POSTER SESSION

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Ca^{2+} IN CELL DIFFERENTIATION, PROLIFERATION AND DEATH

CALCIUM/CALMODULIN KINASE II, A BINDING PARTNER OF THE MULTI PDZ DOMAIN PROTEIN MUPP1 IN MAMMALIAN SPERMATOZOA, REGULATES ACROSOMAL EXOCYTOSIS

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Upon adhesion to the zona pellucida of the oocyte, mammalian sperm undergo acrosomal exocytosis, a process resembling Ca^{2+}-regulated exocytosis in neurons. We have recently observed that the PDZ domain protein MUPP1, which contains 13 potential protein-binding motifs, is expressed in spermatozoa of different mammalian species, where it is exclusively localized to the acrosomal region. In addition, using a functional exocytosis assay combined with a Ca^{2+} chelator strategy, we found that MUPP1 is functionally operative in recruiting molecules which control the initial tethering and/or docking between the acrosomal vesicle and a specific site of the target plasma membrane.

Here we present data which show that MUPP1 plays a functional role in orchestrating signaling molecules operative in acrosomal secretion by controlling a Ca^{2+}-dependent assembly and disassembly of specific binding partners. The results revealed that CaMKII, known to be operative in tethering synaptic vesicle to the active zone, shows a striking co-localization with MUPP1 in the acrosomal region as well as in detergent-resistant membrane fractions of isolated sperm. GST-pull down experiments with solubilized sperm preparations and a panel of various GST-fusion proteins of non-overlapping MUPP1-PDZ domains revealed that the activated form of CaMKII binds to the PDZ domain 10-11 whereas other MUPP1 constructs did not show any binding. To assess whether CaMKII is functionally active in acrosomal secretion, we pretreated isolated epididymal mouse sperm either with the membrane-permeable calmodulin inhibitor W7 or with the CaMKII inhibitor KN93 and subsequently quantified the spontaneous rate of acrosome reaction. The results showed that both inhibitors caused a dose-dependent potentialization of the rate of unstimulated acrosomal exocytosis in mammalian spermatozoa, thus suggesting that CaMKII is involved in preventing spontaneous acrosomal secretion.

Keywords: spermatozoa, acrosome, MUPP1, CaMKII

TRPC1 CHANNEL RECRUITMENT BY THE CaR IN MCF-7 HUMAN BREAST CANCER: ROLE IN PTHRP SECRETION, PROLIFERATION AND CHEMOTAXIS

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The calcium sensing receptor (CaR) is expressed in primary breast cancers and in several breast cancer cell lines including MCF-7. The CaR controls several cellular processes such as proliferation, chemotaxis, migration and parathyroid hormone related protein (PTHrP) secretion which is known to stimulate osteolysis during metastatic bone resorption. Recently, Mihai et al. (2006) have proposed the CaR as a new marker predicting the risk of bone metastases. Transient Receptor Potential Canonical 1 (TRPC1) channels play a critical role in sustaining both proliferation and its required $[Ca^{2+}]_i$ elevation. We previously demonstrated that the activation of the CaR by the increase in $[Ca^{2+}]_o$ induces a cationic current, which is inhibited by 2-APB and La^{3+}, both recognized as inhibitors of the TRPC family. Moreover, we found that MCF-7 express TRPC1 (El Hiani et al., 2006).

Here, we investigated the role of TRPC1 in the PTHrP secretion, proliferation and chemotaxis induced by the CaR activation in MCF-7 cells. Our results show that the CaR activated ERK1/2 via PLC/PKC and Metalloproteinases-EGF receptor dependent pathways. Moreover, down-regulation of TRPC1 by siRNA reduced both Ca^{2+} entry and ERK1/2 phosphorylation induced by CaR. Activation of CaR induced cell proliferation, PTHrP secretion and chemotaxis in an ERK1/2 and a TRPC1-dependent manner. Indeed, treatment by TRPC blockers or by siRNA against TRPC1 failed to activate ERK1/2 after CaR-stimulation and, therefore, to induce PTHrP secretion, cell proliferation and chemotaxis. Moreover, CaR activation had no effect on MCF-7 migration. Our findings provide strong arguments for the recruitment of TRPC1 to generate Ca^{2+} entry that is involved in CaR induced PTHrP secretion, cell proliferation and chemotaxis.

El Hiani et al. (2006) J. Membr. Biol. 211, 127-137.
Mihai et al. (2006) Eur. J. Surg. Oncol. 32, 511-515.

Keywords: calcium receptor, breast cancer, proliferation, chemotaxis, TRPC1

DEREGULATION OF CALCIUM FLUXES IN HTLV-I INFECTED CD4$^+$ T-CELLS PLAYS A MAJOR ROLE IN MALIGNANT TRANSFORMATION

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The CD4$^+$ T-cell malignancy induced by human T-cell leukemia virus type 1 (HTLV-I) infection and termed Adult T-cell Leukemia Lymphoma (ATLL), is caused by defects in the mechanisms underlying cell proliferation and cell death. In the CD4$^+$ T-cells, calcium ions are central for both phenomena. ATLL is associated with a marked hypercalcemia in many patients. The consequence of a defect in the Ca^{2+} signaling pathway for lymphocyte activation is characterized by an impaired NFAT activation and transcription of cytokines, chemokines and many other NFAT target genes whose transcription is essential for productive immune defense. Fresh ATLL cells are present in the absence of the TCR/CD3 and CD7 molecules on their surface. While CD7 is a calcium transporter, reduction in calcium influx in response to T-cell activation was reported as a functional consequence of TCR/CD3 expression deficiency. Understanding this changes and identifying the molecular players involved might provide further insights on how to improve ATLL treatment.

Keywords: lymphoma, T cell activation, CD7, NFAT, CD4$^+$, HTLV-1

CHARACTERIZATION OF THE Ca^{2+}-REGULATED EZRIN-S100P INTERACTION AND ITS ROLE IN TUMOR CELL MIGRATION

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Ezrin is a multidomain protein providing regulated membrane-cytoskeleton contacts that play a role in cell differentiation, adhesion and migration. Within the cytosol of resting cells ezrin resides in an auto-inhibited conformation in which the N- and C-terminal ezrin/moesin/radixin (ERM) association domains (ERMADs) interact with one another. Activation of ezrin's membrane-cytoskeleton linker function requires an opening of this interdomain association that can result from PtdIns(4,5)P_2 binding to the N-ERMAD and threonine-567 phosphorylation in the C-ERMAD. We have shown that ezrin can also be activated by Ca^{2+}-dependent binding of the EF hand protein S100P.

To precisely investigate the regions involved in ezrin-S100P complex formation, we performed different interaction studies with a series of C-terminally truncated N-ERMAD and S100P derivates. These included solid phase binding assays like surface plasmon resonance (BIAcore) approaches and led to the mapping of the respective binding sites and determination of K_D-values of the analyzed interactions.

Phospholipid-binding assays revealed that S100P and PtdIns(4,5)P_2 compete for at least partially overlapping binding sites in N-ERMAD suggesting that the S100P-mediated activation of ezrin could resemble the activation via PtdIns(4,5)P_2 binding. Using different S100P and ezrin mutants we also show that the protein interaction and a resulting activation of ezrin promotes the transendothelial migration of tumor cells. Thus, a prometastatic role of ezrin and S100P that had been proposed based on their overexpression in highly metastatic cancers is probably due to a direct interaction between the two proteins and the S100P-mediated activation of ezrin.

Keywords: membrane/cytoskeleton linkeage, S100P, ezrin, PIP_2, migration, tumor development

CONTRIBUTION OF INTRACELLULAR Ca^{2+} STORES TO Ca^{2+} SIGNALING DURING CHEMOKINESIS OF HUMAN NEUTROPHIL GRANULOCYTES

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Extracellular agonists increase the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) by stimulating Ca^{2+} release from intracellular stores and by Ca^{2+} influx. The endoplasmic reticulum (ER) is the major agonist-sensitive Ca^{2+} store and it accumulates Ca^{2+} via the thapsigargin (TG)-sensitive Sarco/Endoplasmic-Reticulum Ca^{2+} ATPases (SERCAs). In addition to the ER, the Golgi complex may also function as a Ca^{2+}-storage site. The Golgi takes up Ca^{2+} via both SERCAs and the TG-insensitive Secretory-Pathway Ca^{2+} ATPases (SPCAs). Human neutrophil granulocytes are short-lived (about 30 hours) cells that are a major component of the non-specific immune response. Their intracellular signaling route includes an increase in $[Ca^{2+}]_c$ by Ca^{2+} release from intracellular stores and capacitative Ca^{2+} entry.

Along with SERCA2b and SERCA3, the endogenous expression of both SPCA isoforms (SPCA1 and SPCA2) was shown in neutrophils by Western blotting and immunocytochemistry.

Ca^{2+} release from a TG-resistant store was demonstrated in human neutrophils during chemokinesis induced by chemotactic factors (fMLP or interleukin-8) in Ca^{2+}-free solution. Upon fMLP or interleukin-8 stimulation, Ca^{2+} release from intracellular stores was observed in respectively about 40% and 20% of the neutrophils pre-treated with Ca^{2+}-free solution and TG. In these conditions, 20-30% of the cells preserved migratory behaviour. Additionally, F-actin staining suggests that the Ca^{2+} necessary for the reorganization of the actin cytoskeleton is predominantly derived from SERCA-loaded intracellular stores. However, the SPCA-loaded intracellular store mainly contributed to the completion of protrusion formation.

Our results indicate that both SERCA-dependent and SERCA-independent (SPCA-dependent) intracellular Ca^{2+} stores contribute to Ca^{2+} signaling during chemokinesis of human neutrophil granulocytes.

Keywords: Golgi apparatus, SERCA, SPCA, chemotaxis, neutrophils, Ca^{2+} release

CELLULAR PRION PROTEIN INTERACTION WITH AN ACTIVE LAMININ γ-1 CHAIN PEPTIDE TRIGGERS CALCIUM SIGNALING PROMOTING NEURITOGENESIS

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The cellular prion protein (PrPc) is a highly conserved cell surface glycoprotein expressed in the central nervous system. Its conformationally modified isoform, PrPsc, is responsible for prion diseases. In the last years, multiple and diverse functions of PrPc have been reported.

Our group identified and characterized some PrPc partners and cell signaling pathways triggered by these engagements. A peptide at the carboxy-terminal of the laminin (Ln) γ-1 chain (RNIAEIIKDI) binds to PrPc with high affinity and increases the percentage of cells extending neurites and the number of neurites per cell in wild-type neurons but not in those from PrPc-null ($Prnp^{0/0}$) mice. This phenotype was abrogated by specific inhibitors of the phospholipase C (PLC), protein kinase C (PKC) and extracellular regulated kinase (ERK) while a PKA inhibitor has no effect. Using an intracellular Ca^{2+} probe, Fluo-3-AM, we verified that the Ln γ-1 peptide, in particular its KDI domain, increased intracellular Ca^{2+} both by endoplasmic reticulum mobilization and extracellular medium influx in wild-type neurons whereas no effect was observed in $Prnp^{0/0}$ neurons. Ln γ-1 peptide but not a peptide where the KDI domain was changed to GGG promotes activation of PKC and phosphorylation of ERK only in neurons expressing PrPc. The increment of PKC was completely blocked by PLC or PKC inhibitors while blockers of the ERK and PKA pathways have no effect. Additionally, ERK were impaired by PLC, PKC and ERK inhibitors. In other hand γ-1 binding induced PrPc internalization and this event was not necessary for Ca^{2+} signaling or ERK phosphorylation.

Thus, our data suggest that Ca^{2+} is the first intracellular signal activated by PrPc-laminin γ-1 peptide interaction which is followed by consecutive PKC and ERK activation which finally promotes neuritogenesis.

Keywords: prion protein, neurons, laminin, Ca^{2+} signalling, neuritogenesis

ALTERED STRUCTURE OF THE CEREBELLAR GRANULE CELL LAYER OF MICE LACKING CALRETININ

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Ca^{2+}-binding proteins, such as calretinin, are abundantly expressed in distinctive patterns in the central nervous system, but their physiological functions remain poorly understood. Calretinin is highly expressed in cerebellar granule cells and calretinin-deficient mice exhibit alterations in motor coordination (Schwaller et al., 2002).

Using confocal microscopy, we demonstrate that the cerebellar cortex of calretinin-deficient mice exhibit a significantly decreased granule cells density. Interestingly, it has been shown that the migration of granule cells is tightly associated with intracellular Ca^{2+} oscillations (Komuro et al., 2005). Therefore, we hypothesize that the modification of these Ca^{2+} oscillations in calretinin-deficient mice could be involved in the observed morphological alterations.

To test this assumption, we are currently developping two strategies. First, using confocal microscopy and cerebellar microexplant culture, we are studying Ca^{2+} oscillations occuring during granule cell migration in the wild type control and calretinin knock-out mice. This allow us to characterize the impact of variations in Ca^{2+}-buffering capacity over neuronal development and on the generation of the Ca^{2+} oscillations observed during the granule cell migration. On the other hand, using a similar approach as a previous study (Roussel et al., 2006), we are developping a theoretical model based on De Young-Keizer mathematical model for $[Ca^{2+}]_i$ oscillations (De Young and Keizer, 1992). This dedicated computational model will shed light on the possible mechanism responsible for the modulation by calretinin, of Ca^{2+} oscillations during the granule cell migration.

De Young and Keizer (1992) Proc. Natl. Acad. Sci. USA 89, 9895-9899.
Komuro et al. (2005) Cell Calcium 37, 387-393.
Roussel et al. (2006) Cell Calcium 39, 455-466.
Schwaller et al. (2002) Cerebellum 1, 241-258.

Keywords: migration, calretinin, Ca^{2+}-binding proteins, granule cells, Ca^{2+} oscillations, computational modelling

INVOLVEMENT OF ENDOPLASMIC RETICULUM STRESS IN MEGAKARYOCYTE MATURATION, A ROLE FOR SERCA3 PROTEINS?

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The Sarco/Endoplasmic Reticulum Ca^{2+} ATPases type 3 (SERCA3) family which is composed of 6 human isoforms, controls with SERCA2b the intracellular Ca^{2+} stores (meanly the endoplasmic reticulum (ER)) refilling. We have recently shown that the expression of SERCA3 is increased during the maturation of the megakaryocytes (MK) (Nurden et al., 2006) and we have also described a relationship between the overexpression of some SERCA3 isoforms in HEK-293 cells and the induction of ER stress (Chaabane et al., 2006), questioning its occurrence during MK maturation.

Here, we report that a family in which severe thrombocytopenia linked to abnormal thrombopoiesis and abnormal Ca^{2+} ATPase expression, presents platelets with high level of ER stress. Then, using different models of human MK maturation (cultured $CD34^+$ cells & megakaryocytic cell lines), we demonstrate for the first time that the MK maturation involves a transient induction of ER stress markers including processing of X-box-binding protein-1 (XBP-1) mRNA and expression of glucose-regulated protein (GRP) 78. The ER stress induction seems to culminate at the proplatelet formation stage. The relation between ER stress induction and MK maturation was further established with data suggesting that in megakaryocytic cell lines direct ER stress induction by tunicamycin can lead to expression of maturation markers (expression of CTAPIII mRNA and β3 proteins) and that overexpression of some SERCA3 isoforms (3f & 3b) in MEG-01 cells can induce ER stress and some maturation.

This findings suggests that the modulation of Ca^{2+} ATPase expression may account for the activation of ER stress that leads to apoptotic-like phase of the MK maturation process.

Chaabane et al. (2006) Biochem. Biophys. Res. Commun. 345, 1377-1385.
Nurden et al. (2006) Blood,108:2587-2595.

Keywords: megakaryocytes, platelet maturation, ER stress, SERCA

OXIDANT-INDUCED INHIBITION OF THE PLASMA MEMBRANE Ca^{2+} ATPase IN PANCREATIC ACINAR CELLS: ROLE OF THE MITOCHONDRIA

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Impairment of the normal spatio-temporal pattern of intracellular Ca^{2+} ($[Ca^{2+}]_i$) signalling, and in particular the transition to an irreversible "Ca^{2+} overload" response, has been implicated in various pathophysiological states. In some diseases, including pancreatitis, oxidative stress has been suggested to mediate this Ca^{2+} overload and the associated cell injury. We have previously demonstrated that oxidative stress with hydrogen peroxide (H_2O_2), evokes a Ca^{2+} overload response and inhibition of plasma membrane Ca^{2+}-ATPase (PMCA) in rat pancreatic acinar cells (Bruce et al., 2007).

The aim of the present study was to further examine this oxidant-induced inhibition of the PMCA, focussing on the role of the mitochondria. A modified in situ $[Ca^{2+}]_i$ clearance assay was utilised to isolate PMCA activity in which the mitochondrial Ca^{2+} uptake was inhibited with Ru360. The contribution of PMCA activity to this $[Ca^{2+}]_i$ clearance was further validated by the almost complete inhibition with 1 mM La^{3+}. Under these conditions H_2O_2 (50 μM - 1 mM) markedly inhibited the PMCA activity. This H_2O_2-induced inhibition of the PMCA correlated with mitochondrial depolarisation (assessed using tetramethylrhodamine methylester fluorescence) but could occur without significant ATP depletion (assessed using magesium green fluorescence). The H_2O_2-induced PMCA inhibition and mitochondrial depolarisation were sensitive to the mitochondrial permeability transition pore (mPTP) inhibitors, cyclosporin-A and bongkrekic acid.

These data suggest that oxidant-induced opening of the mPTP and mitochondrial depolarisation may lead to an inhibition of the PMCA, that is independent of mitochondrial Ca^{2+} handling and ATP depletion, and we speculate that this may involve the release of a mitochondrial factor. Such a phemonenon may be responsible for the Ca^{2+} overload response, and for the transition between apoptotic and necrotic cell death

Bruce et al. (2007) Am. J. Physiol. Cell. Physiol. 293, C938-950.

Keywords: PMCA, oxidants, pancreatitis, mitochondria, pancreatic acinar cells

A PROTEIN COMPLEX INVOLVING BAX INHIBITOR-1, A CELL DEATH SUPPRESSOR, REGULATES INTRACELLULAR Ca^{2+} DYNAMICS

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Bax inhibitor-1 (BI-1) is an evolutionarily conserved membrane protein first identified in yeast as a suppressor of Bax-induced cell death. BI-1 also prominently protects mammalian cells against ER-stress induced cell death. BI-1 physically interacts and co-localizes with Bcl-2 in the endoplasmic reticulum (ER), where both reduce the Ca^{2+} content. We therefore wondered whether both proteins share similar mechanisms of cell protection, and whether they depend on each other.

In this study, we found that BI-1 links STIM1 to the type 1 inositol 1,4,5-trisphosphate receptor (IP_3R1) and this interaction is strengthened by Bcl-2. Using pull-down assays, we demonstrated that the N-terminal part of BI-1 interacts with Bcl-2 and STIM1, whereas the C-terminal part of BI-1 interacts with IP_3R1. Next, we developed cell-permeable TAT-peptides representing parts of the N- and C-terminal domains of BI-1. The N-terminal peptides were able to dissociate Bcl-2 and STIM1 from the BI-1 protein complex without interfering with its binding to IP_3R1. Addition of these N-terminal TAT-peptides potently induced cell death, which depended mainly on Ca^{2+} influx from the extracellular space. The underlying mechanism involves STIM1 translocation to the plasma membrane in response to cellular accumulation of the N-terminal peptide. Moreover, addition of these N-terminal peptides during $^{45}Ca^{2+}$ fluxes performed on monolayers from permeabilized mouse embryonic fibroblasts induced a potent inhibition of the passive Ca^{2+} leak from the ER and a complete inhibition of IP_3-induced Ca^{2+} release. These results were confirmed by measuring ER Ca^{2+} store content with MagFluo4 in permeabilized DT40 wild type and triple-IP_3R knockout lymphocytes.

Hence, our results point to a critical role for BI-1 in the regulation of the intracellular Ca^{2+} homeostasis by forming with Bcl-2, STIM1 and IP_3R1 a macromolecular signaling complex.

Keywords: IP_3 receptor, apoptosis, Bax inhibitor-1, STIM1, Bcl-2

ROLE OF S100A4 IN SMOOTH MUSCLE CELL PROLIFERATION: IMPLICATIONS FOR ATHEROSCLEROSIS AND RESTENOSIS

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During atherosclerosis and restenosis, smooth muscle cells (SMCs) proliferate and migrate from the arterial media to the intima where they undergo phenotypic changes. We reported that SMC populations isolated from normal porcine coronary artery media exhibited distinct phenotypes: spindle-shaped (S) and rhomboid (R). R-SMCs were characterized by highly proliferative, migratory and proteolytic activities and a dedifferentiated phenotype compared with S-SMCs. They were recovered in higher proportion from stent-induced intimal thickening (IT) compared with media suggesting that they participated in IT formation (Hag et al., 2002).

The comparison of S- and R-SMCs by 2D-gel electrophoresis followed by tandem mass spectrometry allowed us to indentify S100A4 as being a marker of the R-SMC population in vitro. S100A4 was colocalized with alpha-smooth muscle actin of quiescent R-SMCs and was up-regulated during migration. In vivo, S100A4 was absent in the media and was overexpressed in SMCs of experimentally induced IT in porcine coronary artery and of IT, atheromatous plaques and restenotic lesions in humans (Brisset et al., 2007).

We observed that S-SMCs treated with PDGF-BB acquired a R-phenotype associated to increased S100A4 expression and secretion. We further investigated the relationship between S100A4 expression and the PDGF-BB induced phenotypic changes. Blockade of S100A4 expression by using silencing RNA approach decreased PDGF-BB-induced SMC proliferation but did not affect SMC phenotype. Recombinant S100A4 increased S-SMC proliferation, which was associated to increased intracellular S100A4 expression. However, no change in SMC phenotype was observed.

Our results suggest that S100A4 when stimulated with PDGF-BB or recombinant S100A4 plays a key role in SMC proliferation. S100A4 could be a new target to prevent SMC proliferation during atherosclerosis and restenosis.

Brisset et al. (2007) Circ. Res. 100, 1055-1062.
Hao et al. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 1093-1099.

Keywords: S100A4, proliferation, vascular myocytes

PLECKSTRIN HOMOLOGY (PH) DOMAIN OF PLC δ1 COLOCALIZES WITH F-ACTIN AND INTERFERES WITH THE INTRACELLULAR Ca^{2+} SIGNALING IN STARFISH EGGS

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An intracellular Ca^{2+} increase is responsible for triggering egg activation and cortical granule exocytosis. The release of Ca^{2+} from the intracellular stores is mediated by second messengers such as $InsP_3$, cADPr, and NAADP. Recent studies have indicated that plasma membrane-enriched phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is not only a metabolic substrate of phospholipase C (PLC) but also a signaling molecule that regulates diverse actin-binding proteins.

In this study, we have microinjected starfish eggs with the fluorescently tagged pleckstrin homology (PH) domain of PLC δ1 to study the role of PIP_2 during fertilization. The application of the PH domain in our study is twofold. The first aim is to visualize accessible PIP_2 on the plasma membrane and to examine how the PIP_2 level changes as a result of possible PIP_2 hydrolysis during fertilization. Secondly, we have used RFP-PH as a tool to sequester the accessible PIP_2 on the membrane with the attempt to evaluate the roles of PIP_2 in intracellular Ca^{2+} signaling. We have observed that RFP-PH significantly delayed and diminished the sperm-induced Ca^{2+} rise in starfish eggs. RFP-PH also inhibited the release of Ca^{2+} from its stores in response to photoactivation of exogenous $InsP_3$. In contrast, the R40A mutant probe, unable to bind PIP_2, did not display such effects. Hence, the repression of Ca^{2+} signals by RFP-PH is through its binding to PIP_2, which may interfere with the Ca^{2+} release mechanism itself rather than simply preventing the access of PLC to its substrate. In parallel to the changes in Ca^{2+} signaling, RFP-PH labeling has increased in the subplasmalemmal region and the long microvilli where F-actin has newly formed.

Taken together, these results raise the possibility that the PH domain of PLC δ1 may have an effect on intracellular Ca^{2+} signaling in an unconventional mechanism involving PIP2 and the actin cytoskeleton.

Keywords: PH domain, PIP_2, actin, phospholipase C, starfish, oocyte activation, Ca^{2+} signaling

THE MULTI-SERCA SYSTEM OF NON-FAILING AND FAILING HUMAN HEART IN 2008

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Ca^{2+} ions play a key role in cardiac contraction and relaxation. Among the structures involved in the regulation of the $[Ca^{2+}]_i$ are SERCA (Sarco/Endoplasmic Reticulum Ca^{2+}ATPases). They are encoded by 3 genes, SERCA1-3, each of them giving rise to various isoforms. Until recently, it was thought that human heart mainly express two isoforms: SERCA2a & 2b. In 2006, a new SERCA2c isoform was added (Dally et al., 2006).

The aim of the present study was to look for the expression and role of 3 additional SERCA3 isoforms: a, d & f. This hypothesis was based on the previous evidence for these SERCA3 mRNAs in the heart (Martin et al., 2002; Bobe et al., 2004). First, the existence of endogenous proteins was obtained by using isoform-specific antibodies, and either Western Blotting of protein lysates or immunoprecipitation of membrane proteins. An immunolocalisation study of both left ventricle tissue and isolated cardiomyocytes showed a distinct compartmentalization of the SERCA3 isoforms, as a uniform distribution of SERCA3a was detected while -3d & -3f isoforms were observed around the nucleus and at a submembrane region in the close vicinity of the sarcolemma, respectively. Second, we studied their expressions in failing hearts including mixed (MCM) (n = 1) and idiopathic dilated cardiomyopathies (IDCM) (n = 4). Compared with controls (n = 5), similar expressions of SERCA3a & -3d mRNAs were observed in all patients. In contrast, SERCA3f mRNA was found to be up-regulated in failing hearts (125 ± 7%), while SERCA3f overexpressed protein was found to be associated with endoplasmic reticulum (ER) stress in HEK-293 cells (Chaabane et al., 2006). Remarkably, overexpression of SERCA3f paralleled an increase in ER stress markers including processing of X-box-binding protein-1 (XBP-1) mRNA (176 ± 24%), and expression of XBP-1 protein and glucose-regulated protein (GRP)78 (232 ± 21%).

These findings revisit the human heart's Ca^{2+} ATPase system and indicate that SERCA3f may account for the mechanism of ER stress in vivo in heart failure.

Bobe et al. (2004) J. Biol. Chem. 279, 24297-24306.
Chaabane et al. (2006) Biochem. Biophys. Res. Commun. 345, 1377-1385.
Dally et al. (2006) Biochem. J. 395, 249-258.
Martin et al. (2002) J. Biol. Chem. 277, 24442-24452.

Keywords: SERCA, cardiomyocytes, failing heart, ER stress

OXIDATIVE DAMAGE TO THE ENDOPLASMIC RETICULUM BY HYPERICIN-MEDIATED PHOTODYNAMIC THERAPY AS INDUCER OF APOPTOSIS AND AUTOPHAGY PATHWAYS

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Photodynamic therapy (PDT) is an anticancer therapy utilizing cytotoxic reactive oxygen species (ROS) produced after irradiation of a photosensitizer with visible light, to kill the cancer cells (Dolmans et al., 2003). Light activation of the ER-associated photosensitizer hypericin causes immediate photooxidative damage to the SERCA pump, due to the local generation of $^1O_2$, This event results in the rapid loss of ER Ca^{2+} homeostasis which leads to ER stress and a subsequent stress-response, triggering both apoptotic cell death and autophagy.

While a previous study indicated an involvement of autophagy in the execution of apoptosis-incompetent Bax/Bak double knock-out MEFs (Buytaert et al., 2006), the exact role of PDT-induced autophagy in apoptosis-competent cells remains largely unclear.

In this study we show that in apoptosis-competent cells, the induction of apoptotic cell death occurs concomitantly with the onset of autophagy, suggesting that both pathways are simultaneously propagated in response to PDT-mediated ER stress. To study the role of autophagy in apoptosis-competent cells we used MEFs conditionally expressing the essential atg-gene Atg5. Interestingly, a knock-out of Atg5, does not only inhibit autophagy but also hampers the release of cytochrome c and caspase-activation in response to PDT. Intriguingly, Atg5-deficient cells exhibit an increased capacity to remove oxidative damaged proteins as compared to their wild type counterparts. On the other hand, pharmacological inhibition of autophagy with 3-methyladenine, also suppresses autophagy but increases the apoptotic fraction due to a hampered removal of oxidized proteins.

These data point at a complex level of cross-communication between apoptosis and autophagy in PDT-treated cells and suggest that either key autophagy genes have a potential role in propagating apoptosis or that other compensatory and protective mechanisms exist in cells in which autophagy has been permanently knocked down. We are currently elucidating these possibilities.

Buytaert et al. (2006) FASEB J. 20, 756-758.
Dolmans et al. (2003) Nat. Rev. Cancer 3, 380-387.

Keywords: photodynamic therapy, apoptosis, autophagy, ER stress

OVEREXPRESSION OF TRP CALCIUM CHANNELS AND CORRELATION WITH CLINICAL FEATURES IN HUMAN BREAST DUCTAL ADENOCARCINOMAS

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Plasma membrane ionic channels contribute to proliferation, differentiation, and apoptosis processes. The TRP (Transient Receptor Potential) calcium channels are involved in cancer progression and some of them have been proposed as prognostic marker in prostate cancer (TRPV6 and TRPM8) or proliferative marker in head and neck cancer (TRPM7). However, little is known about the expression and the role of TRP channels in breast cancer.

We first investigated, by RT-PCR, the expression pattern of TRPC, TRPV and TRPM channels in three human breast models: cancer tissue samples, primary culture of tumoral epithelial cells (hBCE), and cancerous MCF-7 cell line. Then, we focused our study on TRPC1, TRPC6, TRPV6, TRPM8 and TRPM7 which are well known to be involved in other cancers, particularly in prostate cancer. The statistical analysis, on up to 59 patients, showed an overexpression of these channels in a large majority of the tumoral tissue samples compared to their normal counterparts, and a correlation of the overexpression with some tumor characteristics (grade, size, expression of the proliferative marker Ki67 or the estrogen receptor ERα). TRPC6 is overexpressed in tumors with low Ki67 (<10%). TRPC1 and TRPM8 are overexpressed in small tumors (<2cm) when both grade (grade I) and Ki67 are weakest. In contrast, TRPM7 is overexpressed in large tumors (>2cm) with high Ki67 and grade (grade III). TRPV6 was mainly overexpressed in ER positive invasive tumors, as confirmed by laser capture microdissection. Finally, these five channels are functional in breast cancer cells as demonstrated in the MCF-7 cell line using the whole-cell patch clamp technique.

In conclusion, this work allowed:1) the establishment of the expression pattern of TRP channels in human breast epithelial cells; 2) the identification of the TRP channels involved in breast carcinogenesis; and 3) the initiation of studies about the functionality and the role of these channels in breast cancer.

Keywords: TRP channels, breast cancer

THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR IN AVIAN GRANULOSA CELLS: A KEY TO SURVIVAL DURING FOLLICULOGENESIS

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Introduction: Increasing concentrations of an extracellular calcium load in the culture medium of granulosa explants was able to attenuate granulosa cell (GC) apoptosis in a dose-dependent manner upon 24h of culture, suggesting a receptor-mediated effect (Mussche et al., 2000).

Objective: The current study aimed at investigating the possible involvement of the calcium-sensing receptor (CaR) in quail GC survival signalling.

Methods: The presence of the CaR at the GC surface of quail (Coturnix coturnix japonica) follicles was assessed by western blot and immunohistochemistry. The effect of the polyvalent cations calcium and magnesium, the polycationic molecule spermine, and the positive allosteric modulator R-568 on apoptosis was evaluated in serum-deprived F1 granulosa quail explants after addition of C8 ceramide or LY294002 for 24h at 38ºC. Fluorescence microscopy was used to quantify the percentage of apoptotic cells upon nuclear staining with DAPI.

Results: The CaR was identified as a 130 to 140 kDa protein expressed in quail follicles selected for ovulation and in cultured GC explants. No immunoreactive signal was evidenced at the cohort of previtellogenic or small vitellogenic follicles. Addition of the different CaR agonists caused inhibition of apoptosis elicited by gonadotropin withdrawal or C8 ceramide. Incubation in the presence of LY294002 elicited GC apoptosis, indicating that the PI3-K pathway is involved in GC survival. However, LY294002–induced apoptosis could be attenuated by incubation with CaR agonists suggesting that the PI3-K pathway is not the major survival system activated through the CaR.

Conclusions: This report shows the first direct evidence of the presence of CaR in preovulatory granulosa explants and suggests a pivotal regulatory role of the CaR in follicle selection.

Mussche et al. (2000) Ann. N.Y. Acad. Sci. 926, 101-115.

Keywords: calcium receptor, granulosa cells, folliculogenesis, apoptosis

S100B IN MYOBLASTS COUNTERACTS APOPTOSIS AND STIMULATES THE TRANSITION FROM QUIESCENCE TO PROLIFERATION

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Muscle regeneration is a process by which mononucleated, quiescent satellite cells (SCs) that coexist with myofibers, become activated, proliferate and eventually fuse with each other to form new myofibers and/or fuse with the damaged myofibers to repair them (Charge and Rudnicki, 2004). However, during muscle regeneration a fraction of SCs neither participate in the formation of myotubes nor die; they stop proliferating and reacquire the quiescent state thus reconstituting the SC reserve pool. S100B has been implicated in the regulation of proliferation and differentiation in several cell types (Donato, 2001). We showed that (Tubaro et al., 2008): 1) silencing S100B in myoblasts by RNA interference resulted in stimulation of differentiation via IKKβ / NF-κB-dependent repression of expression of the muscle-specific transcription factor, MyoD, and conversely, overexpression of S100B in myoblasts resulted in inhibition of differentiation; 2) myoblasts in differentiation medium downregulated S100B expression; and 3) S100B was constitutively expressed in muscle tissue SCs.

We show here that overexpression of S100B in myoblasts reduces spontaneous and H_2O_2-induced apoptosis, makes myoblasts less prone to acquire a quiescent state, and accelerates resumption of proliferation of quiescent myoblasts. This latter effect is dependent on a rapid and sustained activation of the mitogenic and pro-survival kinases ERK1/2 and Akt and of NF-κB transcriptional activity. Thus, we propose that intracellular S100B participates in muscle regeneration by modulating differentiation cues (Tubaro et al., 2008) and by regulating the transition from quiescence to proliferation and back to quiescence.

Specifically, S100B might contribute to myofiber regeneration by stimulating the proliferation of activated SCs and protecting them from death-inducing stimuli. On the other hand, upregulation of S100B expression in SCs might adversely affect myofiber regeneration and the reconstitution of the SC reserve pool in case of muscle injury.

Charge and Rudnicki (2004) Physiol. Rev. 84, 209-238.
Donato (2001) Int. J. Biochem. Cell Biol. 33, 637-668.
Tubaro et al. (2008), submitted for publication.

Keywords: S100B, muscle regeneration, myoblast, quiescence, proliferation, apoptosis

ROLE OF S100B IN NEUROSPHERE FORMATION IN THE MIO-M1 MULLER CELL LINE

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S100B has been implicated in the regulation of proliferation, differentiation, apoptosis, Ca^{2+} homeostasis, the dynamics of cytoskeleton constituents, protein phosphorylation and enzyme activities (Donato, 2001). Reducing S100B expression in the GL15 glioma cell line and MIO-M1 Müller cells by RNA interference (RNAi) resulted in disassembly of stress fibers, reduced migration, and enhanced formation of GFAP filaments and acquisition of the stellate morphology typical of differentiated astrocytes (Brozzi et al., 2008). These effects coincided with a reduced activity of the PI3K/RhoA/ROCK module and increased activity of the GSK3β/Rac1 module (Brozzi et al., 2008), suggesting that S100B normally activates the PI3K/RhoA/ROCK module thereby favoring astrocyte migration and modulates the GSK3β/Rac1 module thereby reducing astrocyte differentiation.

We show that rat primary astrocytes transiently downregulate S100B expression when exposed to the differentiating agent, db-cAMP, and re-express S100B at later stages of db-cAMP-induced differentiation. Müller glia have properties of neuronal progenitor cells in the retina (Reh and Fischer, 2006). MIO-M1 Müller cells (Limb et al., 2002) have stem cell properties and differentiate into neuronal-like cells when treated with bFGF (Lawrence et al., 2007). Cultivation of MIO-M1 cells as described by Lawrence et al. (2007) results in formation of neurosphere-like aggregates made for the most part of small S100B-positive cells and virtually devoid of GFAP filament-positive cells. However, reducing S100B levels by RNAi results in reduced migration of MIO-M1 cells, an almost complete loss of their ability to form neurospheres and downregulation of the neuronal marker, calretinin. Also, silencing S100B in MIO-M1 cells significantly attenuates bFGF-induced neurosphere formation.

Our data suggest that S100B might modulate astrocyte differentiation, maintaining the pluripotency of radial glia stem cells and countering differentiation cues, and that downregulation of S100B in these cells concurs to their commitment to astrocytes.

Donato (2001) Int. J. Biochem. Cell Biol. 33, 637-668.
Brozzi et al. (2008), submitted for publication.
Reh and Fischer (2006) Meth. Enzymol. 419, 52-73.
Limb et al (2002) Invest. Ophthalmol. Vis. Sci. 43, 864-869.
Lawrence at al (2007) Stem Cells 25, 2033-2043.

Keywords: S100B, stem cells, differentiation, glial cells

DIFFERENTIAL INVOLVEMENT OF RAGE AND FGFR1 IN S100B EFFECTS ON MYOBLAST DIFFERENTIATION

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Extracellular S100B was shown to inhibit myoblast differentiation by inactivating the promyogenic p38 MAPK and activating the mitogenic ERK1/2 in a receptor-mediated manner (Sorci et al., 2003; Riuzzi et al., 2006). Also, S100B differentially affected C2C12 myoblast differentiation depending on cell density and the duration of treatment of myoblasts with the protein (Riuzzi et al., in preparation). Indeed, a short-term (24 h) treatment with S100B resulted in inhibition and stimulation of differentiation in the case of high-density (HD) and low-density (LD) myoblasts, respectively, during the next 24 h. In both cases, S100B stimulated proliferation, but it activated ERK1/2 and p38 in LD myoblasts and activated ERK1/2 and inhibited p38 in HD myoblasts. In LD myoblasts, the S100B-induced increase in myoblast number and the concomitant activation of p38 accelerated myogenic differentiation, while in HD myoblasts differentiation was interfered with by S100B due to proliferation in the presence of hypoactive p38.

These results pointed to engagement of different receptors in LD and HD myoblasts. Indeed, co-immunoprecipitation studies revealed that S100B engaged RAGE in LD myoblasts and bFGF receptor 1 (FGFR1) in HD myoblasts (Riuzzi et al., in preparation). In LD myoblasts S100B could not bind FGFR1 owing to competing bFGF. By contrast, RAGE and FGFR1 co-immunoprecipitated with S100B in HD myoblasts, while HMGB1 and bFGF only bound RAGE and FGFR1, respectively. However, S100B also bound bFGF, and S100B interaction with FGFR1 in HD myoblasts was mediated by bFGF. Thus, in HD myoblasts a quaternary RAGE-S100B-bFGF-FGFR1 complex forms that results in inability of RAGE to activate p38 (Sorci et al., 2004; Riuzzi et al., 2007) probably due to inability of bFGF-bound S100B to induce RAGE oligomerization (Ostendorp et al., 2007). In LD mouse muscle satellite cells also S100B bound RAGE but not FGFR1 while in LD RAGE null satellite cells S100B bound FGFR1. Thus, S100B-RAGE and S100B-FGFR1 interactions in myoblasts differentially affect myogenic differentiation which might have important implications during muscle regeneration.

Ostendorp et al. (2007) EMBO J. 26, 3868-3878.
Riuzzi et al. (2006) J. Cell. Physiol. 207, 461-470.
Riuzzi et al. (2007) J. Biol. Chem. 281, 8242-8253.
Sorci et al. (2003) Mol. Cell. Biol. 23, 4870-4881.
Sorci et al. (2004) Mol. Cell. Biol. 24, 4880-4894.

Keywords: S100B, RAGE, FGFR1, FGF, myoblast, differentiation

CELL PROLIFERATION AND TRPC6 EXPRESSION IN LIVER CANCER CELL LINE

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We have recently demonstrated that store-operated calcium entry (SOCE) is the main Ca^{2+} influx pathway involved in controlling proliferation of the human hepatoma cell lines HepG2 and Huh-7 (Enfissi et al., 2004). However, the molecular nature of the calcium channels involved in this process remains unknown. Huh-7 and HepG2 cells express TRPC1 and TRPC6 (transient receptor potential canonical 1 and 6), as well as STIM1 and Orai1, and these four channels are the most likely candidates accounting for SOCE in these cells.

Stable TRPC6-overexpressing or TRPC6 knockdown Huh-7 clones were generated to investigate correlations between the presence of the protein, the rate of cell proliferation and SOCE amplitude. Proliferation rate and SOCE amplitude were increased by 80% and 160% respectively in TRPC6-overexpressing Huh-7 cells as compared to control cells. By contrast, proliferation rate and SOCE amplitude were reduced by 50% and 85% respectively in TRPC6 knockdown clones than in untransfected cells. OAG-induced calcium entry was similar in all cells and siRNA against TRPC1 had no effect on SOCE amplitude, highlighting the relationship between SOCE, TRPC6 and cell proliferation in Huh-7 cells. SOCE amplitude was reduced by STIM1 and Orai1 knockdowns, suggesting possible cooperation between these proteins and TRPC6 in these cells. EGF and HGF increased TRPC6 expression and SOCE amplitude in Huh-7 cells and cyclin D1 expression was decreased by STIM, Orai1 and TRPC6 knockdowns. TRPC6 was very weakly expressed in isolated hepatocytes from healthy patients and expressed more strongly in samples obtained from tumoral zones from liver of cancer patients. Immunocytochemical staining indicated that TRPC6 was expressed on hepatocytes solely in the tumoral zones of human liver, strongly supporting a role for these calcium channels in liver oncogenesis.

Enfissi et al. (2004) Cell Calcium 36, 549-467.

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Keywords: hepatoma cells, TRPC6, store-operated Ca^{2+} entry

TRPV CHANNELS EXPRESSION AND FUNCTIONAL ROLE IN TUMOR ANGIOGENESIS

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Growth factor-induced intracellular calcium signals in endothelial cells regulate cytosolic and nuclear events involved in the initiation and progression of angiogenesis. Therefore regulation of changes in $[Ca^{2+}]_i$ is a potent putative molecular target for angiogenesis interference. The molecular nature of the channels involved is still unknown: however recently a large amount of evidence points to the involvement of transient receptor potential (TRP) superfamily of channels as a relevant route of agonist-induced calcium entry, including in vascular endothelium. Interestingly, some authors reported that members of TRP channels could be activated by fatty acids: in particular, TRPV1 and 4 are gated by arachidonic acid (AA) metabolites.

Here we studied the role of TRPV4-mediated intracellular calcium signals in endothelial cells derived from human breast carcinomas (B-TECs). B-TECs express functional TRPV4 channels in the plasma membrane, as shown by the ability of the specific TRPV4 agonist 4αPDD to mediate $[Ca^{2+}]_i$ increase. Recently we reported that AA stimulation promotes B-TECs organization of vessel-like structures in vitro and induces $Ca^{2+}{}_i$ signals in the entire capillary–like structure during the early phases of tubulogenesis in vitro: no such responses are detectable in B-TECs organized in more structured tubules. Analyzing at the single cell level the capillary-like structure we show a “positional” effect of TRPV4 mediated $Ca^{2+}{}_i$ signals: only a low percentage of ‘highly connected’ cells within the tubules respond to AA or 4αPDD, while the same agonists fail to induce any $Ca^{2+}{}_i$ signal in the great majority of ‘less connected’ cells . Similar results were obtained in monolayer cell cultures, suggesting a specific role of these channels during B-TECs migration.

Keywords: angiogenesis, TRPV4, breast cancer, arachidonic acid

Orai1 DOWNREGULATION: A MISSING LINK IN UNDERSTANDING THE PROSTATE CANCER APOPTOSIS RESISTANCE

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Prostate cancer (PCa) is one of the leading threats to men's health. During the early stage, when it depends on androgens for growth and survival, androgen ablation therapy may be effective in causing tumors to regress, while, in the later, androgen-independent, stage there is currently no successful therapy. The progression to androgen independence is associated with the appearance of new cell phenotypes, characterized by a low apoptosis rate. It is, therefore, vital to understand what drives PCa to apoptosis resistance.

We have previously shown that apoptosis inhibition in androgen-independent PCa cells is associated with the downregulation of Store Operated Calcium Entry (SOCE), due to the decrease in the number of functional channels activated following endoplasmic reticulum calcium store depletion. However, the molecular nature of these channels, which play a major role in PCa cell apoptosis induction, has not yet been elucidated.

Here, we show that the recently-identified Orai1/CRACM1 protein is a store-operated calcium channel in androgen-dependent human prostate cancer (LNCaP) cells. The expression of Orai1/CRACM1 and the amplitude of store-operated current decreased dramatically in androgen-deprived cells. This downregulation of the Orai1/CRACM1 channels leads to the inhibition of sustained SOCE and, subsequently, to the decrease in apoptosis. Orai1/CRACM1 knockdown protects LNCaP cells from thapsigargin-induced apoptosis. Thus, our work demonstrates that Orai1/CRACM1 plays a pivotal role in prostate cancer progression to an apoptosis-resistant phenotype. Consequently, it is a potentially attractive target for therapeutic intervention. Furthermore, this study is the first to show the role of Orai1/CRACM1 in physiopathology outside the immune system.

Keywords: store-operated Ca^{2+} entry, apoptosis, prostate cancer, Orai1

INVOLVEMENT OF TRPM7 IN BREAST CANCER CELL PROLIFERATION

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One major clinical problem with breast cancer is the cell's ability to proliferate. Because Transient Receptor Potential (TRP) channels have been implicated in tumour cells, we have investigated the presence of TRPM7 and its role in cell proliferation in the MCF-7 breast cancer cell line, in human breast cancer epithelial primary culture (hBCE) and in breast cancer tissues.

TRPM7 is expressed at both mRNA and protein levels in 26 normal and carcinoma breast tissues and its expression, in breast cancer, depends on the proliferation index (Ki67). TRPM7 is over-expressed in grade III breast cancer tissues when Ki67 is greatest, while its expression is unchanged when Ki67 is lowest. Under the patch clamp technique, a spontaneous Mg^{2+}-sensitive cationic current was developed in MCF-7 cells. This current was characterized by an inward current and a strong outward rectification current, both inhibited by intracellular Mg^{2+} and Mg^{2+}-ATP. The inward current was reduced only by La^{3+}, in contrast to the outward current which was sensitive to 2-aminoethoxydiphenyl borate (2-APB), spermine, La^{3+} and fluflemanic acid. A Mg^{2+}-sensitive current was also recorded in hBCE cells. It was also inhibited by intracellular Mg^{2+}. TRPM7 transcripts were detected in both hBCE and MCF-7 cells. Down-regulation of TRPM7 by siRNA inhibited the currents, particularly the inward one, the basal intracellular Ca^{2+} concentration and MCF-7 cell proliferation. Moreover, cell proliferation decreased when we reduced the extracellular Ca^{2+}. Our findings strongly suggest the importance of TRPM7 in generating spontaneous Ca^{2+} influx that is probably associated with the proliferative potential of breast cancer cells.

* Both authors contributed equally to this work

Keywords: TRPM7, proliferation, breast cancer

SERCA2b IS A KEY ACTOR OF IGF-1 DEPENDENT MCF-7 PROLIFERATION

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The insulin-like growth factor 1 (IGF-1) plays an important role in the normal development and function of the mammary gland. However, accumulating evidence suggests that IGF-1 is also involved in breast cancer development. Indeed, elevated levels of circulating IGF-1 are associated with increased risk of breast, prostate, and colon cancers. IGF-1 is a potent mitogenic agent in MCF-7 human breast carcinoma cells. Maintenance of Ca^{2+} levels within the lumen of the endoplasmic reticulum (ER) is another critical factor for cell growth. Accumulation of Ca^{2+} in the ER is mediated by specific sarco/endoplasmic reticulum calcium transport ATPases (SERCA) and it's been shown that down-regulation of endogenous SERCA expression reduced both prostatic cancer cell proliferation and $[Ca^{2+}]_{ER}$.

The present study evaluates the role of SERCA activity as a key actor for MCF-7 cell growth induced by IGF-1.

For that, we used in a first part a pharmalogical approach : 48h treatment with thapsigargin and cyclopiazonic acid (CPA), two SERCA blockers, reduced in a dose-dependent manner both $[Ca^{2+}]_{ER}$ and MCF-7 cell proliferation induced by IGF-1. Both drugs were without effects when applied alone. Moreover, IGF-1 was able to restore the $[Ca^{2+}]_{ER}$ and cell proliferation induced by SERCA inhibition, when we removed the reversible SERCA inhibitor (CPA).

As SERCA2b constitutes the ubiquitously SERCA isoform, we used in a second part, a small interfering RNA SERCA 2a/b sequence, to down-regulate endogenous SERCA2b expression.We observed in transfected cells a significant reduction in Ca^{2+}-store content leading to a decrease in cell growth induced by IGF-1.

The present study provides direct evidence that SERCA2b activity and $[Ca^{2+}]_{ER}$ are major players in IGF-1 signal transduction controlling epithelial breast cancer cell growth.

Keywords: breast cancer, SERCA, proliferation, IGF-1

SV40- AND ASBESTOS-INDUCED UPREGULATION OF CALRETININ PROTECTS MESOTHELIAL CELLS FROM CYTOTOXICITY AND MAY LEAD TO MESOTHELIOMA CARCINOGENESIS

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Malignant mesothelioma is an aggressive fatal tumor originating from mesothelial cells and is frequently associated with exposure to asbestos. Evidence is growing that SV40 virus might be involved in the etiology of mesothelioma by acting as a co-carcinogen.

MeT-5A GE cells (immortalized cells of mesothelial origin), were transfected with SV40 early region genes. Only in clones with high expression levels of T large antigen, calretinin (CR) expression was considerably upregulated in comparison with the parental cell line. Incubation with asbestos fibers showed that clones with high CR expression levels had a higher survival rate. To directly assess the putative cytoprotective role of CR, cells were transfected with a CR-expression plasmid and selected stably CR-expressing clones were exposed to asbestos. The survival rate was highest in clones with high CR expression levels and lower in the “low CR” and the mock-transfected control groups. Thus, CR appears as a major factor contributing to the resistance to asbestos toxicity.

Perturbations of signaling pathways play an important role in human cancer and the PI3K/AKT pathway is activated in many cancers including human mesotheliomas. Asbestos treatment of SV40-, CR-transfected and control cells resulted in approximately 50% increased pAKT/tAKT levels in all clones. Adding the PI3K/AKT inhibitor PI103 to asbestos-treated cells reduced cell viability significantly in SV40- and CR-transfected clones, but the control group was not affected indicating that only in high CR-expressing clones the AKT pathway is involved in the protective mechanisms against crocidolite. Elevated CR levels in mesothelial cells may be the common underlying cause leading to the increased resistance to signals (e.g. asbestos fibers) normally leading to cell death. By such a mechanism, affected mesothelial cells may escape senescence, accumulate additional mutations finally leading to a fully transformed state.

Keywords: mesothelioma, calretinin, asbestos, protein kinase B

ASSISTED OOCYTE ACTIVATION WITH IONOPHORE-TRIGGERED Ca^{2+} CHANGES DOES NOT GENERATE Ca^{2+} OSCILLATIONS BUT IS SUFFICIENT TO ACTIVATE THE OOCYTE

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At fertilization sperm triggers oscillations in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), that are necessary for oocyte activation and normal embryo development. Egg activation does not occur in case of globozoospermic sperm. However, artificially increasing $[Ca^{2+}]_i$ in oocytes injected with globozoospermic sperm using a Ca^{2+} ionophore is sufficient to achieve normal fertilization and embryo development as evidenced by successful human pregnancies and births. It is nevertheless unclear whether assisted oocyte activation induces Ca^{2+} oscillations. We investigated in mouse oocytes $[Ca^{2+}]_i$ changes triggered by injection of globozoospermic sperm and ionophore treatment.

Injection of human globozoospermic sperm did not trigger $[Ca^{2+}]_i$ changes nor did it activate the oocyte (0/12), in contrast to injection with human fertile sperm where almost all cells that extruded the second polar body (PB2) displayed Ca^{2+} oscillations (17/19). Treatment with ionomycin (twice 10 µM, 10 min) increased $[Ca^{2+}]_i$ during exposure and activated more than 50 % of the oocytes (PB2 extrusion ~2.5 h later, 61 %, 81 % and 83 % for patient A, B and C respectively) but did not trigger Ca^{2+} oscillations (0/14, 0/21 and 0/20 for oocytes injected with sperm from patient A, B and C respectively versus 17/19 for fertile sperm injected oocytes, $p<0.001$). Computational modeling indicated that ionophore treatment (two exposures) was equally successful as a normal Ca^{2+} oscillation pattern to induce release from MII arrest and cell cycle resumption.

Assisted oocyte activation with ionomycin does not induce Ca^{2+} oscillations after ICSI with sperm from globozoospermic men. This, however, does not prevent successful activation of downstream pathways involved in cell cycle resumption as also evidenced by pregnancies.

These findings support the view that artificially induced $[Ca^{2+}]_i$ changes can replace the complex pattern of Ca^{2+} oscillations to attain normal embryo development.

Keywords: oocyte activation, computational modelling, Ca^{2+} signalling, Ca^{2+} oscillations

RESVERATROL MODULATES INTRACELLULAR CALCIUM BY OPENING THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR IN ENDOTHELIAL CELLS

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Resveratrol is a non-toxic polyphenol that has been shown to inhibit tumor growth in various animal models of cancer. One potential mechanism by which resveratrol blocks the growth of tumors is by inhibiting the formation of new blood vessels (angiogenesis).

Calcium signaling in endothelial cells plays a key role in regulating angiogenesis, therefore the objective of the current study was to evaluate whether resveratrol can regulate calcium signaling, inhibit angiogenesis, and thereby modulate tumor growth. Intracellular calcium levels ($[Ca^{2+}]_i$) were measured in a mouse endothelial cell line, SVEC 4-10. Treatment of the SVEC 4-10's with 200 μM resveratrol induced a rise in $[Ca^{2+}]_i$ that peaks at 265±87 nM approximately 25 seconds following resveratrol addition.

Cells were pretreated with thapsigargin and showed a 71% reduction in the peak resveratrol-induced calcium response, indicating that the endoplasmic reticulum (ER) is the intracellular source of calcium. The ryanodine receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP_3R) are channels that control the release of calcium from the ER. To further evaluate the resveratrol-induced rise of $[Ca^{2+}]_i$, inhibitors were used for both the IP_3R and the RyR, 2-APB and Ruthenium Red, respectively. 2-APB inhibited the peak resveratrol-induced rise of $[Ca^{2+}]_i$ by 81%, however Ruthenium Red had no effect.

The release of calcium via the IP_3R is often induced by an increase in cellular IP_3 levels. IP_3 is formed by the cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C (PLC). Therefore, to determine if resveratrol activates PLC, an inhibitor of PLC (U73122) was used. The peak resveratrol-induced rise in $[Ca^{2+}]_i$ was diminished by 91% in the presence of U73122.

In conclusion, the data provided here demonstrate that resveratrol activates PLC-dependent production of IP_3, and that the IP_3 then activates the IP_3R causing the release of calcium from the ER.

Keywords: IP_3 receptor, Ca^{2+} release, resveratrol, endothelium, phospholipase C

MODEL OF MOUSE EMBRYONIC CARDIOMYOCYTE EXCITATION-CONTRACTION COUPLING

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The process linking the electrical activity of the cardiomyocyte membrane to the contraction of the myocyte is referred to as excitation-contraction coupling (ECC). The ECC of adult cardiomyocytes is known in great detail, but the mechanisms regulating the activity of embryonic cardiomyocytes are largely unknown.

In this study we have characterized the function of early mouse embryonic cardiomyocytes (embryonic days 9-11) and developed a mathematical model based on this characterization. The major findings were 1) the spontaneous activity in these cells is based on spontaneous sarcoplasmic reticulum originated Ca^{2+} oscillations, which trigger the action potentials via the Na^{+}/Ca^{2+}-exchanger and 2) the action potentials and Ca^{2+} oscillations can also be synchronized externally to form coordinated contraction in tissue. We validated the mathematical model, which explains the mechanisms underlying this functional versatility by comparing the simulated ECC features to those observed in the experiments. We further simulated the ECCs with genetically engineered expression levels of the Na^{+}/Ca^{2+}-exchanger, hyperpolarization-activated current and calreticulin Ca^{2+} buffer.

Our mathematical model was able to reproduce the experimentally observed unique ECC features of normal and genetically engineered embryonic mouse myocytes. Our model thus explains the major components and contributing roles and interactions that are required for mouse embryonic ECC.

* Both authors contributed equally to this work.

Keywords: computational modelling, embryonic cardiomyocytes, excitation-contraction coupling

ACTIVATION OF DHP-CALCIUM CHANNELS THROUGH FGF SIGNALING DURING NEURAL INDUCTION IN XENOPUS LAEVIS

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In vertebrate, the formation of the nervous system starts at gastrulation with a process called neural induction. This process requires at least partly the inhibition of BMP signalling in the ectoderm by Noggin. However, our studies on Xenopus argue that an increase in internal Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a crucial role. Ca^{2+} imaging of intact Xenopus embryos reveals patterns of Ca^{2+} transients occurring in the dorsal ectoderm where neural induction occurs. Noggin triggers a depolarisation and the activation of dihydropyridine-sensitive (DHP) calcium channels is responsible of the increase in $[Ca^{2+}]_i$. In addition we have demonstrated that these transients of Ca^{2+} are necessary and sufficient to orientate the ectodermal cells toward neural fate (Moreau et al., 1994; Leclerc et al., 2000; 2003), and calcium controls directly the expression of neural genes.

The mechanism by which the DHP calcium channels are activated during neural induction remains unknown. One candidate mechanism is through the activation of FGF signalling which has been shown to be involved in neural induction. We demonstrated, on isolated ectoderm tissue, that FGF4 depolarize the membrane of ectodermal cells and induce an increase in $[Ca^{2+}]_i$. This Ca^{2+} increase can be blocked by SU5402, an inhibitor of FGF receptor, and by DHP Ca^{2+} antagonists. These inhibitions block neural induction.

Here we discuss a gating mechanism for the activation of dihydropyridine-sensitive Ca^{2+} channels during neural induction that involved membrane depolarisation through activation of the FGF signalling pathway.

Leclerc et al. (2000) J. Cell Sci. 113, 3519-3529.
Leclerc et al. (2003) Dev. Biol. 261, 381-390.
Moreau et al. (1994) Proc. Natl. Acad. Sci. USA 91, 12639-12643.

Keywords: L-type Ca^{2+} channels, FGF, neurogenesis

BASAL Ca^{2+} INFLUX CONTROLS NFAT TRANSCRIPTIONAL ACTIVITY AND PROLIFERATION OF HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Basal calcium leak into vascular smooth muscle cells (VSMC) was identified 30 years ago yet remains poorly understood. We have previously detected by using single-channel recording in hamster VSMCs a spontaneous voltage-independent basal Ca^{2+} channel (B-type) activity characterized by high conductance (>20 pS), permeability to Ca^{2+} and Ba^{2+} and inhibition by La^{3+}, AlF_3, eosin and mibefradil (Lipskaia et al., 2007). This basal calcium current was low in quiescent and significantly increased in proliferating VSMC, lacking the sarco/endoplasmic reticulum calcium ATPase, SERCA2a. The B-type channel activity was correlated with transcriptional activation of the calcium-regulated transcription factor NFAT, required for proliferation.

In this study, we tested the hypothesis that in human arteries, SERCA2a controls VSMC proliferation via inhibition of the basal calcium leak. In human atherosclerotic coronary arteries (CA) SERCA2a and the ryanodine receptor (RyR2) were expressed only in differentiated VSMCs from the media but not in the neointima, containing mainly proliferating cells. Primary CA VSMCs had lost SERCA2a and RyR2 within 3 days after induction of proliferation. SERCA2a gene transfer inhibited VSMC migration and proliferation through inhibition of NFAT leading to down regulation of cell cycle controlling proteins cyclin D1 and Erg1. Single-channel patch-clamp recording showed that SERCA2a gene transfer inhibited basal voltage-independent Ca^{2+} influx. Promoter-reporter assay showed that in human VSMCs, NFAT was activated by store-dependent Ca^{2+} influx, inhibited by depolarization-induced Ca^{2+} influx and by several calcium channels inhibitors such as nifedipine (LTCC inhibitor), mibefradil (TTCC inhibitor), carboxyamidotriazole and 2-aminoethoxydiphenyl (SOCC inhibitors). The NFAT transcriptional activity strongly correlated with proliferative state. In conclusion, in human CA VSMC, basal voltage-independent Ca^{2+} influx controls proliferation via the transcription factor NFAT.

Lipskaia et al. (2007) Am. J. Pathol. 171, 162-171.

Keywords: SERCA, proliferation, NFAT, SERCA, vascular myocytes, Ca^{2+} entry

STUDY OF S100 PROTEINS IN HUMAN NORMAL AND MALIGNANT CELLS AND TISSUES

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Aim: S100 proteins play key role in regulation of proliferation of normal and malignant cells. Thus, S100A11-mediated pathway participates in TGFβ-triggered inhibition of cell proliferation (Miyazaki et al., 2004). This study was devoted to comparison of S100A11 protein level in human skeletal muscle myoblasts (MBs), rhabdomyosarcoma (RMS) and prostate cancer (PCa) cells, and prostate tissue.

Methods: MBs were cultured in F-12 media. Differentiation of MBs was induced by incubation in media containing 2% of horse serum. RMS (lines RD and A-204) and PCa (line LNCaP-FGC) were cultured in RPMI-1640 media. Samples of benign and malignant prostate tissue were obtained from Institute of Urology of Russian Ministry of Health (Moscow, Russia). Proteins were fractioned using 2D-PAGE followed by staining with Coomassie dye and silver method. Protein spots of interest were identified using MALDI-TOF mass-spectrometry.

Results: We observed increasing of S100A11 protein level during proliferation followed by its decreasing after induction of myoblasts differentiation. S100A11 protein was not detected in RMS cells, mature skeletal muscle, and MBs after 6 days of incubation in differentiation media. There was no significant difference in level of S100A11 protein between benign and malignant prostate tissue and cultured PCa cells. Nevertheless, in 4 of analyzed samples of benign prostate (n=20) and one sample of PCa tissue (n=34) abnormally high level of S100A9 protein was detected.

Conclusions: It seems high level of S100A11 protein in proliferating myoblasts could play important role in regulation of exit from cell cycle during differentiation. Deterioration in regulation of rhabdomyosarcoma cells proliferation could be caused by low level of S100A11 protein in these cells. Abnormally high level of S100A9 in prostate tissue could be stipulated by inflammation or dysregulation of prostate cell proliferation as S100A9 protein is involved in regulation of both processes.

Miyazaki et al. (2004) Cancer Res. 64, 4155-4161.

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Keywords: S100A11, skeletal muscle, prostate cancer, proliferation

IP_3-INDUCED Ca^{2+} SIGNALLING IS INVOLVED IN BREAST CANCER CELLS PROLIFERATION - REGULATION BY ESTRADIOL

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Breast cancer is a major public health concern and represents the first cause of mortality by cancer among women. The steroid hormone 17β-estradiol (E2) is a key growth regulator of the mammary gland and is involved in normal breast development. However, numerous clinical and experimental data have clearly established that exposure to estrogens is the leading cause of sporadic female breast cancer. In the same way, many recent studies are in favor of the involvement of Ca^{2+} signalling in the control of cell growth through the modulation of cell proliferation and apoptosis (Roderick and Cook, 2008).

The aim of the present study was to investigate the role of Inositol 1,4,5-Trisphosphate (IP_3)-Induced Ca^{2+} Signalling (IICS) in the control of proliferation of the MCF-7 human breast cancer epithelial cell line and its potential regulation by E2. Our results show that the IP_3R inhibitors caffeine and 2-APB inhibited the proliferation of MCF-7 stimulated by 5 % Fetal Calf Serum (FCS) or 10 nM E2. Furthermore, Ca^{2+} imaging experiments showed that FCS treatment is able to trigger, in a Ca^{2+}-free medium, an elevation of internal Ca^{2+} in a 2-APB-sensitive manner. IP_3Rs seem to be expressed ubiquitously in the breast since all three types of IP_3R were detected in tumourous or non-tumourous cell lines (MCF-7 and MCF-10A respectively) and in human breast epithelial tissues. Furthermore, a 48-hours treatment with 10 nM E2 elevated IP_3R3 protein expression level in MCF-7 cells and this effect was counteracted by the pure antiestrogen ICI 182,780.

Altogether, our results are in favor of a role of IICS in MCF-7 cells proliferation, and we can hypothesize that the regulation of IP_3R3 expression by E2 is involved in this effect. This preliminary study will be completed with a RNA interference approach in order to quantify the E2-induced proliferation of MCF-7 cells in which IP_3R3 expression has been silenced.

Roderick and Cook (2008) Nature Rev. Cancer 8, 361-375.

Keywords: breast cancer, IP_3 receptor, estradiol, Ca^{2+} signallling

AMILORIDE DERIVATIVES INDUCE APOPTOSIS BY DEPLETING ER CALCIUM STORES IN VASCULAR ENDOTHELIAL CELLS

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Amiloride and its derivatives are selective blockers of the Na^+/H^+ exchanger (NHE) that at micromolar concentrations have protective effects on cardiac and brain ischemia/reperfusion injury but that at higher concentrations also induce apoptosis by a mechanism that remains to be elucidated.

To investigate the cytotoxic mechanism of amiloride derivatives, we quantified the expression of genes associated with endoplasmic reticulum (ER) stress and measured changes in luminal ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) with a "cameleon" indicator, D1ER. Amiloride derivatives induced apoptosis in vascular endothelial cells, an effect that increased at alkaline extracellular pH. The potency order for cytotoxicity was 5-(N,N-hexamethylene)-amiloride (HMA) > 5-(N-methyl-N-isobutyl) amiloride (MIA) > 5-(N-ethyl-N-isopropyl) amiloride (EIPA) >> amiloride. HMA dose-dependently increased the transcription of the ER stress genes GADD153 and GADD34 and rapidly depleted $[Ca^{2+}]_{ER}$, mimicking the effects of the SERCA inhibitor thapsigargin. The decrease in $[Ca^{2+}]_{ER}$ evoked by amiloride derivatives was confirmed in HeLa cells and was mirrored by an increase in cytosolic Ca^{2+} concentration.

These results show that in addition to inhibiting NHE, amiloride derivatives directly disrupt ER and cytosolic Ca^{2+} homeostasis, most likely by interfering with the activity of sarco/endoplasmic reticulum ATPases (SERCA). We propose that ER Ca^{2+} depletion and subsequent ER stress provide a rationale framework for the apoptotic effects of NHE inhibitors.

Keywords: amiloride, Ca^{2+} stores, apoptosis, ER stress

ALTERATION OF THE CORTICAL ACTIN CYTOSKELETON IN STARFISH EGGS LEADS TO ECTOPIC Ca^{2+} SIGNALING AND PREVENTS MONOSPERMIC FERTILIZATION AND SPERM ENTRY

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Recently, during the course of characterizing meiotic maturation of starfish oocytes, we have observed that the actin cytoskeleton plays important roles in modulating intracellular Ca^{2+} release and cortical granule exocytosis. The actin cytoskeleton also plays a pivotal role in fertilization process, as is exemplified by acrosome reaction. Although mutual activation between the egg and sperm is crucial to successful fertilization, the significance of the dynamic rearrangement of the actin cytoskeleton inside the fertilized egg has been largely unknown.

In this communication, we have studied the roles of the actin cytoskeleton in fertilized eggs by using pharmacological agents that either polymerize or depolymerize actin filaments. During normal fertilization, the sperm can induce Ca^{2+} signals and start vitelline layer elevation in the fertilized egg, while remaining on the surface of jelly coat. We have observed that hyperpolymerization of actin in subplasmalemmal regions with jasplakinolide or heparin abolishes the cortical flash of sperm-induced Ca^{2+} signals.

In addition, the kinetics and other aspects of Ca^{2+} rise in response to $InsP_3$ and cADPr were also significantly affected by the alteration of the cortical actin networks. Furthermore, both hyperpolymerization and depolymerization of subplasmalemmal F-actin blocked the formation of fertilization envelopes, suggesting that fine regulation of the cortical actin cytoskeleton is necessary for cortical granule exocytosis. Alteration of the cortical actin cytoskeleton also prevented normal formation of fertilization cone and sperm entry.

Keywords: actin, fertilization, starfish, Ca^{2+} release

EFFECT OF ANNEXIN 2 MUTANTS ON THE MULTICELLULAR ORGANISATION OF EPITHELIAL CELLS

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Annexin A2 (AnxA2) is a Ca^{2+}- and phospholipid-binding protein that has been linked to cellular functions essential for the development of epithelia, namely the formation of cadherin-based cell-junctions, cell polarization and cell migration. To further understand the role of AnxA2 in these processes we compared wildtype AnxA2 and different AnxA2-mutants in their effect on polarization, three-dimensional organization and tubulogenesis of epithelial cells. As modeling systems, MDCK cells grown as 3D-cysts in a collagen-matrix and as monolayers on microporous filter supports were used.

In our analyses we focused primarily on the tyrosine-23 phosphorylation of annexin A2, recently shown to regulate cell morphology in a Rho-dependent manner downstream of activated insulin receptors (Rescher et al., 2008). Using a phosphorylation-mimicking mutant (AnxA2-Y23E) we show that overexpression of this mutant impairs the formation of hollow cysts. On the other hand, MDCK cells expressing a non-phosphorylated mutant were able to form a central lumen but showed a decrease in HGF-induced tubulogenesis.

Our data indicate that the recently established role of annexin A2 in epithelial morphogenesis (Belmonte et al., 2007) appears to be regulated by tyrosine phosphorylation of the protein.

Belmonte et al. (2007) Cell 128, 383-397.
Rescher et al. (2008) J. Cell Sci. 121, 2177-2185.

Keywords: annexin A2, cell polarity, tyrosine phosphorylation, morphogenesis, renal epithelium

REGULATION OF ADP-RIBOSYL CYCLASES DURING EARLY EMBRYOGENESIS OF THE SEA URCHIN

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Calcium signalling is ubiquitous and indispensable for the regulation of cell functions (Berridge et al., 2000). cADPR and NAADP are recently characterised second messengers involved in the mobilisation of calcium from intracellular stores and are synthesised in vitro by a common enzyme ADP-ribosyl cyclase (ARC) (Lee et al., 2001). The sea urchin has long been used as a model system for the study of calcium signalling and development. An extended family of ARCs has been cloned recently from purple urchin, S. purpuratus (Churamani et al., 2007).

In this study the relative expression patterns of the three identified isoforms SpARC1, SpARC2 and SpARC3 during the embryogenesis of S. purpuratus was analysed by semi-quantitative RT-PCR. Remarkably, all three isoforms were differentially regulated during embryo development. SpARC1 was up-regulated during late blastula while SpARC2 was down-regulated during early blastula. SpARC3, which is present in trace amounts in egg and early embryos, showed a precipitous increase during mid-blastula. NAADP production by whole egg and embryo homogenates was also quantified. NAADP production was high in the egg, decreased until blastula and increased again steadily until pluteus.

These data show that ARCs are developmentally regulated and could play a crucial role in early embryogenesis, possibly during the blastula-gastrula transition.

Berridge et al. (2000) Nat. Rev. Mol. Cell Biol. 1, 11-21.
Churamani et al. (2007) PLoS ONE. 2, e797.
Lee (2001) Annu. Rev. Pharmacol. Toxicol. 41, 317-345.

Keywords: ADP-ribosyl cyclase, sea urchins, embryogenesis

THE SECRETORY PATHWAY Ca^{2+}-ATPase ISOFORM 1 (SPCA1) PARTICIPATES IN DIFFERENTIATION AND MANGANESE METABOLISM OF NEURAL CELLS

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Neural cell differentiation involves complex integration of signal transduction network components, in which Ca^{2+} ions play important tasks. Ca^{2+} transporters are critical in these processes. The Secretory Pathway Ca^{2+}-ATPase (SPCA) is found in the membranes of the Golgi apparatus where it transports Ca^{2+} or Mn^{2+} from the cytosol to the Golgi lumen. It is the most recent Ca^{2+} pump identified in neural tissue. The SPCA1 isoform is found in the main neurons of the mouse central nervous system. It is expressed from the earliest stages of the postnatal development in these neurons and can therefore contribute to neural differentiation.

In the present study, we investigated in vitro the role in differentiation of SPCA1 both in neuroblastoma N2a cells and in primary neuron cultures. As in the brain tissue, also in the cultured cells the protein was localized in the membranes of the Golgi complex close to the nucleus. Knockdown of SPCA1 by RNA interference techniques markedly delayed the process of differentiation, increasing the production of neurites and reducing their elongation compared to those of control cells. In addition, we wanted to analyze the function of SPCA1 as a Mn^{2+}-transporter in neural cells. Mn^{2+} is an important cofactor in many biological reactions, but at higher concentrations it can become toxic leading to manganism (related to parkinsonism) and to embryonic malformations. To address this, we performed Mn^{2+} toxicity assays observing a reversible Golgi stress with fragmentation of the Golgi membranes where SPCA1 is located. These results reveal important roles of SPCA1 in the nervous system.

Keywords: SPCA, neurons, differentiation, manganese

ORIGIN OF FERTILIZATION CALCIUM SIGNAL AND CONTROL MECHANISMS OF APOPTOSIS OF THE SEA URCHIN EGG AND EMBRYO: ROLE OF THE PLCγ AND MEK/ERK PATHWAYS

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Egg activation after fertilization is mediated by a transient rise in intracellular free calcium (Ca^{2+}_i). Other Ca^{2+}_i transients are correlated with cell cycle events, being necessary and sufficient for progress through mitosis. Our aims are 1) to precise the role of phospholipase Cγ (PLCγ) at fertilization and mitosis; 2) to understand how the Extracellular Regulated Kinase (ERK) interacts with Ca^{2+}_i to either control mitosis entry or either direct the cell toward apoptosis.

Methods. Our model is the Paracentrotus lividus egg. Ca^{2+}_i was measured using a fura-2 fluorescent probe. 1) A fusion protein containing the two SH2 domains of the sea urchin PLCγ and a non functional mutated protein (used as control) were injected before fertilization. 2) Unfertilized eggs, that contain high levels of p-ERK, were submitted to various drugs that triggered apoptosis (the MEK inhibitor U0126, protein synthesis inhibitors or the kinase inhibitor staurosporine). Apoptosis was evaluated by: tetramethyl rhodamine (TMRE) to visualize mitochondrial membrane potential, caspase activity, egg phenotypes.

Results. 1) Our results indicate that inhibiting the PLCγ pathway reduced but never totally abolished the fertilization Ca^{2+}_i signal. Moreover, this pathway was clearly implicated during the first mitotic division. All control mutated constructs were without effect. 2) Each drug used induced different morphological hallmarks of apoptosis: change in cytoplasm with spots, blebbing, vesicles, and cellular deformations. Ca^{2+}_i oscillations were triggered by protein synthesis inhibitors or after several hours treatment with U0126. Injection of EGTA buffer prevented these oscillations and inhibited apoptosis.

Conclusion. PLCγ is not sufficient to trigger the fertilization Ca^{2+}_i signal, contrary to what is widely accepted in the litterature. However, PLCγ is absolutely necessary to control progression through the first mitotic divisions. Apoptosis can be triggered in unfertilized sea urchin eggs after protein synthesis inhibition by calcium-dependent mechanism(s). Mechanisms leading to apoptosis induced after protein synthesis inhibition were independent of mitochondria while those triggered after inactivation of the MEK/ERK cascade involved mitochondria.

Keywords: fertilization, apoptosis, sea urchins, phospholipase C, ERK, mitosis

ZEBRAFISH HOMOLOGUE OF SPCA: ROLE IN Ca^{2+}/Mn^{2+} HOMEOSTASIS

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P-type Ca^{2+}-transport ATPases are key regulators of intracellular Ca^{2+} homeostasis. Here, we focus on the SPCA (secretory pathway Ca^{2+} ATPase)-branch of this gene family. The human homologues hSPCA1 and hSPCA2 are encoded by the ATP2C1 and ATP2C2 genes respectively. Both proteins are Ca^{2+}/Mn^{2+}-transport ATPases that localize to the Golgi apparatus. While hSPCA1 is a ubiquitously expressed protein, hSPCA2 is restricted to some secretory cell types. Mutations in the ATP2C1 gene have been reported to cause Hailey-Hailey disease, a blistering skin disease.

We report the identification and functional characterization of the zebrafish SPCA orthologue. In most teleost fish, many genes exist as members of larger gene families. However, the zebrafish genome contains only one SPCA-isoform, termed zfSPCA.

The zfSPCA gene spans a genomic region of 60 kb and consists of 26 exons, encoding a 99 kDa membrane protein. All intron/exon boundaries are conserved between zfSPCA and its human counterparts, illustrating their homology. We cloned the cDNA and demonstrated that the corresponding protein is a functional Ca^{2+}/Mn^{2+}-transporting enzyme. This ion transport is insensitive to the SERCA-specific inhibitor thapsigargin.

Morpholino-knockdown experiments demonstrate the essential role of the zfSPCA gene in embryonic development. Morpholino-injected embryos present an obvious phenotype characterized by severe defects in embryonic axis formation (gastrulation) and increased apoptosis. The phenotype can be rescued by co-injecting the zfSPCA mRNA. To elucidate the relative importance of Ca^{2+} and/or Mn^{2+} transport the rescue experiments are also performed using constructs defective in either Ca^{2+} or Mn^{2+} transport.

Keywords: SPCA, Golgi apparatus, zebrafish, development, manganese

TRPC1 REGULATES SKELETAL MYOBLASTS MIGRATION AND DIFFERENTIATION

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Myoblasts migration is a key step in myogenesis and regeneration. It allows myoblasts alignment and fusion into myotubes. This process has been shown to involve m- or µ-calpains, two calcium-dependent cysteine proteases.

In the present paper, we measured calpain activity in situ (fluorometric measurements) and show, for the first time, a peak of activity at the beginning of the differentiation process. We also observed a concomitant and transient increase of the influx of Ca^{2+} and of the expression of TRPC1 protein. Besides, we recently reported that, in adult skeletal muscle fibres, calpains were specifically activated by a store-operated entry of calcium. In the present study, we therefore repressed the expression of TRPC1 in myoblasts and studied its influence on Ca^{2+} fluxes and on differentiation. TRPC1 knocked-down myoblasts presented a largely reduced store-operated entry of calcium and a significantly diminished transient influx of calcium at the beginning of differentiation. The concomitant peak of calpain activity was abolished. TRPC1 knocked-down myoblasts also presented an accumulation of myristoylated alanine-rich C-kinase substrate (MARCKS), an actin-binding protein, substrate of calpain. Finally, their fusion into myotubes was significantly slowed down, due to a reduced speed of cell migration. Accordingly, migration of control myoblasts was inhibited by 2 to 5 µM GsMTx4 toxin, an inhibitor of TRPC1 or by 50 µM Z-Leu-Leu, an inhibitor of calpain. In contrast, stimulation of control myoblasts with IGF-1 increased the basal influx of Ca^{2+}, activated calpain and accelerated migration. These effects were not observed in TRPC1 knocked-down cells.

We therefore suggest that an entry of calcium through TRPC1 channels induces a transient activation of calpain, a subsequent proteolysis of MARCKS, allowing in its turn, myoblasts migration and fusion.

Keywords: myoblast, calpain, differentiation, TRPC1, Ca^{2+} entry

STIM1 AND ORAI1-DEPENDENT STORE-OPERATED CALCIUM ENTRY CONTROLS EARLY POST-NATAL HUMAN MYOBLAST DIFFERENTIATION

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Our previous work on human myoblasts suggested that a hyperpolarization followed by a rise in $[Ca^{2+}]_{in}$ involving store-operated Ca^{2+} entry (SOCE) channels induced myoblast differentiation.

Advances in the understanding of the SOCE pathway led us to examine more precisely its role in myoblast differentiation. We found that SOCE orchestrated by STIM1, the ER Ca^{2+}-sensor activating Orai Ca^{2+} channels, is crucial.

Silencing STIM1, Orai1, or Orai3, but not Orai2, reduced SOCE amplitude and myoblast differentiation. Conversely, over-expression of STIM1 with Orai1 increased SOCE and accelerated myoblast differentiation. STIM1 or Orai1 silencing decreased resting $[Ca^{2+}]_{in}$ and intracellular Ca^{2+}-store content, but correction of these parameters did not rescue myoblast differentiation.

Remarkably, SOCE amplitude correlated linearly with the expression of an early marker of myoblast differentiation, MEF2, regardless of the STIM or Orai isoform that was silenced. Finally, contrary to our previous model, we found that the hyperpolarization also depends on SOCE, indicating that STIM1 and Orai1 are key molecules for the induction of human myoblast differentiation.

Keywords: store-dependent Ca^{2+} entry, STIM1, Orai1, myoblast, differentiation

cADPR IS SYNTHESIZED INSIDE ACIDIC ORGANELLES AND IS TRANSPORTED TO THE CYTOSOL UPON CELL STIMULATION

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Cyclic ADP-ribose is an important Ca^{2+}-mobilizing cytosolic messenger synthesized from β-NAD^+ by ADP-ribosyl cyclases (ARCs). However, the focus upon ectocellular mammalian ARCs (CD38, CD157) has led to confusion as to how extracellular enzymes generate intracellular messengers in response to stimuli.

We have cloned and characterized three ARCs in the sea urchin egg and found that endogenous ARC β and γ are intracellular and located within the lumen of acidic, exocytotic vesicles where they are optimally active. Intra-organelle ARCs are shielded from cytosolic substrate and targets by the organelle membrane but this barrier is circumvented by nucleotide transport: we show that a β-NAD^+ transporter provides ARC substrate that is converted luminally to cADPR which, in turn, is shuttled out to the cytosol via a separate cADPR transporter. Moreover, nucleotide transport is integral to ARC activity physiologically since three transport inhibitors all inhibited the fertilization-induced Ca^{2+} wave that is dependent upon cADPR.

This represents a novel signalling mechanism whereby an extracellular stimulus increases the concentration of a second messenger by promoting messenger transport from intra-organelle synthesis sites to the cytosol.

Keywords: cADPR, ADP-ribosyl cyclase, acidic organelle