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Review

Thirty years of calcium signals at fertilization

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Abstract

It was discovered about 30 years ago that a dramatic increase in intracellular calcium ion concentration ($[Ca^{2+}]_i$) occurs at fertilization and that this increase acts as the pivotal signal for egg activation. Later, the Ca^{2+} signal at fertilization turned out to be ubiquitous among animal species. Extensive advance has been brought during these 30 years in research on spatiotemporal aspects and signaling mechanisms of the $[Ca^{2+}]_i$ increase, sperm factors that induce the Ca^{2+} response, and cell cycle resumption caused by the $[Ca^{2+}]_i$ rise. I provide a historical account of these advances in mammals, sea urchins, and a few other models.

Keywords: Fertilization mechanism; Sperm–egg interaction; Intracellular calcium; Sperm factor; Egg activation

Contents

1. Introduction

During these 30 years we have become aware that a dramatic increase in $[Ca^{2+}]_i$ occurs at fertilization in eggs of every animal species ever examined [1]; this has been accomplished by invention and the development of the method of $[Ca^{2+}]_i$ measurement and Ca^{2+} imaging. Unfertilized eggs are arrested at a certain stage of meiotic cell division in a species-specific manner and are released from the arrest by fertilization. This phenomenon is referred to as "egg activation". The $[Ca^{2+}]_i$ rise at fertilization is a pivotal signal for egg activation and is responsible for triggering early embryogenesis [1]. Research has focused on the phenomenon and mechanism of the fertilization Ca^{2+} signal. The first subject was to record the $[Ca^{2+}]_i$ rise. A "Ca^{2+} wave" that starts from the site of sperm–egg fusion and propagates across the egg cytoplasm has been extensively analyzed since it was first recorded in eggs of medaka fish in 1978 [2]. On the other hand, the $[Ca^{2+}]_i$ rise was thought to occur synchronously in the eggs of protostome animals [3,4]. Besides the spatial pat-

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tern of $[Ca^{2+}]$ rise represented by the Ca^{2+} wave, the temporal pattern was characterized by repetitive $[Ca^{2+}]_i$ rises designated as "Ca^{2+} oscillations" that were first reported in mammalian eggs in 1981 [5,6]. The next subject was concerned with the mode of Ca^{2+} mobilization: whether it was due to intracellular Ca^{2+} release from the endoplasmic reticulum (ER) or Ca^{2+} influx from outside of the cell. Both pathways are mediated by Ca^{2+}-permeable channels of the plasma membrane or the ER membrane. Research on signal transduction and intracellular second messengers was advanced in parallel with that of Ca^{2+} dynamics and signaling in somatic cells [7]. In the early 1990s, a critical discussion arose about the mechanism by which the $[Ca^{2+}]_i$ rise was induced by the sperm: whether it was mediated by sperm–egg surface interaction (the sperm receptor hypothesis that depended on sperm–egg binding) or was caused by a cytosolic sperm factor driven into the ooplasm (the sperm factor hypothesis that depended on sperm–egg fusion). Candidates of the sperm factor have been proposed in mammals. Another essential subject is the signaling mechanism that is downstream the $[Ca^{2+}]_i$ rise and leads to resumption of cell cycle. This paper presents a synthesis of the data accumulated over the last 30 years dealing with these questions.

2. Early studies that proved the Ca^{2+} hypothesis

The concept of Ca^{2+} as a signal for egg activation arose in the early 1930s from artificial activation experiments suggesting that leak-in of extracellular Ca^{2+} may cause parthenogenetic activation [8]. Mazia measured increased Ca^{2+} in ultrafiltrates of homogenates of sea urchin *Arbacia* eggs immediately after fertilization [9]. However, no further experimental basis for the Ca^{2+} hypothesis had been provided until Steinhardt et al. showed in 1974 that the Ca^{2+} ionophore A23187, a novel tool for Ca^{2+} mobilization, caused activation of sea urchin [10], starfish, toad, and hamster [11] eggs. The source of Ca^{2+} was thought to be intracellular Ca^{2+} release, because egg activation was induced independently of the ionic composition of the external medium [10,11]. The next advance was brought by the use of the Ca^{2+}-sensitive luminescent protein "aequorin" of the jelly fish [12]. In 1977, Ridgeway et al. [13] succeeded in recording an explosive $[Ca^{2+}]_i$ rise at fertilization or upon application of A23187 in aequorin-injected large eggs (diameter, ~1.1 mm) of medaka *Oryzias latipes*. Steinhardt et al. [14] also demonstrated a $[Ca^{2+}]_i$ rise in sea urchin eggs, although they measured total luminescence of aequorin in several eggs. In 1978, Gilkey et al. [2] first presented images of a propagating Ca^{2+} wave in a medaka egg. The wave velocity was ~10 μm/s, consistent with that of a wave of "cortical alveolus breakdown" (i.e., exocytosis) which Yamamoto had already found in 1939 [15]. The Ca^{2+} wave was not affected by external Ca^{2+}, and injection of Ca^{2+} buffer into the egg caused a propagating Ca^{2+} wave as well as egg activation [2]. Therefore, the Ca^{2+} wave was thought to be mediated by a form of "Ca^{2+}-induced Ca^{2+} release" (CICR) similar to that found in the sarcoplasmic reticulum (SR) of muscle cells at that time [17]. Imaging of the Ca^{2+} wave in much smaller sea urchin eggs was acquired by Eisen et al. in 1984 [16]. These pioneering experiments not only substantiated the Ca^{2+} hypothesis but also revealed the Ca^{2+} wave which propagated the signal over the entire egg from the stimulus point of the sperm fusion site.

3. $[Ca^{2+}]_i$ rise in protostome eggs

In the early 1980s, Lionel Jaffe proposed a hypothesis predicting that, in protostome eggs, $[Ca^{2+}]_i$ rise is due to Ca^{2+} influx, unlike deuterostome eggs [3,4]. This idea was based on the slow and non-wave-like pattern of exocytotic secretion, or measurement of $^{45}Ca^{2+}$ influx in animals of which eggs do not exhibit an exocytotic reaction [4]. Direct measurement and/or imaging of the $[Ca^{2+}]_i$ rise was performed after 1990 and revealed that this prediction was basically true for the first Ca^{2+} response [1]. The exception is that additional repetitive Ca^{2+} responses occur in a wave-like fashion due to intracellular Ca^{2+} release in protostome animals such as nemertean worms (Fig. 1H) [18], bivalve *Mytilus* (Fig. 1G) [19], and polychaete worms (Fig. 1F) [20]. Jaffe's prediction stimulated succeeding work on Ca^{2+} signals, which resulted in the universal concept of Ca^{2+}-dependent egg activation.

4. Research of Ca^{2+} signals in mammalian and ascidian eggs

The technology of mammalian in vitro fertilization (IVF) had been established by the early 1970s, but reports on the fertilization potential and Ca^{2+} response first appeared in 1981. Miyazaki and Igusa showed that the fertilization potential of golden hamster eggs was a series of periodic hyperpolarizations, recorded during monospermic fertilization in eggs freed from the zona pellucidae [5]. Since each hyperpolarization was due to Ca^{2+}-activated K^+ conductance increase [5,21], this phenomenon indirectly indicated that repetitive $[Ca^{2+}]_i$ rises likely accompany mammalian fertilization. In the same year Cuthbertson et al. also showed multiple $[Ca^{2+}]_i$ rises using aequorin in mouse eggs [6], although it was unknown whether the experimental condition was normal fertilization or not. In 1986, repetitive Ca^{2+} transients in hamster eggs were recorded by a Ca^{2+}-sensitive microelectrode [22] and aequorin luminescence [23], and Ca^{2+} waves were demonstrated in monospermic eggs with aequorin and a super-sensitive camera system [23]. Thus, research of the fertilization Ca^{2+} signal in mammalian eggs began and advanced several years later than that of sea urchin and medaka eggs. As more data accumulated, a characteristic temporal Ca^{2+} signal, Ca^{2+} oscillations, turned out to be common to mammalian eggs (Fig. 1A) [24]. Furthermore, periodic Ca^{2+} waves originating near the vegetal pole of the egg were found in ascidian eggs (Fig. 1C) [25,26] and in protosome eggs described above [1] (Fig. 1). As imaging technology advanced, it was subsequently observed that all the Ca^{2+} oscillations in mouse eggs were also emanating from the vegetal hemisphere [27,28]. At present, the study on the mechanism of fertilization Ca^{2+} signals is the most advanced in mammals (Table 1).