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HIGHLIGHT

Calcium signaling during the early meroblastic cleavages of zebrafish and medaka embryos

Andrew L. MILLER

Section of Biochemistry and Cell Biology, and State Key Laboratory for Molecular Neuroscience, Division of Life Science, Hong Kong University of Science and Technology, Hong Kong, China

The regulation of cytokinesis in "giant" embryonic cells (i.e., > 500 μm in diameter) presents exacting challenges that include long-range signaling with respect to time and space; the transport and assembly, followed by disassembly, of an extensive contractile apparatus; and the remodeling and addition of new surface membrane to the resulting daughter cells. As methods have been developed to visualize intracellular Ca^{2+} signaling in a non-destructive and reproducible manner, it is becoming clear that this versatile and ubiquitous signaling system (Berridge et al., 2003) plays a necessary and dynamic role in regulating early embryonic cytokinesis (Webb and Miller, 2008). The large embryos of fish of the Cyprinidae family have proved to be excellent systems in which to explore the role of Ca^{2+} during cytokinesis. This is due to a number of complementary factors such as their *ex utero* development, large cell size, ease of manipulation, and optical clarity (Gamo and Terajima, 1963; Kimmel et al., 1995). In these large embryos there are a number of distinct and sequential steps that contribute to successful cytokinesis. These include the initial positioning of the cleavage furrow within the embryonic cell cortex, followed by the propagation (without significant deepening) of the cleavage furrow across the cell surface; the furrow then begins to deepen (i.e., furrow ingression) toward the underlying yolk cell, which results in two daughter cells (and their nuclei) being separated by a distinct extracellular groove; and finally furrow apposition occurs, where the daughter cells "zip together" following the disassembly of the contractile apparatus (Webb et al., 1997; Chang and Lu, 2000). Furrow apposition is a feature of cytokinesis in the early blastoderm of cyprinids, where the daughter cells do not completely separate from each other as in other dividing systems such as bacteria (Uehara et al., 2010) and yeast cells (Roncero and Sánchez, 2010); however, they remain apposed thus forming a classical hemispherical blastoderm. Cleavage furrow apposition thus represents the final step in this distinct type of embryonic cytokinesis (Fluck et al., 1991; Webb et al., 1997; Lee et al., 2003). Owing to the large size of their embryos and the fact that different sequential cytokinetic elements (positioning, propagation, deepening, and apposition) are separated both temporally and spatially, the first a few meroblastic cleavages of zebrafish embryos have provided an excellent opportunity to explore the mechanisms responsible for generating cytoplasmic Ca^{2+} signals (Lee et al., 2003), as well as their relationship to the rearrangements of cytoskeletal elements and membrane components specific to each stage in the embryonic cytokinetic process (Li et al., 2006; Li et al., 2008).

The earliest reports via direct measurement that $[Ca^{2+}]_i$ might increase during embryonic cell division were reported from medaka (*Oryzias latipes*) using both the Ca^{2+}-sensitive bioluminescent reporter aequorin (Ridgway et al., 1977) and Ca^{2+} sensitive microelectrodes (Schantz et al., 1985), respectively. The first direct visualization of Ca^{2+} transients during embryonic cytokinesis was once again obtained from medaka embryos using aequorin (Fluck et al., 1991). This group reported that the cytokinetic Ca^{2+} transients took the form of two sequential propagating Ca^{2+} waves that accompanied, firstly the progression of the cleavage furrow across the surface of the blastodisc, and secondly its subsequent ingression. This groundbreaking report by Fluck et al. was followed by a number of subsequent reports from zebrafish (*Danio rerio*), using either fluorescence-based Ca^{2+} reporters (Chang and Meng, 1995; Chang and Lu, 2000) or a variety of aequorins (Webb et al., 1997; Créton et al., 1998; Lee et al., 2003; Lee et al., 2006). These subsequent reports confirmed that the Ca^{2+} transients associated with embryonic cytokinesis consisted of a number of sequential

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E-mail: almiller@ust.hk

signals that could be associated with each of the major components required to separate daughter cells, i.e., furrow positioning (Lee et al., 2006), propagation (Fluck et al., 1991; Chang and Meng, 1995; Webb et al., 1997), deepening (Chang and Lu, 2000; Lee et al., 2003), and apposition (Fluck et al., 1991; Webb et al., 1997).

In this issue of *Frontiers in Biology*, an article by Guo et al. entitled "The formation of a Ca^{2+} gradient at the cleavage furrow during cytokinesis of zebrafish embryos" adds further weight to the accumulating evidence that the generation of these intracellular Ca^{2+} signals are an essential and required part of the complex signal transduction network that regulates embryonic cytokinesis. Many aspects of cytokinetic Ca^{2+} signaling remain inadequately understood. Two of these, however, are addressed by Guo et al.: The first is the linear (rather than radial) nature of the propagating cytokinetic Ca^{2+} waves, while the second is the relatively long duration of the cytokinetic Ca^{2+} signals themselves. In the case of the former, Guo et al. confirm earlier reports (Lee et al., 2003; Lee et al., 2004) that during cytokinesis the endoplasmic reticulum (ER) in the blastomeres of cleavage period zebrafish embryos is rearranged into linear arrays that reflect the plane of the cleavage furrow. As a result, any inositol 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} transient will propagate as a linear Ca^{2+} wave along the plane of the furrow, rather than a radial wave as seen during activation/fertilization of many eggs, including zebrafish (Lee et al., 1999; Stricker, 1999). In the case of the zebrafish cytokinetic Ca^{2+} signal durations, it has been previously reported that the propagation and deepening/apposition of Ca^{2+} transients last for ~6 min and ~10 min, respectively (Webb et al., 1997, 2008). Thus, there must be a mechanism present not just to propagate the cytokinetic Ca^{2+} waves, but also to maintain the standing Ca^{2+} gradients that result from them. Guo et al. have suggested that a specific spatial distribution of the ER Ca^{2+} leaks and pumps, i.e., IP_3Rs and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCAs), might contribute to establishing and maintaining localized Ca^{2+} gradients within the cytoplasm of blastomeres during early embryonic cytokinesis. Models have been proposed where the regions of active Ca^{2+} release are concentrated at the leading edge of the propagating furrow, while the elevated cytosolic Ca^{2+} is restored to cytoplasmic resting levels in regions more distal and lateral to the leading edge, thus generating a localized Ca^{2+} gradient (Lee et al., 2006; Webb et al., 2008). This concept fits in nicely with Gou et al.'s proposition and numerical simulation.

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