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Review

Calcium oscillations and mitochondrial enzymes in stem cells

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ABSTRACT

Calcium oscillations are rhythmic fluctuations of the intracellular concentration of calcium ions (Ca^{2+}). As Ca^{2+} evokes various cellular processes, its intracellular concentration is tightly regulated. Ca^{2+} oscillations control biological events, including neuronal differentiation and proliferation of mesenchymal stem cells. The frequency and pattern of Ca^{2+} oscillations depend on cell type. Researchers have studied Ca^{2+} oscillations to better understand how cells communicate and regulate physiological processes. Dysregulation of Ca^{2+} oscillations causes health problems, such as neurodegenerative disorders. This review discusses the potential functions of Ca^{2+} oscillations in stem cells.

Contents

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Abbreviations

ATP	adenosine triphosphate
Ca^{2+}	calcium ion
CaMKII	calcium–calmodulin-dependent protein kinase II
CS	citrate synthase
eCS	extramitochondrial citrate synthase
ER	endoplasmic reticulum
eTCA cycle	extramitochondrial TCA cycle
GPCR	G protein-coupled receptor
HDAC4	histone deacetylase 4
HIF1α	hypoxia-induced factor 1α
IDH	isocitrate dehydrogenase
IP_3	inositol 1,4,5-triphosphate;
KATP	ATP-sensitive potassium
KDH	α-ketoglutalate dehydrogenase
MFN2	mitofusin 2
MSCs	mesenchymal stem cells
NFATc1	nuclear factor of activated T cells, cytoplasmic 1
NSCs	neural stem cells
ORAI	a membrane protein encoded by drosophila *olf186-F* gene
PAWP	postacrosomal sheath WW domain-binding protein
PLC	phospholipase C
RANK	receptor activator of nuclear factor-κB
RANKL	receptor activator of nuclear factor-κB ligand
SR	sarcoplasmic reticulum
STIMs	stromal interaction molecules
TCA cycle	tricarboxylic acid cycle
TET2	ten-eleven translocation 2
VGCCs	voltage-gated Ca^{2+} channels

1. Introduction

Calcium ion (Ca^{2+}) release and clearance from the cytoplasm are finely regulated by various regulators, leading to repetitive changes in intracellular Ca^{2+} concentration in differentiated cells (Table 1) and stem cells (Table 2). Primarily, plasma membrane Ca^{2+}-ATPases maintain cytoplasmic Ca^{2+} concentrations by serving as Ca^{2+} pumps on the plasma membrane [1]. Sarcoendoplasmic reticulum Ca^{2+}-ATPases act as Ca^{2+} pumps in the endoplasmic reticulum (ER) [2]. The mitochondrial Ca^{2+} uniporter regulates Ca^{2+} transport to the inner mitochondrial membrane. The electrogenic Na^{+}–Ca^{2+} exchanger is involved in Na^{+} influx and Ca^{2+} release into the plasma membrane [3,4].

Upon stimulation with hormones, phospholipase C (PLC) γ generally produces inositol 1,4,5-triphosphate (IP_3). IP_3 binds to the IP_3 receptors on the ER membrane, causing Ca^{2+} release from the ER [5]. To replenish Ca^{2+} levels in the ER, Ca^{2+} release-activated Ca^{2+} channels, composed of stromal interaction molecules (STIMs) and a membrane protein encoded by drosophila *olf186-F* gene (ORAI), are involved in store-operated Ca^{2+} entry [6–9]. Decreased Ca^{2+} levels inside the ER initiate the translocation of STIMs to the ER membrane for interaction with the plasma membrane [10]. Translocated STIMs directly interact with ORAI channels in the plasma membrane to regulate Ca^{2+} influx [10]. In addition, the family of transient receptor potential channels and other Ca^{2+} channels in the plasma membrane contribute to maintaining the intracellular Ca^{2+} concentration via store-operated Ca^{2+} entry [11,12]. Increased cytoplasmic Ca^{2+} concentration controls exocytosis and cellular functions. Ca^{2+} signaling is critical for various biological processes because a sufficient Ca^{2+} concentration is continuously needed to perform cellular functions, including cell proliferation and cytokine production. Remarkably, Ca^{2+} signaling in each organelle regulates organelle-specific functions, leading to gene regulation in the nucleus and oxidative metabolism in the mitochondria [10,13]. To utilize Ca^{2+} signaling in these processes, the Ca^{2+} concentration is translated into cellular signals, and

Table 1
Regulatory factors of Ca^{2+} oscillations in differentiated cells.

Gene name	Coding protein	Localization	Cell type	References
Atp2b1	Plasma membrane Ca2+-ATPase	Plasma membrane	Broad cells	[1]
Atp2a2	Sacro-endoplasmic reticulum Ca2+-ATPase	Sacro-endoplasmic retuculum	Broad cells	[2]
Calm1	Calmodulin	Cytoplasm	Broad cells	[1,14]
Cs	Citrate synthase	Mitochondria	Broad cells	[50]
IP3Rs	Inositol 1,4,5-triphosphate receptors	Endoplasmic reticulum	Broad cells	[31]
Glut2	Glucose transporter 2	Plasma membrane	Broad cells	[34]
Csl	Citrate synthase	Extramitochondrial region	Sperm and neurons	[51]
Kit	Truncated and ctytoplasmic form of c-kit receptor	Plasma membrane	Sperm	[28]
NFATc1	Nuclear factor of activated T cells, cytoplasmic 1	Nucleus	Osteoclast	[31]
Ncx1	Sodium-calcium exchanger 1	Plasma membrane	Cardiac muscle cell	[3,4,37]
Mcu	Mitochondrial Ca2+ uniporter	Mitochondria	Broad cells	[2]
Orais	ORAI calcium release-activated calcium modulators	Plasma membrane	Broad cells	[6–10]
Plcg2	Phospholipase C-γ	Cytoplasm	Broad cells	[5]
Plcz1	Phospholipase C-ζ	Cytoplasm	Sperm	[19–21, 23, 26, 29, 30]
RANK	Receptor activator of nuclear factor-kppa κB	Plasm,a membrane	Osteoclast	[31–33]
RANKL	Receptor activator of nuclear factor-kppa κB ligand	Plasma membrane and lysosome	Osteoblast	[31–33]
Ryrs	Ryanodine receptors	Endoplasmic reticulum	Broad cells	[37]
Ppp3ca	Calcineurin	Cytoplasm	Broad cells	[14]
Slc8b1	Electrogenic Na + -Ca2+ exchanger	Mitochondria	Broad cells	[3,4]
Stims	Stromal interaction molecules	Endoplasmic reticulum	Broad cells	[6–10]
TRPCs	Transient receptor potential channels	Plasma membrane	Broad cells	[11,12]
PAWP	Postoacrosomal sheath WW domain binding protein	Plasma membrane	Sperm	[26]

Table 2
Regulatory factors of Ca^{2+} oscillations in stem cells.

Gene name	Coding protein	Localization	Cell type	References
Atp2b1	Plasma membrane Ca2+-ATPase	Plasma membrane	Pluripotent stem cell	[1]
CaMKII	Calcium–calmodulin-dependent protein kinase II	Cytoplasm	Pluripotent stem cell	[48, 49]
HDAC4	Histone deacetylase 4	Nucleus	Pluripotent stem cell	[48, 49]
HIF1α	Hypoxia-induced factor 1α	Cytoplasm and nucleus	Various types of stem cells	[45]
IDH	Isocitrate dehydrogenase	Mitochondriia	Various types of stem cells	[47]
KDH	α-ketoglutalate dehydrogenase	Mitochondriia	Various types of stem cells	[47, 69]
MFN2	Mitofusin 2	Mitochondria	Hematopoietic stem cell	[48]
TET2	Ten-eleven translocation 2	Nucleus	Pluripotent stem cell	[48]

proteins with Ca^{2+}-binding motifs play a role in such translation [10]. These motifs are common in Ca^{2+} channel proteins, proteins mediating Ca^{2+}-regulated cell functions, and Ca^{2+}-sensing proteins [10]. In particular, Ca^{2+}-sensing proteins play an important role in transducing Ca^{2+} concentration changes to calmodulin or calcineurin, cooperatively [14].

Many studies have provided compelling evidence that sperm contain soluble factors (sperm factors) that initiate Ca^{2+} oscillations in eggs after sperm fusion [15–21]. In frog and sea urchin eggs [22–25], cyclic adenosine dinucleotide phosphate-ribose, nicotinic acid-adenine dinucleotide phosphate, cyclic guanosine monophosphate, inositol 1,4,5-triphosphate, and nitric oxide have been identified as candidate soluble sperm factors. Nitric oxide also functions as a sperm factor in sea urchin eggs [24]. In mammals, the postacrosomal sheath WW domain-binding protein (PAWP) has been suggested as a sperm factor [26]. Furthermore, Ca^{2+} oscillations were shown to be triggered by recombinant PAWP injections into cow and pig eggs [27].

In mice, the truncated cytoplasmic form of the c-kit receptor has been proposed as a potential sperm factor [28]. Ca^{2+} oscillations are also triggered by the activation of IP_3 signaling, implying that PLC may be a predominant candidate sperm factor. A novel testis-specific PLCζ1 (PLCz1) was identified, and two recent studies have reported that *Plcz1*-deficient (*Plcz1*-KO) mice are drastically subfertile but not completely infertile, albeit with defects in triggering Ca^{2+} oscillations [29,30]. These findings suggest the presence of other sperm factors.

In this review, we discuss the biological importance of Ca^{2+} oscillations in stem cells.

2. Ca^{2+} oscillations in differentiated cells

2.1. Osteoclasts

Ca^{2+} oscillations play roles in osteoclast differentiation and bone resorption [31–33]. Osteoclasts are specialized cells responsible for breaking down bone tissue, and Ca^{2+} signaling regulates their function [31]. Osteoclast differentiation begins with the activation of osteoclast precursor cells. This activation involves the binding of the receptor activator of nuclear factor-κB ligand (RANKL) to its receptor RANK on the surface of precursor cells. Binding of RANKL to RANK triggers Ca^{2+} oscillations in osteoclast precursor cells which are mediated by the release of Ca^{2+} from intracellular stores, particularly the ER, through the activation of Ca^{2+} release channels, such as inositol trisphosphate receptors (IP3Rs) [31].

Ca^{2+} oscillations also activate the transcription factor nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) through the Ca^{2+}-dependent phosphatase, calcineurin [31]. NFATc1 is a key regulator of osteoclast differentiation and is responsible for the expression of genes involved in osteoclast formation and function. Ca^{2+} oscillations also affect the cytoskeletal reorganization of osteoclast precursor cells [31]. This reorganization is essential for formation of the sealing zone, a specialized structure in which osteoclasts attach to bone surfaces during resorption.

Overall, Ca^{2+} oscillations play a pivotal role in osteoclast differentiation and bone resorption. They regulate the expression of genes involved in osteoclast formation, influence cytoskeletal changes required for bone attachment, and contribute to the acidic and proteolytic environment necessary for efficient bone resorption.

2.2. Pancreatic β cells

Calcium oscillations refer to rhythmic fluctuations in Ca^{2+} concentration in pancreatic islet cells and play a role in regulating insulin secretion [34]. Pancreatic islets, also known as the islets of Langerhans, contain different types of cells, including β cells that secrete insulin. The primary trigger for insulin secretion in pancreatic β cells is an increase in blood glucose levels. When glucose enters β cells through glucose transporters, it undergoes glycolysis and generates adenosine triphosphate (ATP).

Increased ATP levels lead to the closure of ATP-sensitive potassium (KATP) channels in the cell membrane [35]. These channels are normally open when ATP levels are low, allowing potassium ions (K^+) to flow out of the cell and leading to membrane hyperpolarization. The closure of KATP channels causes membrane depolarization, leading to the opening of voltage-gated Ca^{2+} channels (VGCCs). These Ca^{2+} channels allow Ca^{2+} to enter β cells. The influx of Ca^{2+} through VGCCs leads to a rapid increase in the intracellular Ca^{2+} concentration. Ca^{2+} influx is essential for triggering insulin secretion. Rather than a sustained elevation in Ca^{2+} levels, β cells often exhibit Ca^{2+} oscillations in response to glucose stimulation. Each Ca^{2+} spike triggers the fusion of insulin-containing vesicles with the cell membrane, thereby releasing insulin into the bloodstream. In addition to glucose, various factors, such as hormones (incretins-like glucagon-like peptide-1) and neural inputs, modulate Ca^{2+} oscillations in β cells [36].

Dysregulation of Ca^{2+} signaling in pancreatic β cells leads to impaired insulin secretion and is associated with type 2 diabetes. Studying Ca^{2+} oscillations in the islets is essential for understanding the physiology of insulin secretion and may contribute to the development of new therapeutic strategies for diabetes management.

2.3. Cardiac muscle cells

Ca^{2+} oscillations are a key mechanism for maintaining the cardiac action potential, an electrical signal that controls the contraction of cardiac muscle cells (cardiomyocytes) [37]. Ca^{2+} is essential for the initiation and regulation of muscle contraction in the heart [38]. At rest, cardiomyocytes maintain low intracellular Ca^{2+} concentrations. This is mainly achieved by actively pumping Ca^{2+} out of the cells using a sodium-Ca^{2+} exchanger and storing it

in the sarcoplasmic reticulum (SR), a specialized organelle within the cells.

When cardiac pacemaker cells in the sinoatrial node generate an electrical impulse, they travel through the conduction system of the heart and reach the cardiomyocytes. This electrical signal causes the depolarization of the cardiomyocyte membrane, leading to the opening of voltage-gated Ca^{2+} channels (L-type) in the cell membrane. The opening of these Ca^{2+} channels allows the influx of extracellular Ca^{2+} into cardiomyocytes, which enhances the intracellular Ca^{2+} concentration.

Ca^{2+} influx activates the ryanodine receptors in the SR. This activation causes the SR to release a larger amount of stored Ca^{2+} into the cytoplasm in a process known as Ca^{2+}-induced Ca^{2+} release. This sudden increase in the intracellular Ca^{2+} concentration is critical for the initiation of muscle contractions. Ca^{2+} binds to troponin, a component of myofilaments in cardiomyocytes. This binding causes a conformational change in the troponin-tropomyosin complex, allowing actin and myosin to interact and initiate muscle contractions.

The cyclical changes in the intracellular Ca^{2+} concentration that occur during each cardiac cycle can be referred to as Ca^{2+} oscillations. The timing and magnitude of these oscillations are tightly regulated and are critical for maintaining the rhythmic beating of the heart and effective pumping of blood throughout the body.

2.4. Uterine endometrium

Protease-induced Ca^{2+} oscillations in endometrial epithelial cells are a specific cellular response observed in the endometrium, inner lining of the uterus [39]. Ca^{2+} oscillations are triggered by the action of proteases. This phenomenon is particularly relevant during the menstrual cycle and embryo implantation.

The endometrium undergoes cyclic changes during the menstrual cycle in preparation for embryo implantation [40]. The lining thickens in the anticipation of pregnancy. If fertilization occurs, the embryo must attach to endometrial epithelial cells for implantation. During the menstrual cycle, endometrial epithelial cells and surrounding tissues release proteases, such as matrix metalloproteinases and tissue plasminogen activators. These proteases play a role in tissue remodeling and facilitate embryo implantation. Proteases released by endometrial cells activate cell surface receptors or cleave extracellular matrix components [39]. This activation leads to the generation of intracellular signaling molecules and initiation of downstream signaling pathways.

Protease-induced signaling enhances intracellular Ca^{2+} levels in endometrial epithelial cells [39]. These increases are often observed in oscillatory patterns. Protease-induced Ca^{2+} oscillations trigger various cellular responses in endometrial epithelial cells. These responses may include changes in gene expression, alterations in cell adhesion properties, and the secretion of factors that facilitate embryo implantation. Dynamic changes in Ca^{2+} levels and associated cellular responses help prepare the endometrial epithelium for embryo implantation [39]. Adequate Ca^{2+} signaling is crucial for proper embryo attachment and successful pregnancy.

Protease-induced Ca^{2+} oscillations in the endometrial epithelial cells are tightly regulated and coordinated by hormonal fluctuations during menstruation [41]. Hormones (estrogen and progesterone) influence the expression and activity of proteases and their receptors, thereby contributing to the timing and effectiveness of Ca^{2+} oscillations [41]. Understanding protease-induced Ca^{2+} oscillations in endometrial epithelial cells is essential to elucidate the mechanisms involved in embryo implantation and female reproductive health. Dysregulation of these processes causes reproductive disorders and infertility.

3. Ca^{2+} oscillations in stem cells

3.1. Neural stem cells

Neural stem cells (NSCs) are responsible for generating new neurons and glial cells throughout their lifetime [42]. To maintain stem cell identity, NSCs must carefully regulate their behaviors, including self-renewal and differentiation into specialized cell types.

Ca^{2+} oscillations regulate NSC proliferation and self-renewal. Intracellular Ca^{2+} levels affect the activity of various signaling pathways, transcription factors, and cell cycle regulators that determine whether NSCs divide or remain quiescent [42]. Ca^{2+} oscillations in NSCs are crucial for neural circuit formation during embryogenesis. NSCs give rise to neurons that establish functional connections and synaptic contacts with other neurons. Ca^{2+} signaling helps orchestrate the precise timing and guidance of neurite outgrowth and axon pathfinding [42].

In the adult brain, certain regions, such as the hippocampus and subventricular zone, maintain NSCs that continue to produce new neurons [42]. Ca^{2+} oscillations in adult NSCs are essential for regulating the integration of new neurons into existing neural circuits, and memory and learning processes [42]. Understanding the precise mechanisms of Ca^{2+} oscillations in NSCs is crucial for the development of potential therapeutic interventions.

3.2. Mesenchymal stem cells

Ca^{2+} oscillations play a role in regulating various cellular processes in mesenchymal stem cells (MSCs), which are multipotent cells that can differentiate into various cell types, including osteoblasts, adipocytes, and others [43]. Ca^{2+} oscillations help maintain MSCs in an undifferentiated state, thus preserving their stem cell properties [43]. Low levels of intracellular Ca^{2+} are often associated with the maintenance of stem cell phenotypes. Ca^{2+} oscillations are involved in MSC differentiation into specific cell lineages [43]. The pattern and frequency of the Ca^{2+} oscillations dictate the direction of differentiation.

Various extracellular signals, including growth factors, hormones, and cytokines, can trigger Ca^{2+} oscillations in MSCs [43]. These signals often act via G protein-coupled receptors or tyrosine kinases to initiate intracellular Ca^{2+} release.

Ca^{2+} oscillations are involved in regulating MSC migration. Changes in calcium levels affect cytoskeletal dynamics and cell motility, allowing MSCs to respond to chemotactic signals during tissue repair and regeneration [43]. MSCs play a role in tissue repair and wound healing. Ca^{2+} oscillations in MSCs are implicated in their ability to sense and respond to injury signals and initiate healing.

In tissue engineering and regenerative medicine, understanding and manipulating Ca^{2+} oscillations in MSCs are important for directing their differentiation into specific cell types for therapeutic purposes.

3.3. Hypoxia and Ca^{2+} oscillations

Metabolic profiles in pluripotent stem cells are related to their self-renewal and cell fate decision [44]. Hypoxia-induced reactive oxygen species (ROS) accumulation leads to apoptosis in various types of cells. Hence, metabolic switching prevents the excessive ROS production and maintains the physiological states of stem cells [45]. The expression and nuclear localization of hypoxia-induced factor 1α (HIF1α) are tightly regulated, whereas HIF1β is constitutively expressed [45]. The increase of intracellular Ca^{2+} concentration is an intrinsic response

exposed to hypoxia in many cell types. Hypoxia-mediated Ca^{2+} increase enhances *HIF1A* transcription, and HIF1α translation and stabilization. Moreover, MSCs primed with both hypoxia and Ca^{2+} enhance their stemness and capacity for immunomodulatory activity, thereby attenuating graft-versus-host disease [46].

3.4. Epigenetic regulation of Ca^{2+} oscillations and pluripotency

The epigenome, a set of modifications comprising DNA methylation and hydroxymethylation, and histone modifications, controls cellular identity. Ten-eleven translocation (TET) enzymes play essential roles in the early development and differentiation [47]. TET2 plays a role in stem cells by suppressing the expression of lineage-specific genes. The post-translational histone modifications are regulated by the balanced action of histone acetyltransferases and deacetylases (HDACs) [48]. Ca^{2+}-calmodulin-dependent protein kinase II (CAMKII) activation facilitates self-renewal of human embryonic stem cells [48], suggesting that the CAMKII works in self-renewal of pluripotent stem cells in humans and mice. CaMKII phosphorylates HDAC4, which forms complexes with HDAC5, promoting HDAC4 transportation to the cytoplasm [49].

4. Contribution of mitochondrial enzymes to Ca^{2+} oscillations

4.1. Possible involvement of citrate synthase

Citrate synthase (CS), a rate-determining enzyme in the tricarboxylic acid (TCA) cycle, has been reported to function as a sperm factor in newts [50], raising the possibility of a similar mechanism in mammals. A recent study provided evidence of a defect in the initiation of Ca^{2+} oscillations in mouse eggs fused with extra-mitochondrial citrate synthase (*eCs*)-knockout (KO) sperm [51]. The initiation of the first spike of Ca^{2+} oscillations was markedly delayed in eggs fused with *eCs*-KO sperm, despite normal expression of the sperm factor PLCz1, implying that eCS independently triggers the first spike of Ca^{2+} oscillations. In particular, *eCs*-KO sperm revealed that the first spike and frequency of Ca^{2+} oscillations were significantly delayed and reduced compared to that in wild-type sperm. Thus, these findings clarify the existence of at least two sperm factors in mice (Fig. 1), implying that Ca^{2+} oscillations are divided into two processes, namely, initiation and persistence.

4.2. Age-dependent eCS function

Mitochondria, the energy powerhouses of cells, are important organelles for ATP production. ATP supply is essential for the success of fertilization and early embryonic development [52], as the midpiece of the sperm is packed with mitochondria that supply energy for tail motility, capacitation, and the acrosome reaction. Hence, problems in sperm mitochondrial function directly cause sperm dysfunction and male infertility [53].

Mitochondria are also involved in important cellular functions, such as homeostasis, defense against oxidative stress, and apoptosis [54]. Mitochondria undergo fusion and fission to maintain normal cellular function [55]. Mitochondria are increasingly being recognized as important organelles in the aging process. Diseases and aging greatly disturb in mitochondrial function; neurodegenerative diseases with mitochondrial involvement are well-known aging-related diseases [56].

Similarly, a decline in Ca^{2+} signaling with age affects the regulation of cellular functions [57]. The amplitude of the Ca^{2+} rise is negatively related to age [58], leading to a decrease in ATP production. Ca^{2+} signals are important factors in neurodegenerative and aging processes [56] because alterations in Ca^{2+} signals contribute to cell death. Alterations in Ca^{2+} signals may affect metabolite and mitochondrial functions and, consequently, may contribute to dysfunction and impaired cellular function.

As mentioned above, *eCs*-KO male mice are initially fertile and exhibit declining fertility by only six months after birth (corresponding to the age of 30 years in humans), suggesting that eCS plays a role in aging-related male infertility [51]. It is likely that the CS and eCS ratios for citrate synthesis change with age, resulting in an aging-dependent decline in mitochondrial function. In addition, testicular size has been reported to be related to sperm production [59]. Although there were significant differences in testis size between wild-type and *eCs*-KO male mice, there were no significant

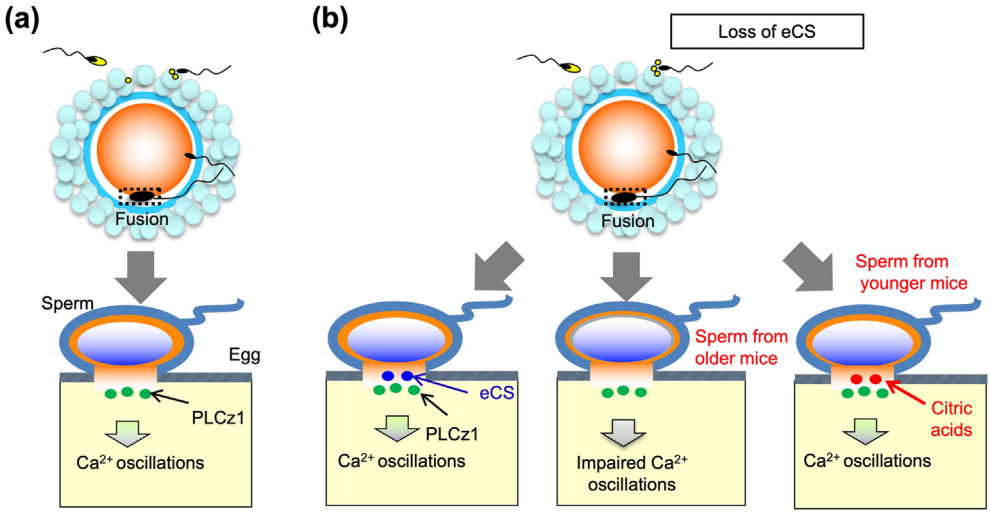

Fig. 1. Schematic model of Ca^{2+} oscillations in eggs after sperm fusion. **(a)** The conventional theory. After sperm-egg fusion, the sperm-derived factors trigger Ca^{2+} oscillations in the egg. Phospholipase C zeta 1 (PLCz1) is considered to be a sperm-derived factor responsible for successful mammalian oocyte activation. **(b)** A new theory based on a recent study [57]. Two sperm-derived factors, PLCz1 and eCS, are involved in triggering Ca^{2+} oscillations in the mouse egg. eCS may function to initiate Ca^{2+} oscillations, especially the first spike, alone and/or assisting PLCz1 to induce Ca^{2+} oscillations. Impressively, *eCs*-KO male mice exhibit impaired initiation of Ca^{2+} oscillations, leading to late-onset male infertility. This may be due to insufficient citrate synthesis due to mitochondrial dysfunction with aging. This figure was modified from Figure 3 in Ref. [65].

differences in sperm function, such as motility and morphology, between the two groups.

4.3. Predicted existence of extramitochondrial TCA cycle

Changes in metabolism regulate molecular pathways, such as apoptosis and angiogenesis. The therapeutic approach to metabolic regulation attempts to treat pancreatic cancer in preclinical studies [60], indicating that energy metabolism is one of the tools for controlling the molecular function of cells.

Even among the same type of cells, an individual cell has a unique ability and is capable of playing different roles depending on the situation. Since recent advances have enabled us to perform omics analysis using a single cell [61], individual expression variability has been demonstrated in the same cell type. If there are some functional differences among cells of the same type, cell function may depend on the status of the cell as well as changes in metabolic conditions with aging. In fact, rat testes and mouse sperm exhibit a remarkable aging-dependent alteration in metabolites [62], suggesting that changes in metabolic conditions may be attributed to a fertility decline in older *eCs*-KO male mice. Thus, in combination with aging, the loss of eCS enhances the TCA cycle, leading to a shortage of extramitochondrial citrates and critical sperm dysfunction at the initiation of Ca^{2+} oscillations in the egg. This finding raises the possibility that eCS could compensate for the decline in energy metabolism that accompanies aging. Accordingly, energy metabolism regulated by eCS may be involved in the decline in fertility of older *eCs*-KO mice.

Citrate is one of eight acids that operate in the TCA cycle and functions in the mitochondria. From yeast to humans, all eukaryotic cells have mitochondria and produce ATP as an energy source at the beginning of citrate synthesis via the TCA cycle. However, before the TCA cycle, glycolysis occurs in the cytoplasm to produce ATP, albeit less efficiently than in the TCA cycle.

Generally, ATP is produced using the TCA cycle under aerobic conditions. However, even under aerobic conditions, cancer cells rely on glycolysis for ATP production [63]; this is also known as the Warburg effect (aerobic glycolysis) [63,64]. Although cancer cells mainly generate ATP via this process, its benefits for their function remain unclear [64]. This suggests new possibilities for the Warburg effect caused by the dysfunction or gain-of-function of citrate synthase, as citrate synthesis is essential for the switch from glycolysis to the TCA cycle.

Owing to the lack of a mitochondrial targeting sequence in eCS, it is predominantly present in the acrosome of sperm and not in the mitochondria. So, eCS may be involved in energy production for sperm function via the extramitochondrial TCA (eTCA) cycle [65] (Fig. 2). In addition, *eCs*-KO male mice exhibited decreased fertility with age, suggesting an increasing contribution of eCS to sperm function in older male mice. This finding implies the possibility of the existence of the eTCA cycle in the extramitochondrial space.

5. Relation between stem cell biology and mitochondrial research

Stem cells primarily rely on glycolysis for energy production, while differentiated cells often shift towards oxidative phosphorylation for ATP synthesis [66]. Mitochondria influence the stem cell fate decision from self-renewal to differentiation by modulating signaling pathways and gene expression [66,67]. Mitochondria are also key regulators of cell death through the release of pro-apoptotic factors [66]. In stem cells, this function is crucial for maintaining the proper cell population during development and tissue homeostasis.

Various types of stem cells depend on glycolysis for ATP production to a larger extent than differentiated cells [48]. Mitochondria affect the epigenome through intermediates of the TCA cycle. For example, increased TCA metabolites, including a-ketoglutarate, fumarate, succinate, and L(S)-2-hydroxylate, are associated with histone hypermethylation and hypoacetylation, and DNA hypermethylation in hematopoietic stem cells [68].

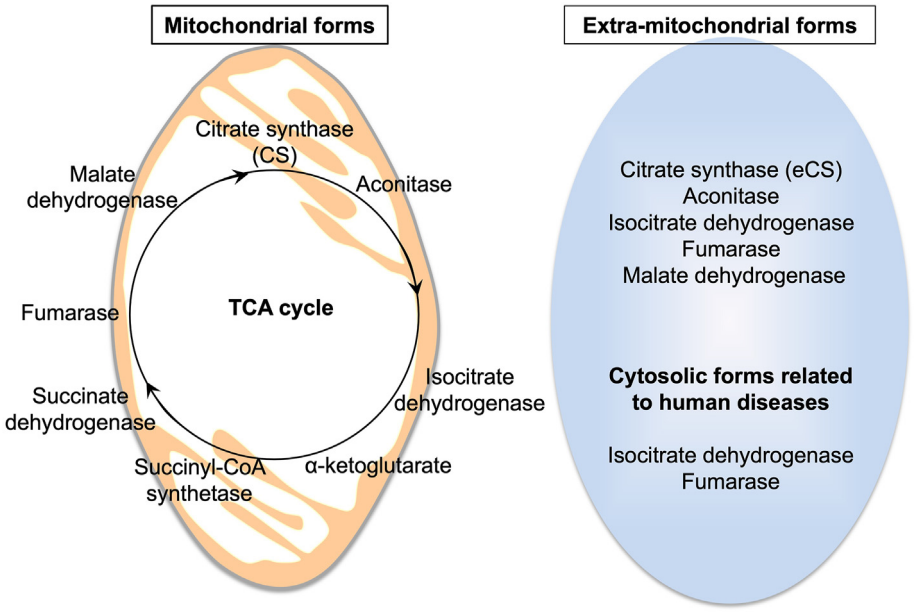

Fig. 2. TCA cycle-related enzymes and human diseases. This cycle occurs in the matrix of mitochondria and is catalyzed by eight enzymes. Interestingly, enzymes function in extramitochondrial forms, such as eCS, aconitase, isocitrate dehydrogenase, fumarase, and malate dehydrogenase. Particularly, isocitrate dehydrogenase and fumarase were reported as human disease-related enzymes. This figure was modified from Figure 4 in Ref. [65].

The Ca^{2+}-mediated enhancement of mitochondrial respiration is expected to be associated with exit from the stem cell state. Two enzymes of the TCA cycle, a-ketoglutarate dehydrogenase (KDH) and isocitrate dehydrogenase are increased by Ca^{2+} stimulation [48]. Nuclear KDH is also involved in histone succinylation [69]. Mitofusin 2 (MFN2) is one of two mitofusins that promote mitochondrial fusion. *Mfn2* transcripts are dominantly expressed in adult hematopoietic stem cells, which have highly fused mitochondria, and intracellular Ca^{2+} concentration is elevated In the absence of *Mfn2 mRNA* [48].

Mitochondria are central to cellular redox balance, producing ROS as natural byproducts of metabolism [67]. Proper regulation of ROS levels is critical in stem cells to maintain signaling pathways that regulate self-renewal, differentiation, and apoptosis. Understanding how mitochondrial enzymes regulate these metabolic transitions is crucial for optimizing stem cell culture conditions and enhancing cell differentiation efficiency.

6. Conclusion

Ca^{2+} oscillations play a critical role in various types of cells. TCA cycle-related enzymes are also essential for maintaining cell functions. The two mechanisms may be very closely related. Ca^{2+} oscillations have been thought to occur in limited types of cells. However, considering its relationship with the TCA cycle, it may be a universal event in almost all cells, including stem cells. Manipulating mitochondrial enzymes and metabolism holds promise for enhancing stem cell-based therapies and regenerative medicine. Strategies aimed at optimizing mitochondrial function, such as mitochondrial transplantation or pharmacological interventions targeting mitochondrial enzymes, are actively being explored to improve stem cell survival, integration, and therapeutic efficacy.

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Declaration of competing interest

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