cardiomyocytes, wild-type canine CSQ2, TRD, and JCT were overexpressed in cultured adult rat cardiomyocytes using recombinant adenoviruses. The overexpressed proteins were detected using either species-specific antibodies, or by fusion with fluorescent proteins. After 24h and 48h, cardiomyocytes were examined using confocal fluorescence microscopy. By the time overexpressed CSQ2 was first detected by immunofluorescence (24h), it was already distributed across jSR sites. In contrast, TRD and JCT exhibited patterns of perinuclear accumulation after 24h. By 48h, they became increasingly concentrated along a transverse pathway that extended from perinuclear puncta towards the cell surface. This novel transverse jSR trafficking pathway co-localized with desmin, a prominent protein component of cardiac intermediate filaments. It was previously found that CSQ2-DsRed forms a polymeric complex that prematurely polymerizes at perinuclear sites of rough ER. We reasoned that this proximal CSQ2 polymer should then bind to newly synthesized TRD and JCT in situ, leading to their enhanced concentration around the nucleus. Indeed, when canine TRD and JCT were co-overexpressed with CSQ2-DsRed, both of these small transmembrane proteins bound to perinuclear CSQ2-DsRed, and failed to exhibit anterograde trafficking to peripheral jSR puncta. In contrast, TRD in which CSQ2 binding sites were deleted readily trafficked towards the cell periphery. Rates of anterograde trafficking of CSQ2 and other jSR proteins may depend upon the assembly of CSQ2 into polymers, which may be regulated by luminal Ca^{2+} concentrations.

1502-Pos Board B394

Calibration of Ca^{2+} Transients Obtained with the Fast Ca^{2+} and Mg^{2+} Dye Magfluo-4

Juan C. Calderon[1], Daniel Raigosa[1], Marco Giraldo[1], Pura Bolaños[2], Carlo Caputo[2].
[1]University of Antioquia, Medellin, Colombia, [2]Venezuelan Institute for Scientific Research, Caracas, Venezuela, Bolivarian Republic of.

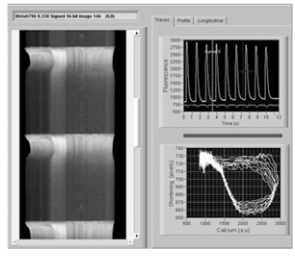
Since there are uncertainties about the concentration of free cytosolic Ca^{2+} reached in a muscle fiber during a transient elicited by electric stimulation, and about the $Kd_{Ca}{}^{2+}$ of the fast dye Magfluo-4, we performed two groups of experiments aimed at calibrating the Ca^{2+} transients obtained with this dye. For the fiber experiments, we used murine single skeletal muscle fibers obtained from enzymatically dissociated *flexor digitorum brevis*, loaded with 8-10 μM of the dye in its acetoxymethyl ester form, during 40 min at 21-22°C, to determine F_{max}, F_{min} and dye compartmentalization. The cells were permeabilized with saponine and exposed to 50 mM of free calcium to intracellularly saturate the dye. Compartmentalization was assessed using digitonin and triton-X100. For our conditions, F_{max} and F_{min} were 160 ± 14 (n=9, mean ± SEM) and 3.4 ± 0.7 (n=6). Dye compartmentalization was found to be less than 10% (n=1). For the *in vitro* experiments, $Kd_{Ca}{}^{2+}$ (20°C) values of 21.6 μM, 27.8 μM and 29.1 μM were obtained in presence of 0, 1 and 2 mM free Mg^{2+}, respectively. To calculate Ca^{2+} concentration from the fluorescence signals, we used the methodologies proposed by Grynkiewicz *et al.* (1985) and by Caputo *et al*. (1994). Preliminary results, taking into account our data or some values from the literature (Baylor et al. 2009), show single Ca^{2+} transient's peak between 2 and 7 μM (n=4). We are now calculating the kinetics of Ca^{2+} transients and the quantitative changes induced by modulating Ca^{2+} release and sequestration mechanisms in different fiber types. In conclusion, we have calculated the peak Ca^{2+} concentration after single stimulation in fibers loaded with Magfluo-4. Our preliminary results are comparable to those obtained with other fast Ca^{2+} dyes, such as Magfura-5, but lower than those reported with Magfura-2.

1503-Pos Board B395

Cell Edge Detector: Recovering Length Information from Line-Scan Confocal Images

Jose L. Puglisi, Leighton T. Izu, Ye Chen-Izu, Donald M. Bers.
University of California, Davis, Davis, CA, USA.

Confocal microscopy has greatly enhanced our understanding of cardiac excitation-contraction coupling. Linescan images have been used to obtain detailed information about intracellular calcium concentration disregarding the mechanical activity of the cell. In order to fill this gap we have designed an algorithm to recover the cell length information from those images. We implemented this program using LabVIEW language. An intuitive user interface allows the researcher to load the image; scan the longitudinal axis; select portion of the file; detect the borders and plot the calcium and cell shortening traces with the corresponding shortening versus calcium loop. The software also calculates and plots into a table the following parameters : maximum fluorescence (F/F0), decay time (tau), peak fractional shortening (L%),half time to relaxation (t1/2), maximum dL/dt, and from the loop curve the calcium concentration corresponding to half shortening (EC50). This new approach allows us to obtain richer information from the linescan images by extracting the corresponding shortening traces from the fluorescence signal. This software is already operational in our labs and has been used to analyze confocal images of calcium transients and myocyte contraction in cardiac cells.



1504-Pos Board B396

TIRFM Imaging of PTRF and MG53 Translocation to Cell Surface Membrane following Osmotic-Stress Induced Injury

Hua Zhu, Ki Ho Park, Jianjie Ma.
Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA.

Plasma membrane damage repair is an intrinsic physiological process for maintenance of integrity of eukaryotic cells under a variety of insults. Osmotic-shock has been widely used to induce cell membrane injury and has become a useful tool to study the mechanisms of cell membrane repair. It is known that following a series of hypo-osmotic stress, intracellular vesicles can traffic to and fuse with the cell surface membrane. These vesicles are labeled with caveolin-1 and PTRF, two essential proteins of the caveolae membrane structure. We have previously shown that PTRF can interact with MG53, a main component of the cell membrane repair machinery, for anchoring of the repair patch at the acute membrane injury site. In this study, we sought to explore the trafficking behavior of PTRF and MG53 during osmotic shock using two-colorTotal Internal Reflection Fluorescence Microscopy (TIRFM). We found that rapid translocation of MG53 toward the cell surface membrane could be triggered upon return of the cell to an isotonic environment following pre-exposure of the cell to a hypotonic solution (150 mOsm, 1 min). This process is PTRF dependent, as MG53 cannot translocate to cell membrane in HepG2 cells which lack endogenous PTRF expression. These data support our previous finding that PTRF is an indispensable component of the MG53-mediate membrane repair process. We are currently in the process of defining the kinetics and temporal relationship for the movement of PTRF/MG53-containing vesicles after osmotic shock, toward understanding the role for MG53/PTRF in facilitating the membrane fusion and fission processes during cell membrane repair.

1505-Pos Board B397

MG53 can Function in Keratinocyte Membrane Repair and Contribute to Excisional Wound Healing in Rodent Skin

Mattew Orange[1], Christopher Ferrante[1], Rosalie Yan[2], Tao Tan[2], Norio Takizawa[2], Pei-Hui Lin[1,3], **Haichang Li**[3], Hiroshi Takeshima[4], Noah Weisleder[1,5], Jianjie Ma[1,3].
[1]Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Protein Therapeutics Division, TRIM-edicine, Inc, New Brunswick, NJ, USA, [3]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA, [4]Department of Biological Chemistry, Kyoto University, Kyoto, Japan, [5]Department of Physiology and Cell Biology, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA.

Plasma membrane repair is a dynamic cellular process observed in many cell types where membrane disruptions are actively repaired by a vesicle-mediated mechanism. Mitsuguimin 53 (MG53) is a novel member of tripartite motif (TRIM) family of proteins and an important component of the membrane repair machinery. In skeletal muscle, MG53 protects against eccentric contraction related damage while in cardiac muscle, MG53 protects against ischemia-reperfusion injury. While previous studies illustrate MG53 function in striated muscles, little is known about MG53 function in other tissues. The skin is an organ that covers the entire body and is constantly exposed to various environmental factors that can induce membrane disruptions. Here we investigated the function of MG53 in the skin and its contribution to wound healing. The presence of extracellular recombinant human MG53 (rhMG53) protected primary human keratinocytes from mechanical damage. Expression of GFP-MG53 in cultured human keratinocytes confirmed that the protein responded to membrane damage in the same fashion as in muscle fibers and other non-muscle cells. Excisional wounding revealed delayed healing of mg53-/- mice when compared to wild-type. While western blotting showed MG53 expression in extracts of mouse skin, MG53 was not expressed in human or mouse cultured keratinocytes. Immunohistochemistry revealed that expression of MG53 in mouse skin was specific to the panniculus carnosus, a subdermal striated muscle layer common in rodents that has limited distribution in human skin. Together these data suggest that MG53 has potential as a therapeutic agent to increase wound healing in a variety of tissues.