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Brief Review

Developmental regulation of intracellular calcium homeostasis in early cardiac myocytes

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Abstract: The proper intracellular Ca^{2+} signaling is essential for normal cell functions and organ development, and the maintaining Ca^{2+} homeostasis in cardiac myocytes is of functional importance for the intact heart. As the first functional organ in the vertebrate embryo, the heart is continuously remodeled and maintains its physiologic pumping function in response to increasing circulatory demands. The expressions of Ca^{2+} handing proteins in the embryonic heart, however, are different from those in neonatal and adult hearts, which means that the regulation of Ca^{2+} transients in embryonic cardiomyocytes is different from that in adult cardiac myocytes. Recent advances in molecular and cellular biology, as well as the application of embryonic stem cell differentiation system, have made progress in uncovering the regulation of Ca^{2+} homeostasis during cardiomyogenesis. This paper briefly summarizes the Ca^{2+} homeostasis during early development of cardiomyocytes and reviews current knowledge of the regulatory mechanisms controlling Ca^{2+} homeostasis during cardiomyocyte development.

Key words: Ca^{2+} homeostasis; cardiomyocytes; development

心肌细胞发育过程中胞浆内钙稳态的调控

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摘　要：Ca^{2+} 信号是细胞和各器官生长发育、行使其生理功能的基础，维持心肌细胞的钙稳态是保持正常心脏功能的先决条件。作为在胚胎发育过程中最早出现并行使功能的器官，胚胎期心脏的形态结构发生了明显的变化，泵血功能不断增强，以适应不断增强的机体的生理需求。从胚胎到成年，心肌细胞的功能有非常大的改变，各钙离子通道的表达也发生明显变化。因此，发育早期心肌细胞的钙稳态调控与成熟心肌细胞有明显的不同，在发育过程中引起细胞收缩的 Ca^{2+} 来源也有明显的变化。随着分子和细胞生物学研究的发展，以及胚胎干细胞体外分化模型的应用，人们对心肌细胞发育过程中钙稳态的调控有了进一步的认识。本文综述了早期心肌细胞发育过程中胞浆内钙稳态的变化，总结了早期心肌细胞钙稳态调控机制的最新研究进展。

关键词：钙稳态；心肌细胞；分化发育
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Ca^{2+} ions are major players in an intracellular signaling system that translate extracellular stimuli into the regulation of a bewildering number of phenomena such as muscle contraction, neurotransmitter release and other secretion processes, cell proliferation, gene expression and cell death. Therefore, the proper intracellular Ca^{2+} signaling is essential for normal cell functions and organ development[1]. Ca^{2+} homeostasis in cardiac myocytes is of functional impor-

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tance for at least three reasons[2]. First, cardiac myocytes must achieve a resting cytosolic free calcium ion concentration ($[Ca^{2+}]_i$) of <200 nmol/L if the contractile elements are to relax. With extracellular Ca^{2+} concentration at 1 mmol/L, this low $[Ca^{2+}]_i$ must be maintained in the presence of a 5 000-fold gradient for Ca^{2+} across the sarcolemma. Second, contraction in cardiomyocytes arises by the Ca^{2+} induced Ca^{2+} release (CICR) mechanism, i.e. Ca^{2+} entering through sarcolemmal specific ion channels activates Ca^{2+} release channels (RyRs) in the sarcoplasmic reticulum (SR). This is the base of cardiac excitation-contraction (E-C) coupling. Upon the extrusion of cytosolic Ca^{2+} back to the SR and out of the sarcolemma, relaxation occurs. The fundamental principle of this process is to maintain a steady Ca^{2+} homeostasis, the amount of Ca^{2+} entering the cell with each contraction must be extruded before the subsequent contraction. Third, the contractile force of cardiac myocytes is modulated by variations in the magnitude and rise or decay of the Ca^{2+} transients. Therefore, any alteration of Ca^{2+} homeostasis would cause abnormal contractions and drugs that modify Ca^{2+} homeostasis may significantly alter the contractile force of an individual cardiomyocyte and thus of the intact heart.

Heart is the first organ that becomes functional in the vertebrate embryo. Approximately embryonic day (E) 7.25 in mouse, the precardiac mesoderm forms a primitive tubular heart, which starts beating at E8. Heart is continuously remodeled until the four-chambered organ is formed, and maintains its physiologic pumping function in response to increasing circulatory demands[3]. The ensuing development of E-C coupling is fundamental to the embryonic cardiac function during embryogenesis. The expression of Ca^{2+} handing proteins in embryonic heart, however, is different from that in the neonatal and adult heart[4,5], which means that the process of Ca^{2+} transients in embryonic cardiomyocytes may be different from that in adult cardiac myocytes. Because of the known difficulties to obtain cardiomyocytes from the very early mammalian embryos (e.g., before day 12 to 13 of gestation in mouse), there is only limited knowledge on the developmental aspects of cardiomyocyte functions. Recent studies have established the embryonic stem (ES) cell *in vitro* differentiation system as a tool for the investigation of early cardiomyogenesis[6-11]. The ES cell-derived cardiomyocytes (ESCMs) represent specialized cell types of the heart, such as atrial-like, ventricular-like, sinus nodal-like, and Purkinje-like cells[6]. During differentiation, the ultrastructural[7], molecular biological[9] and electrophysiological[6,12] studies have demonstrated that within ES cell-formed embryoid bodies (EBs), the various stages of cardiomyogenesis parallel the murine heart development. This *in vitro* model system is especially useful to identify gene function during cardiac development when "loss of function" studies result in early embryonic lethality[6,9,13]. Furthermore, the development of the new technology allows observing intracellular Ca^{2+} movement and, thus, provides the new knowledge on the regulation of early cardiac Ca^{2+} homeostasis. The present paper reviews the new interpretation on the developmental regulation of the Ca^{2+} homeostasis in early cardiomyocytes.

Developmental changes of Ca^{2+} homeostasis

During the heart remodeling process, regular heart beating is already observed in the very early period of heart development, which indicates that an intracellular system of contraction, as well as cardiac automaticity, establishes in the early period. With regard to the diastolic $[Ca^{2+}]_i$ of (102±1) nmol/L in 12-day fetal and (151±4) nmol/L in adult cardiomyocytes[14], it is reasonable that there is an increasing change in the rest $[Ca^{2+}]_i$ during the cardiac maturation. During the cardiac differentiation from ES cells *in vitro*, the basal $[Ca^{2+}]_i$ rises significantly from (94±7) nmol/L at early developed stage to (135±11) nmol/L at late developed stage[15]. Ca^{2+} sparks, which reflect the Ca^{2+}-dependent activation of RyRs, are rarely detected in the early fetal cardiac myocytes, and Ca^{2+} transients in the fetal myocytes are characterized by a slower upstroke and decay of the $[Ca^{2+}]_i$ compared to those in adult myocytes[14]. In early to late differentiated cardiomyocytes derived from ES cells, the more rapid and higher spontaneous Ca^{2+} transients are developed gradually[15,16].

Control of resting $[Ca^{2+}]_i$

In the adult cardiomyocytes, the resting $[Ca^{2+}]_i$ is determined by a Ca^{2+} leak that is compensated for by the ATP-dependent sarcolemmal Ca^{2+} pump (sarcolemmal Ca^{2+}-ATPase) and the sarcolemmal Na^+-Ca^{2+} exchanger (NCX). The cardiac NCX contains 970 amino acids and has a molecular mass of 108 kDa[17]. The NCX is reversible and carries out ion transport in a consecutive or "ping-pong" reaction mechanism. NCX transports 3 Na^+ inwardly and releases an intracellular Ca^{2+} ion to the extracellular space after completing a cycle. This would result in movement of one net charge inward per cycle and thus Ca^{2+} extrusion constitutes an inward current of NCX ($I_{Na/Ca}$). This electrogenicity also indicates that NCX is sensitive to membrane potential as well as intracellular and extracellular $[Ca^{2+}]$ and $[Na^+]$[18]. It is identical to the reversal potential of NCX (around -40 mV) in resting cardiomyocytes. If membrane potential depolarizes to more positive than the reversal potential, the NCX will function as the "reverse" (Ca^{2+} in/ Na^+ out) mode. The membrane potential in the normal resting myocytes is known to be more negative than the reversal potential, therefore, the NCX functions in a "forward" (Na^+ in/Ca^{2+} out) mode and extrudes Ca^{2+} from the cells.

The other Ca^{2+} transport system that contributes to the maintenance of low cytosolic Ca^{2+} concentration in ventricular myocytes is the sarcolemmal Ca^{2+} ATPase. The purified protein was cloned with a 108 kDa molecular mass, and a central stretch of ~80 kDa is all required for Ca^{2+} transport[19] that contains the regulatory calmodulin binding domain and has regulatory sites phosphorylated by PKA and PKC[20]. One Ca^{2+} ion seems to be transported per ATP hydrolyzed and Ca^{2+} extrusion by this pump appears to be coupled to proton influx ($1Ca^{2+}$:$1H^+$). Although the sarcolemmal Ca^{2+}-ATPase probably does contribute to the maintenance of $[Ca^{2+}]_i$ in resting myocytes, its importance seems to be minor in comparision with the NCX. In cultured guinea-pig adult ventricular myocytes, the rate at which the sarcolemmal Ca^{2+}-ATPase extrudes the Ca^{2+} from the myocytes appears to be about 1/10 that of the NCX over the range of physiological cytosolic Ca^{2+} concentrations[21].

It is still unclear whether the mechanisms to modulate the rest $[Ca^{2+}]_i$ in embryonic cardiomyocytes is different from that in adult cardiac myocytes. However, NCX is homogenously expressed in early embryonic and fetal heart, and the expression level is high in fetal heart but decreases postnatally[22]. The NCX current density is also declined rapidly after birth[23]. The highly expression of NCX in cardiomyocytes is thought to be required to maintain a low $[Ca^{2+}]_i$. NCX1-null mouse embryos lack a heartbeat and have abnormal myofibrillar organization, and die before E10.5[24-26]. Therefore, NCX is more important in developing hearts and may play a pronounced role in maintaining a low $[Ca^{2+}]_i$ during the development of cardiomyocytes. Recently, we observed that both ryanodine, a specific inhibitor of RyRs, and thapsigargin, a specific SR Ca^{2+}-pump ATPase inhibitor, significantly elevate resting $[Ca^{2+}]_i$ in cardiomyocytes derived from ES cells at early, intermittent and late differentiation stages, demonstrating that functional SR also takes part in maintaining basal $[Ca^{2+}]_i$[15]. With regard to the increasing rest $[Ca^{2+}]_i$, both SR and the sarcolemmal Ca^{2+} transport proteins may contribute to the maintenance of the resting $[Ca^{2+}]_i$ in early developing cardiomyocytes.

Regulation of Ca^{2+} influx in the E-C coupling

In adult cardiac myocytes, the increase of $[Ca^{2+}]_i$ from the 100 nmol/L at diastole to a peak of ~10 μmol/L at maximal contraction occurs because a small quantity of Ca^{2+} enters the sarcolemma and triggers a much larger release of Ca^{2+} from the SR Ca^{2+} release channel RyRs by the process of CICR, which leads to a dramatic increase of $[Ca^{2+}]_i$[2]. For the contraction of the hearts, a Ca^{2+}-influx from extracellular space is required because the removal of Ca^{2+} from extracellular solution abolishes cardiac contraction. This phenomenon is also observed in embryonic cardiomyocytes. Therefore, the developmental expressions of those sarcolemmal Ca^{2+} channels have a direct influence on the modulatory mechanisms of E-C coupling.

The L-type Ca^{2+} channel is an oligomeric complex of five subunits (α1, α2/δ, β and γ) and can be phosphorylated by cyclic AMP (cAMP)-dependent protein kinase (PKA)[27]. L-type Ca^{2+} channels are thought to be the main transporter for trans-sarcolemmal Ca^{2+} influx in adult cardiomyocytes[28]. In the early (3-day) stage of development, the L-type Ca^{2+} channels appear in embryonic chick heart cells, and the L-type Ca^{2+} current density in 3-day is higher than that in 17-day cells[29]. In mouse and rat hearts, the density of Ca^{2+} currents increases during fetal development and reaches its maximal level at about the time of birth[30,31]. In the 9.5-day postcoitum mouse heart[32] and in early-stage ESCMs[33], β-adrenergic receptor (β-AR) stimulation already modulates L-type Ca^{2+} channel currents. In brief, L-type Ca^{2+} channels are considered to be of great importance in E-C coupling in early embryonic cardiomyocytes.

Ca^{2+} currents in the cardiomyocytes generally consist of two components: a high threshold slow (L-type) Ca^{2+} current and a low threshold early transient (T-type) Ca^{2+} current. The T-type Ca^{2+} current is also a trigger for the Ca^{2+} release from the SR, although its function is much less compared to that of the L-type current[34,35]. The expression pattern of T-type Ca^{2+} current depends on species, tissues, and developmental stages. The T-type Ca^{2+} currents is relatively abundant in the conduction system, such as sinoatrial nodal cells and Purkinje cells, whereas it is negligible in some ventricular cells (e.g., rat)[28]. It has been documented that T-type Ca^{2+} currents present in early fetal and ES cell-derived early-stage cardiomyocytes[36,37] and that the mRNA expression of T-type channel increases significantly following a decline during the cardiomyocyte differentiation from ES cells[37]. T-type Ca^{2+} channels also present in embryonic human cardiomyocytes and the mRNA expression decreases with development[38]. In chick-ventricular development, a Ca^{2+} entry via the T-type channels plays a significant role in E-C coupling in the developing heart through the stimulation of CICR[39]. Pharmacological approaches also suggest that the Ca^{2+} entered via the T-type Ca^{2+} channels contributes to excitability in midgestational fetal mouse myocardium[36].

In developing cardiomyocytes, the Ca^{2+} influx can also be mediated via NCX "reverse" mode (Ca^{2+} in/Na^+ out). In theory, when the membrane depolarizes to the reversal potential of the NCX, a Ca^{2+} influx should occur as the exchange reverses. This process may be further stimulated by subsarcolemmal rises in sodium concentration caused by the sodium influx via the cardiac sodium channels[40]. The reverse NCX Ca^{2+} current can trigger SR Ca^{2+} release in adult rabbit ventricular myocytes[41]. The expressions of NCX mRNA and protein are maximal near the time of birth and decline postnatally in chick, rat and rabbit hearts[22,42,43]. Similarly, the density of the outward NCX current (the reverse mode) is highest at birth and decreases postnatally[23,44]. The high expression of NCX in developing cardiomyocytes is thought to be required to maintain a low intracellular Ca^{2+} concentration, and may play a role in E-C coupling[26,28]. Furthermore, such a Ca^{2+} influx appears to be sufficient to produce contraction in neonate cells, but not in adult cells[44]. It is quite likely that the Ca^{2+} efflux mediated by NCX1 might compensate for the low Ca^{2+} uptake by the SR in immature cells and its significance in the regulation of the contraction and relaxation becomes less upon the maturation of the SR.

SR regulation and CICR

The RyR, as the major Ca^{2+} release channel in the SR, is a very large protein (565 kDa) and it exists as a homotetramer (2.2 MDa). The large size of this homotetramer has helped to identify it ultrastructurally as the junctional "foot" process, and the majority of the RyR molecular mass is located between the SR and sarcolemmal membranes where it forms a 28 nm×28 nm structure that extends ~12 nm above the SR membrane. This is the region where numerous ligands interact with RyRs, such as calmodulin, Ca^{2+}, FK-506-binding proteins, PKA, phosphatases 1 and 2A, imperatoxin A[45]. There are three RyR isoforms in mammals, and the main isoform in cardiac cells is RyR2[46]. The contraction of the adult myocardium is highly dependent on SR function and less dependent on trans-sarcolemmal Ca^{2+} influx when compared with the neonatal myocardium[47]. Some studies have shown that in fetal heart cells, the SR is scarce when observed with electronmi croscope[48], and isolated SR vesicles from the fetal heart have lower volume, lower density, and a decreased capability to load Ca^{2+} compared to those isolated from the mature heart[49-51]. The expression levels of RyR2 and cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2) mRNAs and proteins in fetal cardiomyocytes are obviously lower than those in adult cells[4]. Ryanodine, a specific inhibitor of RyR, has little to no effect on Ca^{2+} transients in fetal cells[4,48,52]; and RyR2 has been postulated to act as a Ca^{2+} leak channel and not as a functional Ca^{2+} release channel in embryonic cardiomyocytes. Therefore, it is proposed that the contraction of fetal cardiomyocyte is regulated predominantly by sarcolemmal Ca^{2+} influx rather than Ca^{2+} release from SR.

However, this view of the embryonic cardiomyocytes without functional SR has been challenged by recent reports. The recent observations demonstrate that SR RyR2[9,53] and SERCA2a[54,55] mRNAs/proteins are already abundantly expressed from the earliest stages of beating myocardium and functional SR Ca^{2+} stores are present in E13 rat ventricle. Early stage ESCMs continuously contract by the spontaneous intracellular $[Ca^{2+}]_i$ oscillations, which are from RyR sensitive Ca^{2+} stores[56]. Moreover, the amplitude of Ca^{2+} transients in fetal cardiomyocytes is inhibited by both ryanodine and thapsigargin, a specific SERCA2 inhibitor[14,15]. Takeshima *et al*.[57-59] demonstrated that RyR2 deficient and the junction protein (JP-2) null cardiomyocytes have slow, weak, and irregular Ca^{2+} transients with early embryo lethality, indicating that RyR2 released Ca^{2+} is critical for Ca^{2+} homeostasis in early embryonic cardiomyocytes. The SR is functional in the embryonic chick heart before hatching, although most of the Ca^{2+} associated with the transients comes through the sarcolemmal Ca^{2+} channels[60]. To specifically test whether SR Ca^{2+} release directly contributes to or regulates Ca^{2+} homeostasis and contraction in early developing cardiomyocytes, we have compared the dynamics of $[Ca^{2+}]_i$ transients and its relationship to cardiomyocyte contraction in cells differentiated from wild-type ($RyR2^{+/+}$) and RyR2 deficient ($RyR2^{-/-}$) ES cells both with time of *in vitro* differentiation and following pharmacological intervention. With time of $RyR2^{+/+}$ ESCM differentiation, SR function develops progressively with characteristics of an enhanced frequency and amplitude and a progressive decrease in the duration of Ca^{2+} transients that could be inhibited by ryanodine and thapsigargin. $RyR2^{-/-}$ ESCMs, comparatively, demonstrate a significantly prolonged time-to-peak and reduced frequency of Ca^{2+} transients and contractions. β-adrenergic stimulation of $RyR2^{+/+}$ ESCMs increases the frequency and amplitude of Ca^{2+} transients in a differentiation-dependent manner, but β-adrenergic responses are much weaker in RyR2$^{-/-}$ ESCMs. We also demonstrate that SR load and RyR2 are essential for upstroke and frequency of Ca^{2+} transients, and consequently contraction and beating rate even at an early developmental stage and that RyR2 and SR are important for β-AR stimulation in these cells[13,15]. These results firmly establish that SR and RyR2 are not only essential for determining the beating rate and the rate of diastolic depolarization[13], but also for regulating the contraction through more rapid and robust Ca^{2+} transients in early cardiac development[15]. Moreover, the SR function in regulation of Ca^{2+} homeostasis increases with cardiomyocyte development[13,15]. With the SR structure and function maturation, the $[Ca^{2+}]_i$ fluctuations in cardiac precursor cells at very early developmental stage develops gradually into the Ca^{2+} sparks and the global $[Ca^{2+}]_i$ transients with a marked increase in the frequency and the amplitude in late maturation cardiomyocytes[16].

In adult cardiomyocytes, Ca^{2+} influx through L-type Ca^{2+} channels activates RyR2 and triggers SR Ca^{2+} release at T-tubule-SR junctions. This CICR process is the predominant mechanism of E-C coupling and it is structurally required that the sarcolemmal Ca^{2+} channels and SR Ca^{2+} release channels are located in close proximity. The T-tubule network is absent in the early fetal myocytes[14], which is one of the reasons that have led to the assumption that CICR is not critical to E-C coupling in embryonic cardiomyocytes. However, Lohn *et al*.[61] reported a distance of 20~50 nm between the abundant caveolae and SR, and this close proximity could be responsible for the localized Ca^{2+} sparks observed in neonatal myocytes. Two types of junctional membrane complexes between the cell-surface membrane and the SR with gap size of ~12 and ~30 nm in cardiomyocytes were observed from E9.5 mouse embryos[58]. Because of the ~12 nm junctional gap in mature mouse myocytes, fetal cardiomyocytes might also possess functional peripheral couplings as do in adult cells. This is supported by that JP-2 is expressed at time coincident with the expression pattern of RyR2 in RyR2$^{+/+}$ ESCMs but decreased in RyR2$^{-/-}$ cells[15], and that JP-2 null E9.5 cardiomyocytes have slow, weak, and irregular Ca^{2+} transients[58]. In addition, the recent study in cat atrial myocytes also demonstrate that subsarcolemmal junctional SR sites are activated in a stochastic fashion by the opening of voltage-dependent sarcolemmal Ca^{2+} channels, and subsequently, central nonjunctional SR sites are activated by Ca^{2+}-induced Ca^{2+} release propagating from the periphery[62]. During the development of cardiac myocytes, the gradual development of the T-tubule network, the maturation of the SR system, and the spatial colocalization of L-type Ca^{2+} channel and RyR2 lead to more and more Ca^{2+} sparks triggered simultaneously. Therefore, the SR is becoming more important in the regulation of $[Ca^{2+}]_i$ homeostasis during the cardiomyocyte development[14].

Decay of $[Ca^{2+}]_i$ during the relaxation

The amount of Ca^{2+} which enters the cytosol of myocytes at each steady state twitch must also be extruded from the cytosol; otherwise the $[Ca^{2+}]_i$ homeostasis will alter. The decay of the Ca^{2+} transients occurs in adult cardiomyocytes because of the reuptake of Ca^{2+} into the SR through the Ca^{2+}-pump ATPase and the extrusion of Ca^{2+} from the myocyte by the NCX[28], as discussed above.

The cardiac SERCA2, a 100~115 kDa protein, is concentrated in the longitudinal component of the SR[63]. The SERCA transports two Ca^{2+} ions for each ATP molecule consumed in cardiac SR. Phospholamban (PLB) is an endogenous inhibitor of SERCA in cardiac myocytes. While the level of SERCA2a mRNA remains unchanged, its protein level increases with human heart development[38]. It has also been proven that the abundance of SERCA2 gene products is regulated primarily though post-transcriptional mechanisms during the rat perinatal period[64].

A Ca^{2+} extrusion from the cells via the NCX1 also occurs during the decline phase of the Ca^{2+} transients. The two fundamental principles to maintain steady-state contraction of cardiac physiological function are[2], (i) a balance exists between the amount of Ca^{2+} entering the cell via the Ca^{2+} currents and the amount of Ca^{2+} extruded by the NCX; and (ii) the amount of Ca^{2+} released from the SR equals that sequestered by the SR Ca^{2+}-pump ATPase. If an abrupt change in this balance of fluxes takes place, the Ca^{2+} content of the SR will be affected. There is a dynamic competition between NCX1 and SERCA2 during relaxation, and both of them contribute to a variable amount toward $[Ca^{2+}]_i$ decline depending on species, developmental stages and physiologic conditions. The expression of SERCA2 and PLB increase gradually during the cardiac development, and the NCX expression level in early developed cells is twice as in adult myocytes[4,5,22,43]. These complementary temporal gradients in SERCA and NCX expression suggest a role for NCX activity to compensate for less SERCA expression in the embryonic/early postnatal state of development. Pharmacological researches also demonstrated that in rat cardiomyocytes the contributions of the SERCA2 uptake to the twitch relaxation associated with $[Ca^{2+}]_i$ decline increase from ~75% to 92%, and the contributions of NCX decrease from ~24% to 5% from birth to adulthood[65].

The role of inositol-1,4,5-trisphosphate receptor (IP_3R) in Ca^{2+} homeostasis

Except RyRs, there is an inositol-1,4,5-trisphosphate (IP_3) sensitive Ca^{2+} release channel on the SR membrane. Three IP_3R subtypes are expressed in a wide variety of tissues, and only the type-2 IP_3R is expressed in isolated ventricular myocytes[66]. The IP_3R released Ca^{2+} plays an important regulatory role in the cellular proliferation and apoptosis, whereas RyR-released Ca^{2+} is required for muscle contraction[66]. The IP_3R mRNA expression and the IP_3-induced intracellular Ca^{2+} release are detected as early as 5.5-day postcoitum in the mouse embryo, which is earlier than the expression of RyR2 mRNA[67]. In adult guinea pig ventricular myocytes, the low concentrations of IP_3 (1~10 μmol/L) transiently increase isotonic contractions which accord with the receptor-initiated SR Ca^{2+} release[68]. The complementary temporal changes in RyR2 and IP_3R function in the regulation of Ca^{2+} transients indicate that IP_3R has a critical role in the regulation of Ca^{2+} homeostasis in early developed cardiomyocytes. A recent study showed that IP_3-dependent shuttle of free Ca^{2+} in and out of the SR is essential for a proper generation of pacemaker activity during early cardiomyogenesis and fetal life[69]. Therefore, the possible contribution of IP_3R to Ca^{2+} homeostasis in early developed cardiomyocytes should be considered and explored further.

Summary

Dynamic changes in the regulation of Ca^{2+} homeostasis occur during the development of the heart, and the source of Ca^{2+} for producing contraction is especially altered during development (Fig.1).

In embryonic developing cardiac cells, most Ca^{2+} required for contraction is derived from the Ca^{2+} influx through the voltage-dependent sarcolemmal Ca^{2+} channels (L-type and T-type), and RyR2-mediated SR Ca^{2+} release is critical and irreplaceable in Ca^{2+} homeostasis. Ca^{2+} entering through the sarcolemmal Ca^{2+} channels and then being released from Ca^{2+}-release channels in peripheral SR may diffuse into the intracellular space, which in turn stimulates Ca^{2+}-release channels in the deep space. However, as the absence of the T-tubule and the immature SR Ca^{2+} store, the whole linkage of CICR produces a slow kinetics of Ca^{2+} transients in the fetal myocytes.

In neonatal developing cardiac cells, the relative mature SR plays a role as the main source of Ca^{2+} required for contraction compared to that in the fetus. Especially, the

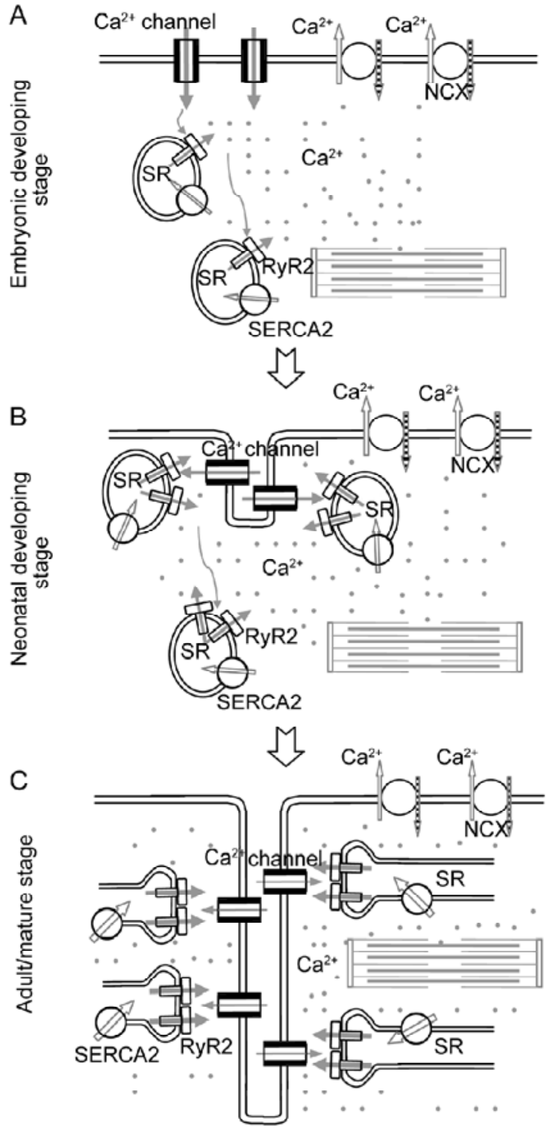

Fig. 1. Schematic model of regulating Ca^{2+} homeostasis during cardiomyocyte development. *A*: In the embryonic developing stage, Ca^{2+} enters into cytoplasm through the L-type/T-type Ca^{2+} channel, which activates RyR2 in immature SR by CICR. Meanwhile, the contraction is also triggered by the increased $[Ca^{2+}]_i$ from the trans-sarcolemmal Ca^{2+} influx and RyR2. During relaxation, Ca^{2+} decline depends on both the sarcolemmal NCX and SERCA2 in the SR. The RyR2-mediated SR Ca^{2+} release is critical and irreplaceable in Ca^{2+} homeostasis and in maintaining physiological beating rate and contraction. *B*: In the neonatal developing stage, the formation of the T-tubular system starts and the SR develops. L-type Ca^{2+} channel receptors are close to RyRs and can evoke the Ca^{2+} sparks in the periphery of cardiomyocytes. *C*: In the adult/mature stage, concomitant with the formation of the T-tubular system and the maturation of the SR, each sarcolemmal Ca^{2+} channel is close to RyR2 and evokes large Ca^{2+} released from SR, and the decay of Ca^{2+} transients mainly depends on SERCA2. Therefore, the SR plays more important roles in the regulation of the Ca^{2+} homeostasis, producing fast and large Ca^{2+} transient in the adult cardiomyocytes via CICR.

SR has been well organized and the T-tubules start to develop in the neonatal period. These morphological changes produce a short distance between the sarcolemmal Ca^{2+} channels and SR Ca^{2+} release channel, and Ca^{2+} influx through the sarcolemmal can activate Ca^{2+} release from the SR by CICR. Thus the kinetics of Ca^{2+} transient becomes faster.

During the process of the cardiac maturation, the SR develops gradually into a major mechanism to regulate cardiac Ca^{2+} homeostasis, which becomes the physiologic basis for the cardiac E-C coupling, such as, the proteins expression of RyR2 and SERCA2 and their functions increase, the T-tubule network develops, the junctophilin-2 links the SR membrane with the T-tubule wall membrane, and the Ca^{2+}-binding protein calsequestrin expresses in the SR lumen.

As discussed above, the source of Ca^{2+} for producing contraction is altered during cardiac development, and the function of SR is critical for cardiac Ca^{2+} homeostasis. Hence, functional abnormalities or gene mutation of RyR2[57] and SERCA2[70] during development may be involved in the genesis of cardiac arrhythmias and sudden deaths. Furthermore, the failing heart has an altered program of gene expression that with embryonic characteristics[71,72], and the expression of RyRs and SR Ca^{2+}-ATPase are decreased[73,74] with defective RyR2 channel function[72]. Moreover, transplantation of exogenous cells into injured myocardium, such as fetal cardiomyocytes[75], bone marrow cells[76] and ESCMs[77], has emerged for the regeneration of damaged myocardium and for the improvement of cardiac function in post infarcted hearts in recent years. Therefore, it is significant to further investigate the detailed knowledge about the establishment of the E-C coupling and the altered regulation of Ca^{2+} homeostasis during development, which is also important for a better understanding of the normal development aspects and the abnormalities in cardiac diseases.

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