Calcium in development: from ion transients to gene expression

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A scientific symposium on "**Calcium in Development: from Ion Transients to Gene Expression**" was held at the Hong Kong University of Science and Technology from October 9–11, 2000. Renowned for both its natural and architectural beauty, the modern campus of HKUST was a perfect location for bringing together scientists from both France and Hong Kong to share their latest research in calcium signalling during animal and plant development. This first joint Hong Kong/France calcium conference was divided into three sessions on Signal Transduction, Fertilization and Early Development, and Calcium and Gene Expression. **Marc Moreau** (CNRS, Université Paul Sabatier, Toulouse) opened the conference with an overview of calcium in development. He started by describing a brief history of calcium research and then, bringing us up to the present day, he introduced the other speakers and their research interests. He concluded by defining the important questions that face us in linking calcium signals with specific targets in order to determine their role in development.

The first session was devoted to signal transduction. **Mingjie Zhang** (HKUST) started by describing the structural properties of calmodulin (CaM) and how on binding to calcium a dramatic molecular surface change occurs to expose a hydrophobic cavity in each of its two structurally similar domains. The unique flexibility and high polarizability of the methionine residues at the entrance of each cavity along with other hydrophobic amino acid residues create adjustable sticky interaction surfaces, which enable the CaM to bind with wide range of different proteins and enzymes.

Continuing on the subject of CaM, **Donald C. Chang** (HKUST) described the use of a CaM–GFP fusion gene technique to demonstrate the presence of a submembrane fraction of CaM at the equator of HeLa tissue culture cells just before the formation of the cleavage furrow during cytokinesis. He demonstrated, using a TA–CaM probe, that this submembrane fraction of CaM is selectively activated near the cleavage furrow during cytokinesis and suggested that CaM may play an active role in determining the positioning and timing of the cleavage furrow during cell division. Donald also described his new findings regarding the possible roles of Ca^{2+} signalling in apoptosis.

In the final CaM-related presentation, **Mary M.Y. Waye** (Chinese University of Hong Kong; CUHK) reported on the potential role of the ε isoform of the 14-3-3 protein family in modulating a Ca^{2+}-activated Cl^- channel. This isoform, which is known to associate with CaM, is endogenously expressed in *Xenopus* oocytes. Using voltage clamp techniques, Mary demonstrated that injection of 14-3-3 antisense oligodeoxynucleotides resulted in potentiation of the ionomycin-induced Cl^- current, while both 14-3-3 peptide and the CaM inhibitor, W13, suppressed the antisense-potentiated current.

Noting that, in 1998, Professors Murad, Furchgott and Ignarro were awarded the Nobel Prize in Medicine or Physiology for their pioneering studies on nitric oxide (NO)-induced vasodilation and the involvement of cyclic GMP (cGMP), **Ronald R. Fiscus** (CUHK) gave a most topical presentation on the involvement of cGMP in nitric oxide (NO)-induced vasodilation and how cGMP regulates calcium via activation of protein kinase G (PKG). He described how, in vascular smooth muscle cells, agents such as NO that elevate cGMP levels, lower the cytosolic levels of calcium and cause relaxation, and presented results to demonstrate a similar mechanism in primary cultures of hippocampal neurons. For example, when these neurons are placed under stressful conditions, (i.e., glutamate toxicity or glucose deprivation), the associated rise in calcium and cell death is reduced when either cGMP is elevated or PKC is activated.

Continuing the theme of the role of calcium in the development and function of neurons, **H. Benjamin Peng** (HKUST) described the localization of calcium channels at developing neuromuscular junctions (NMJ) in culture. He addressed the hypothesis that the aggregation of both calcium channels and synaptic vesicles are necessary to mediate fast neurotransmitter release at presynaptic active zones (AZs). Using a combination of immunofluorescence to label synaptic vesicles, a localized calcium detection method to identify the

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Ca^{2+} channels, and rhodamine-conjugated α-bungarotoxin to label the acetylcholine receptors (AchR), he described putative synaptic contacts in cultured NMJ where Ca^{2+} channels, synaptic vesicles, and postsynaptic receptors are colocalized. He concluded by suggesting that the enhanced clustering of functional Ca^{2+} channels at the AZs is likely due to a contact-dependent signalling mechanism possibly similar to that known to mediate AChR clustering.

In the final presentation of the conference on signal transduction, **Lucette Pelletier** (INSERM U28, Toulouse) described her exciting research on calcium signalling in T-lymphoctyes. CD4+ T cells are divided into functionally different subsets, including Th1 and Th2 cells. While Th1 cells produce IL-2 and IFNγ, and are involved in cell-mediated immune responses, Th2 cells synthesise IL-4 and IL-5, and are involved in allergic reactions. The signal transduction mechanism is well characterised in Th1 but not in Th2 cells. Using a T cell hybridoma, which produces both IFNγ and IL-4, Lucette described a signalling pathway which she proposes is characteristic of Th2 cells, whereby protein kinase C (PKC) triggers a calcium influx through L-related calcium channels, rather than through the mobilization of calcium stores via IP_3, which is characteristic of Th1 cell transduction.

The second conference session focussed on the role of calcium in fertilization and early development. **Michel Villaz** (INSERM E9931, Grenoble) described both the diverse and selective roles of calcium channels during fertilization in ascidian oocytes thus nicely illuminating the subtle complexities of calcium signalling. Using whole-cell patch clamp recording and fura-2 calcium imaging, he reported that, during fertilization, there was a downregulation of the low-threshold-activated inward calcium current and, specifically during meiosis, an opening of a calcium-release-activated calcium current. He also described the selective contribution of different calcium stores and intracellular channels to different functions, with the inositol 1,4,5-trisphosphate receptor and the ryanodine receptor being involved in the completion of the meiotic cell cycle and in the membrane exocytosis that occurs after the onset of fertilization, respectively.

Continuing on the theme of fertilization but moving from animals to plants, **Mireille Rougier** (Ecole Normale Supérieure, Lyon) described the role of calcium during fertilization in maize. In higher plants, fertilization is a complex process, involving the fusion of sperm to both the egg cell and to the central cell, which initiates the development of the embryo and the endosperm, respectively. Mireille reported that a transient elevation of cytosolic calcium is triggered after sperm–egg fusion. She then described an elegant series of experiments to investigate the contribution of the plasma membrane to this primary calcium elevation as a first step to elucidate its origin. Using a calcium-selective vibrating probe to record calcium fluxes during in vitro fertilization in maize, she presented results to show that immediately after sperm–egg fusion there is an influx of calcium, which propagates as a wave front from the fusion site. The influx was inhibited by gadolinium. In addition, certain aspects of egg activation could be mimicked by creating artificial influxes using calcium ionophores.

Once again switching kingdoms, and moving on now from fertilization to look at later stages in development, **Andrew L. Miller** (HKUST) described an intriguing pattern of calcium signalling that occurs during gastrulation and segmentation in zebrafish embryos. Using the calcium-sensitive bioluminescent reporter *f*-aequorin, which was loaded into zebrafish embryos at the single-cell stage, and an ultrasensitive photon-imaging microscope, a complex array of both intracellular and intercellular calcium signals was visualized throughout gastrulation and segmentation. During the gastrula period, the dramatic global cellular rearrangements that occur are accompanied by a series of intercellular calcium waves, the most prominent being a rhythmic series of waves that traverse the margin of the advancing blastoderm. During the segmentation period, as the embryo undergoes a series of more localized morphogenetic cell movements, the patterns of calcium signalling also reflect this and likewise change from global to being more localised.

The theme of the final conference session was the possible targets of developmental calcium signalling, the main focus being calcium-regulated gene expression. **Jean-Marie Blanchard** (CNRS, Institut de Génétique Moléculaire, Montpellier) opened this session by describing the induction of the c-*fos* gene in mouse Ltk^- fibroblasts. C-fos expression can be induced by an increase in intracellular calcium in many cell types. While different upstream promoter elements have been implicated in this response, in Ltk^- fibroblasts, a novel calcium signalling pathway was described that mediates the intragenic regulation of c-*fos* expression via the suppression of a transcriptional pause site.

Returning to the subject of gastrulation, **Catherine Leclerc** (CNRS, Université Paul Sabatier, Toulouse) described the control of neural-specific gene expression by calcium during gastrulation in *Xenopus laevis* embryos. In amphibian embryos, neural induction results from a specific tissue interaction occurring between the dorsal mesoderm (Spemann organiser) and the dorsal ectoderm at gastrulation. The organiser secretes factors such as noggin, chordin and follistatin that are able to induce neural tissue of anterior character by chelating BMP4, a potent epidermal inducer and repressor of neural determination. Using excised dorsal ectoderm, Catherine had already shown that an increase in intracellular free calcium occurring via L-type calcium channels (LTCs) is a necessary and sufficient requirement for neural induction. From intact gastrula stage *Xenopus* embryos, data were presented to show that noggin stimulates the expression of *Zic3* and *geminin*, two pre-pattern genes that are known to confer a neural rather than epidermal fate to the

dorsal ectoderm. In addition, when calcium transients are abolished by LTC antagonists, the expression of these two early genes is downregulated.

Valérie Baubet (Institut Pasteur, Paris) described the exciting new development of a bifunctional calcium-sensitive bioluminescent reporter gene by fusing green fluorescent protein and aequorin. She then proceeded to describe how this gene might be applied to study the activity of calcium in the neural networks located in the spinal cord of null mutant mice that exhibit a congenital prehensile deficiency of the forepaw following the targeted replacement of the homeobox gene *hoxc8* with the *lacZ* gene.

Finally, **Julian A.J.H. Critchley** (CUHK) presented a more 'downstream' view of the clinical applications of basic calcium research. He emphasized, however, that the pharmacological modulation of calcium signalling can be both difficult and costly due to the diversity in calcium's cellular functions. For this reason, drug development has not kept pace with basic research and still remains one of the great challenges for calcium-related therapeutics.

The conference was brought to a close with a summing up and discussion session led by **Andrew L. Miller**. During this final session, participants unanimously agreed to hold a second Hong Kong/France Calcium Meeting in 2002.