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Short Review | Open Access

A Role for Proton Signaling in the Induction of Somatic Cells to Pluripotent Embryonic Stem Cells

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Abstract

The mechanisms of embryonic stem (ES) cell establishment and egg cell fertilization have been studied without examining the interactions of these cell types, perhaps because of the lack of consideration for the similarities between ES cells and fertilized egg cells, especially the lack of calcium signaling. Here we unify the various concepts developed separately so fat in ES cells and fertilized eggs, although both types of cells have the same fate (i.e., to become somatic cells). We discuss the concept of water property changes in the cytoplasm that occur along with the cell cycle, which was initially introduced in the field of biophysics. With this aspect, the similarities and differences between ES cells and fertilized eggs are understandable, and the water property changes help explain why ES cells can have pluripotency, as fertilized eggs do, following a de novo stimulus such as acid treatment. Obokata et al. recently discovered a shortcut for generating pluripotent stem cells which they named “stimulus-triggered acquisition of pluripotency (STAP)” cells from somatic cells at the transient low-pH stimulation [1]. This report has had a significant impact on many developmental biologists and clinicians, because the low-pH treatment seemed an unexpectedly easy way to change somatic cells to pluripotent embryonic stem (ES) cells [2]. However, this method is not so surprising to biophysicists, because several types of cellular stress have been found to induce egg activation associated with a Ca^{2+} transient increase, such as the change in cytoplasmic pH caused by applying NH_4Cl and/or CO_2, or even mechanical pricking with a needle [3].

In 2002, Burdon et al. reported that the epiblast cells, which have pluripotency, were obtained from a prolonged culture of ES cells [4]. ES cells have an unusual cell cycle in which the G1 phase that operates in other types of cells is reduced [5]. Such features of ES cells are associated with the deregulated characteristics of the proliferation of tumor cells. Unlike these types of cells, a fertilized egg also starts its own cell cycle with Ca^{2+} oscillations [6]. This Ca^{2+} oscillation can be explained by a one- or two-calcium-pool model with the positive feedback of increased Ca^{2+} and inositol 1,4,5- trisphosphate (IP3) during the rising phase of each Ca^{2+} increase. It is thus reasonable to speculate that the cell cycle is the time keeper in Ca^{2+} oscillation in both cultured ES cells in vitro and circadian calcium rhythms in the somatic cells in the body, which were also found to be associated with a slow frequency of Ca^{2+} oscillation, with 1 cycle/day [7].

The property changes of water state in the cell are another critical issue. Mantré described that the state of water in the somatic cells in the body has ordered-structured bound water (like ice), whereas ES cells as well as cancer cells have free (normal) water in the G1 and G2 phases [8]. Only protons, not other ions, are able to move on the surface of bound water, whereas in free water all types of ions (including protons) can move by diffusion. In this review article, we describe the differences between somatic cells and ES cells regarding proton signaling, which regulates Ca^{2+} oscillation, and the change in water properties associated with cell cycle. Proton signaling plays a critical role in the nuclear reprogramming to the pluripotent stage.

Cell Cycle-dependent Calcium Oscillation in Stem Cells

In 2008, Klevecz et al. introduced a new concept of a ”cell attractor” which tunes deterministic cellular noises to gate cells into the S phase, to explain how the cell cycle is timed and how stable cellular phenotypes are maintained [9]. The Ca^{2+} oscillation in the cell may be the most likely candidate of cell attractor, because Ca^{2+} oscillation can regulate both cell cycles and circadian rhythms. In the case of cultured mouse ES cells, the spontaneous Ca^{2+} oscillation is generated by an IP3-mediated Ca^{2+} release [10]. A large portion of Ca^{2+} oscillations was found to continue from the G1 to the S phase of the cell cycle, and a smaller portion was detected in the G2/M phase [10].

Although this Ca^{2+} oscillation during the cell cycle is clear, the triggering signal for this oscillation had not been identified. If the Ca^{2+} oscillation of the cells is triggered by released IP3, the oscillation is quite similar to that of fertilization. In the case of mammalian eggs and ascidian oocytes, it was established that Ca^{2+} oscillation is triggered by IP3 [11]. The difference in the Ca^{2+} oscillation of ES cells and that of fertilized eggs is in the trigger signal. Mammalian eggs and ascidian oocytes can be activated by sperm, an injection of sperm factor, an injection of IP3, an application of calcium ionophores and pricking [6], whereas the cell cycle-dependent Ca^{2+} oscillation of ES cells is always observable. The amplitude and wave shape of Ca^{2+} oscillation are affected to some extent by treatment with Ca^{2+}-free medium with 10 μM ryanodine [3]. These findings indicate that ES cells have always been activated by an unidentified stimulation.

What type of stimulation is likely to activate somatic cells to generate pluripotent ES cells from somatic cells? Gilkey examined the

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roles of Ca^{2+} and pH in activation of Medaka fish eggs [3]. His findings can be summarized as follows, based on the results of the microinjection of pH buffers into the Medaka fish eggs and those of exposing the eggs to reagents that should alter cytoplasmic pH:

1) A transient increase in calcium is necessary for egg activation.
2) Altering the cytoplasmic pH does not affect the egg activation per se.
3) Altering the cytoplasmic pH does change the threshold free-calcium concentration required to activate the egg.

According to his results, Gilkey found that the threshold concentration of free calcium to elicit the Ca^{2+} oscillation/wave was pH dependent. These threshold values are fairly below the estimated value, 30 μM from Ca^{2+} wave; the rate of propagation of the Ca^{2+} wave through the egg's cytoplasm is a function of cytoplasmic pH. Accordingly, Gilkey concluded that if the proton concentration increases by 10-fold (i.e., pH decreases by 1 unit), the amplitude of the Ca^{2+} wave will be amplified by 10-fold [3].

Why does cytoplasmic pH alter Ca^{2+} wave amplitude? Gilkey proposed that "perhaps the calcium binding site for calcium-induced calcium release (CICR) can also bind one proton, and proton binding competitively inhibits calcium binding to this site". His model postulated a two-calcium-pool model: Ca^{2+} is released from the IP3 receptors (IP3Rs) and the ryanodine receptors equipped endoplasmic reticulum, for example. This was the first proposal of a two-calcium-pool model based on the experimental results. However, Gilkey did not consider the contribution of water properties in Ca^{2+} oscillations.

A Role for Cell's Water Property in Proton/Calcium Signaling

Only a few biologists have theorized that the intracellular water is different from general bulk (free) waters [8], because the interior of a cell is overcrowded with relatively huge macromolecules, leaving little space for water [12]. Water molecules are in contact with macromolecule patchwork of the macromolecular surface domain, regardless of whether the macromolecules are hydrophobic or hydrophilic [8]. When water molecules bound to consecutive polar amino acids (e.g., Asn, Cys, Gln, Ser, The, Tyr) in a polypeptide chain, they can bind to form H-bonded lines. These lines of water molecules are good conductors of protons, but not good conductors of electrons. In this model, protons can move by a successive flip-flop step from the first water molecule to the last water molecule of the chain, rather than migrating along the water chain [13]. This process was eventually named "the proton wave."

This domino-like wave motion is faster than diffusion. Only the Ca^{2+} wave observed in living cells is always accompanied by a proton wave signal which is more rapid than diffusion, whereas the proton wave is transferred according to the Coulomb interaction on the water surface. In contrast to positively charged Ca^{2+}, negatively charged molecules such as IP3 and/or cyclic nucleotides will move by the diffusion process in the small gap under the plasma membrane. It can thus be concluded that Ca^{2+} is a good second messenger which can reach the mitochondria and other endoplasmic reticula to produce Ca^{2+} oscillations.

The following question remained: "Does the Ca^{2+} oscillations change with cell cycle or not?" Larman et al. addressed this question in a 2004 study [14]. They observed that Ca^{2+} oscillation was obtained in the M phase but not in the G1 or G2 phase. What is the difference between the two (M vs. G1/G2)? A few hypotheses have been put forth [14,15], but in the next section we propose a new model based on the water state in the cell.

Cell-cycle Changes in the Intracellular Water Properties

Changes in water properties and water content during cell cycle were initially investigated by Beall et al. using nuclear magnetic resonance relaxation time (T1) in synchronized HeLa cells [16]. The T1 in the M phase was found to be the highest, and that in the S phase was the lowest. In the G1 and G2 phases, the T1 values showed a transient state; gradual decreasing in the G1 and recovering in the G2 phase. These data could be understandable if low T1 values correspond to free water and the high T1 values correspond to bound water. This explanation can also be supported by the variation of the water content in the cell cycle [16]. Such changes in water properties according to the cell cycle were further investigated by other groups using fertilized sea urchin eggs [17,18]. This changing of water properties along with the cell cycle completely corresponds to Ca^{2+} oscillations described above.

The residual question is why bound water in the M phase starts to be free water in the G1 phase and vice versa in the G2 phase. Important hints for approaching this problem are in the Hoffmeister series, as was suggested by Wiggins [19]. In brief, bound water can be changed to free water when Na^+ enters the cytosol. As was observed previously, s study of *Xenopus* oocytes showed a depolarization of the membrane potential by fertilization, which will induce Na^+ entry into the cell [20]. It was also found that the proton current can flow through (amiloride-sensitive) Na^+ channels in the hamster taste cells [21]. Taken together, these findings lead us to speculate that a protocol of acidic stimulation (as Obokata et al. did for reprogramming [1]) will induce Na^+ entry, which will induce an increase in intracellular proton level initially; then the water state will be changed gradually from the bound state to the free state, which will initiate a new cell cycle as described above.

Aged Somatic Cell Cannot have Pluripotency

Before an elucidation of the aging effect on the induction of

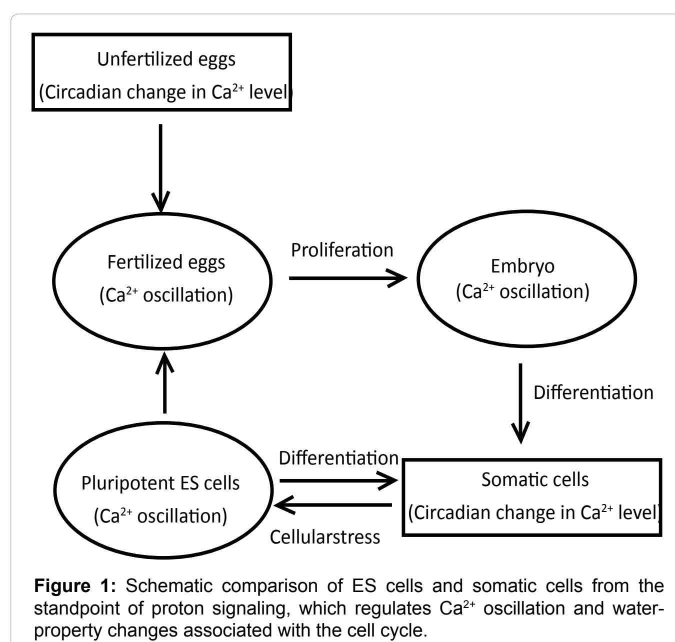

Figure 1: Schematic comparison of ES cells and somatic cells from the standpoint of proton signaling, which regulates Ca^{2+} oscillation and water-property changes associated with the cell cycle.

pluripotency, we must understand the two-calcium-pool model of Ca^{2+} oscillation, because Ca^{2+} oscillation is thought to be a necessary and sufficient condition in the creation of pluripotency. In the two-calcium-pool model, the functioning of both the IP3R and the ryanodine receptor are essential [22]. Cell cycle-dependent Ca^{2+} oscillation was observed and established not only in theoretical models for intracellular Ca^{2+} oscillation [23], but also in mammalian eggs at fertilization [24], in mouse embryos [14] and in mouse ES cells. Therefore it is reasonable to assume that the IP3R is involved in pluripotential ES cells. If such an assumption is correct, it may explain why aged somatic cells cannot have pluripotency by acidic stimulations.

We recently observed that 24-h H_2O_2-treated human keratinocyte showed poor Ca^{2+} responses (i.e., < 10% of the normal response) for purinergic G protein-coupled receptor (P2Y receptor) activation by ATP, but the responses completely recovered when we reduced the treatment period with hydrogen gas-saturated phosphate-buffered saline solution for 1 h (Hsu et al., unpublished data). These poor responses were found to be due to the oxidation of the IP3R. Since the IP3R is essential to the Ca^{2+} oscillation in ES cells, the IP3R in aged somatic cells cannot continue Ca^{2+} oscillation, resulting in these cells' failure to have pluripotency.

Concluding Remarks

A summary of the information presented in this review is given in Figure 1 Unfertilized eggs, a type of somatic cells, will be changed to embryos after fertilization with the Ca^{2+} oscillation-associated cell cycles. Similarly, somatic cells obtained from a young body will have pluripotency by acidic stimulation, whereas somatic cells from an aged body cannot have pluripotency because of the deletion of Ca^{2+} oscillation by oxidized (aged) IP3Rs.

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