$  is still unclear, as binding partners of this protein within the sperm or the fertilizing egg have not yet been identified. Calmodulin (CaM) is a ubiquitous  $Ca^{2+}$  sensor in eukaryotic cells. A previous study has reported that CaM directly interacts and regulates the activity of PLC delta 1 protein, a

somatic PLC isoform with structural similarities to sperm PLC $\zeta$ . Bioinformatics analysis revealed putative CaM-binding sites on PLC $\zeta$  sequence. In the present study, we have used co-immunoprecipitation analysis and we show that in the presence of Ca<sup>2+</sup>, human PLC $\zeta$  directly interacts with CaM. Isothermal titration calorimetry (ITC) experiments were performed to map the interaction. Three different peptides corresponding to disparate sequences within human PLC $\zeta$  were used and it was shown that PLC $\zeta$  interacts with CaM via one region of the molecule. In addition, recombinant proteins corresponding to the N- and C-lobe of human CaM were used for ITC experiments, which revealed that CaM interacts with PLC $\zeta$  in the presence of Ca<sup>2+</sup>, only through one of its lobe domains. *In vitro* PIP<sub>2</sub> hydrolysis assays revealed that CaM alters PLC $\zeta$  PIP<sub>2</sub> hydrolytic activity at high Ca<sup>2+</sup> concentrations and, as suggested by liposome binding assays, this appears to be due to CaM binding to PLC $\zeta$  affecting proper access of the enzyme active site to its substrate PI(4,5)P<sub>2</sub>.

#### 1969-Pos Board B289

##### Amino Acid Contributions to Binding across Paired EF-Hands in Calmodulin

Suzanna Bennett<sup>1</sup>, Margaux Miller<sup>2</sup>, Thomas Middendorf<sup>1</sup>, Richard Aldrich<sup>1</sup>.

<sup>1</sup>Neuroscience, University of Texas at Austin, Austin, TX, USA, <sup>2</sup>School of Medicine, University of Utah, Salt Lake City, UT, USA.

The ubiquitous calcium-binding protein calmodulin participates in a network of interactions influencing cellular processes like gene regulation and ion channel modulation. The immediate function of calmodulin is to bind calcium to its four EF-hand calcium binding domains grouped into two pairs, each pair residing in either lobe of the dumbbell-shaped protein. Ligand binding involves a constellation of amino acid interactions localized within the binding site as well as across paired sites. Our previous work has shown that each of the four binding sites has a unique apparent affinity that appears to have been selected for during evolution. We have investigated how the differences in binding loop amino acid sequences cause differences in binding affinity of the individual EF-hands, as well as how the amino acid sequence of one site influences binding at its paired site through cooperative mechanisms. Between the two paired N-lobe EF-hands, all but five positions within twelve residue binding loops are identical. Each of these five loop positions were individually mutated to its counterpart from the paired site and site-specific binding measurements were performed on N-lobe fragments to explore the two roles each position plays to determine binding in the paired EF-hand system: locally within their own site (*cis*-effects) and at the paired site (*trans*-effects). Several mutations had both *cis*- and *trans*- effects whereas others had predominantly *cis*- or *trans*-effects. The complexity of the mutant effects on binding of both sites suggests that the N-lobe has been conserved to act as a complex multiple site calcium binding unit, rather than two independent EF-hands.

#### 1970-Pos Board B290

##### Automated Characterization of Dynamic Parameters of Intracellular Calcium Signals

Laurent MacKay.

Physiology, McGill University, Montreal, QC, Canada.

Dynamic processes, such as intracellular calcium signaling, are hallmark of cellular biology. As real-time imaging modalities become widespread, a need for analytical tools to reliably characterize time-series data without prior knowledge of the nature of the recordings becomes more pressing. We develop a signal-processing algorithm that autonomously computes the parameters characterizing prominent single transient responses (TR) and/or multi-peaks responses (MPR). The algorithm corrects for signal contamination and decomposes experimental recordings into contributions from drift, TRs and MPRs. This is achieved by sequentially decomposing the signal into its individual components, characterizing them individually, and finally combining the distinct components to yield a coherent representation of the entire recording. It subsequently provides numerical estimates for the following parameters: time of onset after stimulus application, activation time (time for signal to increase from 10% to 90% of peak), and amplitude of response. It also provides characterization of the (i) TRs by quantifying their area under the curve, response duration (time between  $\alpha$  amplitude on ascent and descent of the transient), and decay constant of the exponential decay region of the deactivation phase of the response, and (ii) MPRs by quantifying their number of peaks, mean peak magnitude, periodicity, oscillatory persistence (time between first and last discernable peak) and duty cycle (fraction of period during which system is active). We validate the outputs of the algorithm through comparison with user-mediated measurements of the recordings, and demonstrate that parameter estimates obtained manually and algorithmically are correlated. This opens the possibility for such an algorithm to be used as a general framework for comprehensively analyzing dynamic time-series with transient or multi-peaked responses.

#### 1971-Pos Board B291

##### Novel Approach to Study SERCA Function *In Situ*

Elisa Bovo, Siddharth Bhayani, Olga Raguimova, Seth L. Robia, Aleksey V. Zima.

Cell and Molecular Physiology, Loyola University Chicago, Maywood, IL, USA.

Previously used techniques to measure the sarcoplasmic/endoplasmic reticulum Ca-ATPase (SERCA) function either are cell destructive or lack sensitivity. We designed a new approach that allows studying SERCA function in the cellular environment under well-controlled conditions. We used the genetically encoded Ca sensor CEPIA-1er to directly measure ER [Ca] ([Ca]<sub>ER</sub>) in HEK293 cells expressing SERCA2a. These cells were also expressing ryanodine receptor (RyR2) for pharmacological control of ER Ca content. The plasma membrane was permeabilized with saponin to control cytosolic [Ca] and energy supply. HEK293 cells expressing wt-SERCA2a manifested periodic [Ca]<sub>ER</sub> depletions (Ca waves) due to spontaneous activation of RyR2. Ca waves were rarely seen in cells expressing only RyR2, because endogenous SERCA was unable to increase [Ca]<sub>ER</sub> to a critical level that triggers spontaneous Ca-induced Ca release (CICR). Application of 10 mM caffeine completely depleted [Ca]<sub>ER</sub>. Once caffeine was removed, RyR2 inhibitors were applied to measure the rate of [Ca]<sub>ER</sub> recovery. Thus, ER Ca uptake can be analyzed throughout the whole physiological range of [Ca]<sub>ER</sub>. ER Ca uptake was progressively decreased with increasing cytosolic [ADP] and can be blocked by thapsigargin. At the end of each experiment, the CEPIA-1er signal was calibrated with ionomycin. Using this approach, we characterized properties of wt- and AAA-SERCA2a (partial loss-of-function SERCA2a mutant). ER Ca uptake as a function of [Ca]<sub>ER</sub> was analyzed to estimate maximum ER Ca uptake rate and maximum ER Ca load generated by wt and the SERCA mutant. We found that AAA-SERCA2a had slower Ca uptake rate than wt-SERCA2a, as a result HEK293 cells expressing AAA-SERCA2a were not effective to increase ER Ca load to the level that triggers Ca waves. Thus, this new approach can be used as a sensitive screening tool to study different drugs and mutations that affect SERCA function.

#### 1972-Pos Board B292

##### Stimulatory and Inhibitory Effects of PKC Isozymes are Mediated by Serine/Threonine PKC Sites of the Cav2.3 $\alpha_1$ Subunits

Ganesan L. Kamatchi<sup>1</sup>, Senthilkumar Rajagopal<sup>2</sup>, Brittney K. Burton<sup>1</sup>.

<sup>1</sup>Biology, Norfolk State University, Norfolk, VA, USA, <sup>2</sup>Biotechnology, Rayalseema University, Kurnool, India.

Protein kinase C (PKC) isozymes are known to potentiate voltage-gated calcium (Cav) currents through Cav2.2 and Cav2.3 channels by targeting the serine/threonine (Ser/Thr) phosphorylation sites of Cav $\alpha_1$  subunits. While some of these Ser/Thr sites of Cav2.2 $\alpha_1$  subunits mediated the stimulatory action (Thr-422, Ser-2108 and Ser-2132), others facilitated the inhibitory action (Ser-425) of PKCs  $\beta$ II and  $\epsilon$ . In the current study, it was investigated if the homologous sites of Cav2.3 $\alpha_1$  subunits (stimulatory: Thr-365, Ser-1995 and Ser-2011; inhibitory: Ser-369) behaved in a similar manner. In this regard, several Ala and Asp mutants were constructed in Cav2.3 $\alpha_1$  subunits in such a way that the Ser/Thr sites can be examined in isolation or in selected combination. These mutants or WT Cav2.3 $\alpha_1$  along with auxiliary  $\beta_{1b}$  and  $\alpha_2/\delta$  subunits were expressed in *Xenopus* oocytes and the effects of PKCs  $\beta$ II and  $\epsilon$  studied on the barium current (*I*<sub>Ba</sub>). The results showed that Thr-365 and Ser-1995 mediated the stimulatory and Ser-369, the inhibitory effects of PKCs  $\beta$ II and  $\epsilon$ . While these sites behaved similar to their homologs, Ser-2011 differed from its homolog Ser-2132 as it failed to mediate the stimulatory effects of these isozymes. Furthermore PKCs produced neither stimulation nor inhibition when stimulatory Thr-365 or Ser-1995 and inhibitory Ser-369 were present together. However, the PKCs potentiated the *I*<sub>Ba</sub> when two stimulatory sites, Thr-365 and Ser-1995 were present together, thus overcoming the inhibitory effect of Ser-369. Taken together net PKC effect may be the difference between the responses of the stimulatory and inhibitory sites.

#### 1973-Pos Board B293

##### Dynamic Substrate Gating in CaMKII by Autophosphorylation

Derrick E. Johnson, Swarna S. Ramaswamy, Andy Hudmon.

Biochemistry and Molecular Biol, Indiana University, Indianapolis, IN, USA. Calcium/calmodulin (CaM)-dependent protein kinase II (CaMKII) regulates diverse substrates in cellular processes ranging from metabolism and cell cycle control to calcium homeostasis, excitable cell activity and plasticity. CaMKII is a multimeric holoenzyme composed of 8–14 subunits from four closely related isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ). The calcium sensor CaM binds to a target sequence within the autoregulatory domain (ARD) of each CaMKII subunit. Coincident CaM binding within the holoenzyme supports intersubunit autophosphorylation (T<sup>286</sup>  $\alpha$ -isoform and T<sup>287</sup>  $\beta$ -,  $\gamma$ -, and  $\delta$ -isoforms) within