

Introduction. Calcium signals and developmental patterning

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Calcium ions generate ubiquitous cellular signals. Calcium signals play an important role in development. The most obvious example is fertilization, where calcium signals and calcium waves are triggered by the sperm and are responsible for activating the egg from dormancy and cell cycle arrest. Calcium signals also appear to contribute to cell cycle progression during the rapid cell cycles of early embryos. There is increasing evidence that calcium signals are an essential component of the signalling systems that specify developmental patterning and cell fate. This issue arises from a Discussion Meeting that brought together developmental biologists studying calcium signals with those looking at other patterning signals and events. This short introduction provides some background to the papers in this issue, setting out the emerging view that calcium signals are central to dorsoventral axis formation, gastrulation movements, neural specification and neuronal cell fate.

Keywords: calcium signals; development; patterning; axis formation; cell fate; organogenesis

1. INTRODUCTION

Calcium signals contribute to all phases of development. Their central role at fertilization is well known. Put simply though, the further in development we go, the less clear we are about how calcium signals are generated and what they do (Whitaker 2006). We will briefly describe our knowledge of the fertilization calcium signalling mechanisms as a way of introducing the salient points about calcium signalling in embryos. We will then set each of the articles in this issue in context.

2. CALCIUM SIGNALS AT FERTILIZATION

Calcium signalling mechanisms at fertilization conform to the general pattern of calcium signalling. At fertilization, calcium is released from internal storage in the endoplasmic reticulum (ER) through the activation of inositol trisphosphate receptor (InsP₃R) channels in the ER membrane by the second messenger InsP₃. A second ER channel, the ryanodine receptor, also contributes in some cases, as do the alternate calcium-releasing messengers, cyclic adenosine diphosphate ribose and nicotinic acid adenine dinucleotide phosphate. Calcium signals exert many of their effects by activation of kinases and phosphatases such as calmodulin-dependent kinase II (CaMKII) and calcineurin. Fertilization calcium signals are relatively well understood and form much of the basis for our understanding of developmental calcium signals (Whitaker & Swann 1993; Stricker 1999; Runft *et al.*

2002; Santella *et al.* 2004; Galione & Ruas 2005; Whitaker 2006, 2008).

A striking feature of the fertilization calcium signal is that it often takes the form of a wave. Calcium waves of different speeds and generated by different mechanisms are important in development, not least because calcium, though a small ion, has a very low diffusion constant inside cells because it is strongly bound by cellular calcium buffers (Baker 1972). Calcium waves are a means of making local calcium signals spread, as Jaffe's paper discusses (Jaffe 2008).

3. CALCIUM SIGNALS AND THE CELL DIVISION CYCLE

Fertilization calcium signals act to overcome the cell cycle arrest of the unfertilized egg or oocyte. There are indications that some embryos use calcium signals to control the onset and progress of mitosis (Wilding *et al.* 1996; Groigno & Whitaker 1998; Parry *et al.* 2005; Whitaker 2006), though others may not (Fitzharris *et al.* 2005). The contribution that calcium signals make at cell division is represented in this issue by the work of Miller and colleagues on cleavage in very early zebrafish embryos (Webb *et al.* 2008).

4. CALCIUM SIGNALS AND DEVELOPMENT

The work of Miller and others in zebrafish has revealed that calcium signals are present throughout early development, to neurulation and beyond (Webb & Miller 2000, 2003; Webb *et al.* 2001). The transparency of the zebrafish embryo and its rapid development make it especially suited to calcium imaging studies. For the most part, the precise function attached to these signals is unknown.

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One contribution of 11 to a Discussion Meeting Issue 'Calcium signals and developmental patterning'.

5. CALCIUM SIGNALS AND AXIS FORMATION

A striking proof of the importance of calcium signals to dorsoventral axis formation is the observation that microinjection of an inhibitory antibody of the InsP_3R into *Xenopus* embryos leads to very dorsalized, two-headed embryos (Kume *et al.* 1997a). This observation was the first of a series of studies that showed that G-protein-coupled signalling specified ventral cell fate in *Xenopus* (Kume *et al.* 1997b, 2000a,b; Saneyoshi *et al.* 2002). Plasma membrane calcium channels also contribute to ventral specification (Palma *et al.* 2001).

6. CALCIUM AND THE Wingless PATHWAY

Wingless (Wnt) signalling is central to dorsoventral patterning. Parallel experiments in zebrafish and *Xenopus* have demonstrated a strong link between the Wnts and calcium signalling. Zebrafish offered optical transparency and mutants while *Xenopus* is well suited to microinjection of agonists and dominant negative constructs (Moon *et al.* 1997; Slusarski *et al.* 1997a,b; Sheldahl *et al.* 2003; Veeman *et al.* 2003; Westfall *et al.* 2003a,b). Wnt-mediated ventral specification is linked to calcium activation of CaMKII (Kühl *et al.* 2001) and calcineurin (Saneyoshi *et al.* 2002). In this issue, Slusarski and colleagues tell this story in zebrafish and *Xenopus* (Freisinger *et al.* 2008), Kestler & Kühl (2008) give us a taxonomy of the Wnt signalling network complete with an advanced mathematical model and Smith *et al.* (2008) show how extracellular morphogens such as Wnts and TGF- β may form extracellular morphogenetic gradients within embryos.

7. CALCIUM AND CELL MOVEMENTS

Calcium signals have been observed during convergent extension movements in Keller explants from *Xenopus* embryos and are suppressed by inhibition of Wnt signalling (Wallingford *et al.* 2001). Current evidence suggests that movements are regulated by Dishevelled modulating calcium through Xwnt 11 (Tada & Smith 2000; Wallingford *et al.* 2000; Kühl *et al.* 2001; Tada & Concha 2001). Wnt/Ca signalling is also necessary for the development of Brachet's cleft (essential for involution) by modulating ectoderm–mesoderm adhesion (Winklbauer *et al.* 2001). Keller & Shook (2008) describe in this issue how convergent extension movements induce gastrulation in *Xenopus*.

Neural crest cell migration is a key and puzzling event in vertebrate development, akin as Kuriyama & Mayor (2008) point out in this issue to metastasis of cancer cells. They provide evidence that Wnt signalling is a key contributor to the direction of neural crest cell migration.

8. CALCIUM AND NEURAL INDUCTION

There appear to be two sets of calcium signals active at around the time of gastrulation in *Xenopus*. The first set comprises those associated with convergent extension that arise in mesoderm close to the dorsal lip and propagate through the mesoderm (Wallingford *et al.* 2001). The second set originates in the dorsal ectoderm in the animal hemisphere and propagate through ectoderm towards the blastopore, antiparallel

to the mesodermal calcium waves in the underlying tissue (Leclerc *et al.* 2000). L-type calcium channel blockers prevent the second set of calcium signals (Leclerc *et al.* 1997, 2000; Palma *et al.* 2001). Although neural cell fate has been thought to be the default pathway for ectodermal differentiation, Moreau *et al.* (2008) present evidence in this issue that these ectodermal calcium signals specify neuronal differentiation.

9. CALCIUM AND LEFT–RIGHT AXIS FORMATION

Left–right axis formation appears to involve microscopic fluid flow driven and sensed by cilia (Essner *et al.* 2002; Tabin & Vogan 2003). This appears to lead to a calcium gradient that drives left–right gene expression (Raya *et al.* 2003, 2004; Tanaka *et al.* 2005). Slusarski and colleagues also tackle this in their article here (Freisinger *et al.* 2008).

10. CALCIUM AND NEURONAL SPECIFICATION

Later, as the neural plate develops, calcium signals can nudge and alter cell fate. Spitzer shows here how the precise pattern of calcium signals can alter the neurotransmitters expressed by developing neurons and turn excitatory into inhibitory neurons (Spitzer 2008).

11. A PLETHORA OF CALCIUM SIGNALS

As this issue illustrates, there are two main challenges. The first is work to link calcium signals to the genes whose pattern of expression shapes the embryo. The second, perhaps even more challenging but linked to the first, is to understand how the calcium signalling pathway, whose fingers seem to poke into a wide variety of pies during development, is configured to carry out so many different jobs during early development (Whitaker 2006).

The authors would like to thank Hannah Green, Chloe Sykes and James Joseph at the Royal Society for their help with the meeting and for producing this issue.

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