Excitation-contraction coupling in fish cardiomyocytes

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This is an update of the equivalent articles from the previous edition of Encyclopedia of Fish Physiology (First Edition), Edited by Anthony P. Farrell, Academic Press, 2011 are: H.A. Shiels, DESIGN AND PHYSIOLOGY OF THE HEART | Cardiac Excitation–Contraction Coupling: Routes of Cellular Calcium Flux, https://doi.org/10.1016/B978-0-12-374553-8.00175-1. T.E. Gillis, DESIGN AND PHYSIOLOGY OF THE HEART | Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element, https://doi.org/10.1016/B978-0-12-374553-8.00176-3. H.A. Shiels, DESIGN AND PHYSIOLOGY OF THE HEART | Cardiac Cellular Length–Tension Relationship, https://doi.org/10.1016/B978-0-12-374553-8.00177-5.

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Key points

- Contraction of a cardiac myocyte is initiated when an action potential electrically excites the sarcolemmal membrane leading to the activation of L-type Ca^{2+} channels and the Na^{+}–Ca^{2+} exchanger in the sarcolemmal membrane; and the ryanodine receptor in the sarcoplasmic reticulum membrane.
- The resultant increase in intracellular Ca^{2+} activates the myofilament via troponin C, a Ca^{2+} binding protein.
- The binding of Ca^{2+} to troponin C activates myocyte contraction by initiating a series of conformational changes through the components of the thin filament leading to increased interaction between actin and myosin and the formation of force generating cross-bridges.
- Myocyte relaxation occurs when Ca^{2+} is transported back into the sarcoplasmic reticulum via sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) and out of the cell across the sarcolemmal membrane via the Na^{+}–Ca^{2+} exchanger (NCX).
- The above series of actions leading to contraction of the myocyte is called excitation-contraction coupling

Glossary

ATPase is a protein whose activity is fueled by the hydrolysis of ATP. Ca^{2+}-pumps are examples of ATPases.

Buffers are proteins that bind other molecules. They can bind a given molecule strongly (high affinity) or bind a large number of a given molecule (high capacity). The affinity and capacity are determined by the type of buffer and by physical variables such as temperature and pH.

Ca^{2+} transient is the term used to describe the transient rise and fall of cytosolic Ca^{2+} with each contraction-relaxation cycle.

Ca^{2+}-Pump (like SERCA) is a protein that uses the energy of ATP to pump Ca^{2+} across a membrane, usually against a concentration gradient. These are also called Ca^{2+}-ATPases.

Cardiac troponin complex is a group of three proteins, cardiac troponin I (*c*TnI), cardiac troponin C (*c*TnC), and cardiac troponin T (*c*TnT), that is attached to the actin thin filament and that triggers the formation of cross-bridges during contraction.

Contractile element is the components of the sarcomere that are responsible for the contractile reaction—the series of biochemical and biophysical reactions that result in the formation of cross-bridges and the generation of force by the cardiomyocyte.

Contractility is a measure of the power and/or the capacity of myofilament contraction.

Cross-bridge A physical connection that forms between the myosin thick filament and the actin thin filament. The formation of cross-bridges causes the sarcomere to shorten during a contraction.
Cytosol is the fluid part of the intracellular space which exists between the cell surface membrane and the membrane-bound organelles.
Depolarization is the reduction in electrical potential difference across a cell membrane. This means the inside of the cell becomes less negative with respect to the outside of the cell. Depolarization occurs during the upsweep of the action potential (opposite of repolarization).
Excitability is the capability of a living cell to respond to stimuli and is often related to membrane voltage.
Excitation-Contraction Coupling (e-c coupling) is the term used to describe the progression from membrane excitation with an action potential to the rise of the Ca^{2+} transient to cell contraction.
Myofilaments are the contractile apparatus of the myocyte.
Muscle contraction is when a muscle shortens and generates force.
SERCA stands for sarco(endo)plasmic reticulum Ca^{2+}-ATPase. This Ca^{2+}-pump is responsible for pumping Ca^{2+} out of the cytosol and into the sarco(endo)plasmic reticulum.
Repolarization is the increase in electrical potential difference across a cell membrane. This means the inside of the cell becomes more negative with respect to the outside of the cell. Repolarization occurs during the final phase of the action potential to return excitability to the resting membrane potential (opposite of depolarization).
Sarcomeres are the functional elements of muscle cells responsible for shortening during a contraction.
Steady-state is achieved when Ca^{2+} flux routes are balanced so that the same amount of Ca^{2+} enters the cytosol during contraction and leaves the cytosol during relaxation from beat to beat.
Surface Area to Volume ratio is a mathematical expression. In this context, it is the amount of cell membrane (area) divided by the amount of intracellular space (cytosolic volume). Fish cardiac myocytes are spindle shaped, and therefore have a high surface area to volume ratio relative to mammalian cardiac myocytes which are more brick shaped.
Thick filament is the structural element of a sarcomere that is composed of the protein myosin.
Thin filament is the structural element of a sarcomere that is composed of the protein actin.
Troponin complex is a protein complex, associated with the thin filament, that when activated by Ca^{2+} triggers the contractile reaction.
Working myocardium refers to the regions of the heart that contract and propel the blood when excited via excitation-contraction coupling. These regions are made up of the contractile myocytes of the atrium and ventricle.

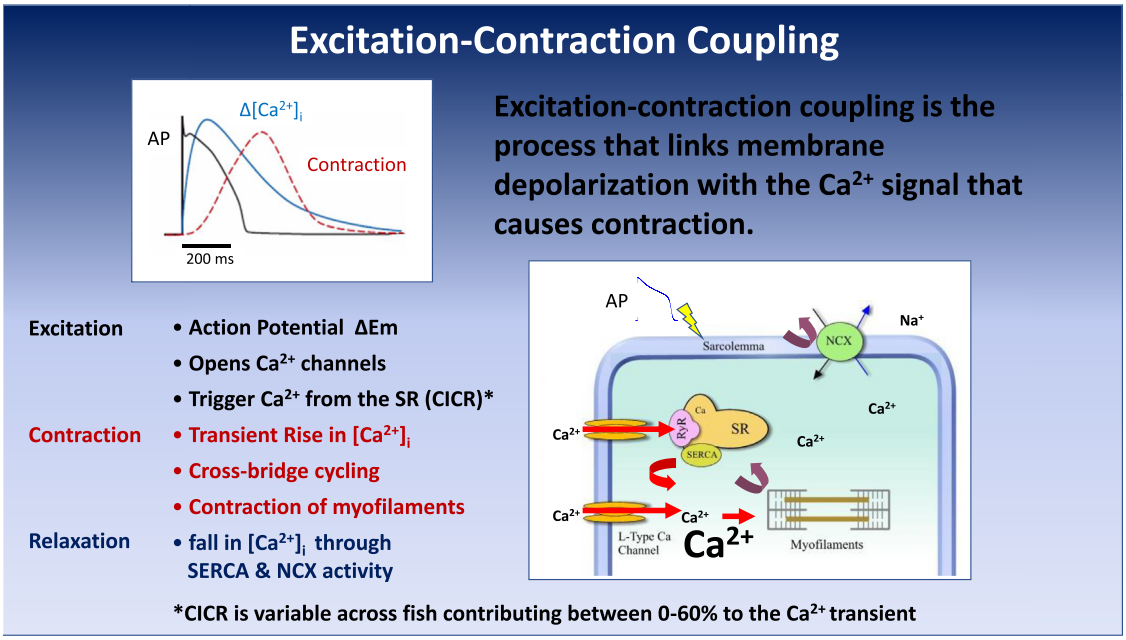
List of abbreviations

AP action potential
Ca^{2+} calcium
CICR Ca^{2+}-induce Ca^{2+}-release
e-c - excitation-contraction
LTCC L-type Ca^{2+} channel
Na^{+} sodium
NCX Na^{+}—Ca^{2+} exchanger
SERCA sarco(endo)plasmic reticulum Ca^{2+} ATPase
SR sarcoplasmic reticulum
cTn cardiac troponin
cTnC cardiac troponin C
cTnI cardiac troponin I
cTnT cardiac troponin T
TM tropomyosin
SR sarcoplasmic reticulum
$KF1/2Ca^{2+}$ Ca^{2+} concentration required to generate half maximal force
$K1/2Ca^{2+}$ Ca^{2+} concentration required to half saturate the protein

Abstract

Excitation of the fish cardiac myocyte with an action potential causes intracellular Ca^{2+} levels to rise, which initiates contraction. This sequence of events is termed "excitation-contraction coupling". Cardiomyocytes contract when intracellular Ca^{2+} concentrations rise and Ca^{2+} binds to the contractile element, the cellular machinery responsible for powering the contraction. This binding allows the formation of cross-bridges between the actin thin filament and the myosin thick filament under the regulation of the troponin complex (troponin I, troponin T, and troponin C) and tropomyosin. To end contraction and begin relaxation, Ca^{2+} levels inside the myocyte must fall. The Ca^{2+} transient is the rise and fall of Ca^{2+} with each contraction-relaxation cycle. In fish, the Ca^{2+} fluxes which generate the Ca^{2+} transient can be of both extra- or intracellular origin. The rate and magnitude of the Ca^{2+} transient determines the rate and strength of heart contraction. The contraction-relaxation cycle is the cellular correlate of the heartbeat.

Teaching slide



Excitation Contraction coupling is the process that links myocyte excitation with myocyte contraction in vertebrate striated muscle. It begins with an action potential (AP) that in cardiac muscle initiates in the pacemaker of the heart. The AP depolarises the myocyte membrane causing L-type Ca^{2+} channels to open and for Ca^{2+} to entre the myocyte down its concentration gradient. This extracellular Ca^{2+} directly contributes to the rising phase of the intracellular Ca^{2+}transient. Ca^{2+} influx through the L-Type Ca^{2+}channel can also trigger the release of the Ca^{2+} stored in the sarcoplasmic reticulum (SR) though a process called Ca^{2+} -induced Ca^{2+} release (CICR). The efficacy of CICR various across vertebrates and is higher in mammals than fish. Within fishes, the general trend is for active fish to have greater CICR and thus a greater amount of intracellular Ca^{2+} contributing to a larger and faster Ca^{2+} transient. However, there are many exceptions. The amplitude of the Ca^{2+} transient determines the number of active cross-bridges which determines the force of contraction. The rate at which Ca^{2+} is cycled to and from he cross-bridges determines the rate of myocyte contraction and relaxation. Intracellular Ca^{2+}levels must fall by the combined action of pumping Ca^{2+} back into the SR through the sarcoendoplasmic reticulum Ca^{2+} ATPase pump (SERCA) and across the myocyte membrane via the sodium (Na^{+})- calcium (Ca^{2+}) exchanger (NCX). The process of excitation-contraction-relaxation is the cellular correlate of the heartbeat.

Introduction

The previous article discussed the cardiac action potential (AP) and the ion channels that open and close to excite the fish cardiac myocyte. This article focuses on the process of excitation-contraction (e-c) coupling. This is the process that links excitation of the cell membrane following an AP with a rise in intracellular Ca^{2+} and the contraction of the cell. The article will start by explaining the "Ca^{2+} transient" and the sources and routes through which Ca^{2+} levels rise and fall inside the myocyte with every heartbeat. It will then describe the series of events termed "cross-bridge cycling" that lead to contraction of the myofilaments initiated by the rise in Ca^{2+}. The article will end by considering how myofilament relaxation occurs to allow the excitation-contraction-relaxation cycle to continue into the next heartbeat.

The excitation-contraction-relaxation cycle is the cellular correlate of the heartbeat. Video 1 shows the contraction and relaxation cycle of a trout ventricular myocyte. Routes of cellular Ca^{2+} flux associated with the contraction and relaxation cycle are very important for understanding heart contractility because the rate of (how fast) and magnitude (how much) $-Ca^{2+}$ cycles inside of the myocyte directly determines the rate and strength of myofilament activation and thus myocyte contraction. The contraction and relaxation of the myocytes that compose the fish heart, determine how strongly and how quickly the fish heart can beat. There are different types of myocytes that make up the fish heart and each are specialized for different functions. For example, some cells do not contract very much but are very important in electrical conduction (i.e., pacemaker cells). The process of e-c coupling usually refers to the cardiac myocytes that are powering cardiac contraction. These are the myocytes of the atrium and the ventricle. Thus, for this article, the term cardiac myocyte or cardiomyocyte refers to an atrial or ventricular myocyte that is part of the "working myocardium."

Excitation-contraction coupling

The progression from membrane excitation with an AP to the rise of intracellular Ca^{2+} to cell contraction is termed excitation-contraction (e-c) coupling. The time course of e-c coupling can be seen for a mammalian myocyte in **Fig. 1** (Bers, 2002). Notice the central blue line. This is the Ca^{2+} transient and it is the link between cardiac excitation (black line showing the AP) and cardiac contraction (dotted red line). Each of these processes proceed in sequence within each myocyte during each heart heartbeat. **Fig. 2**A is an image of a bluefin tuna (*Thunnus orientalis*) ventricular myocyte. The myocyte membrane delineates the cell and is the site of membrane depolarization and Ca^{2+} entry into the cell. The contractile elements are visible as the striations throughout the myocyte which interact with Ca^{2+} and cause contraction.

The Ca^{2+} transient

The term Ca^{2+} transient is often abbreviated $\Delta[Ca^{2+}]_i$, where Δ is a change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during a single contraction and relaxation cycle. The mammalian Ca^{2+} transient shown in **Fig. 1** rises quickly (~100 ms) and then decays more slowly (~400 ms), thus causing a transient change in intracellular Ca^{2+} concentration (Bers, 2002). The Ca^{2+} transient in fish cardiac myocytes has the same components and a similar shape but is slower. When thinking about routes of cellular Ca^{2+} flux during e-c- coupling it is perhaps best to have a schematic model of a cardiac myocyte in your head through which you can track Ca^{2+} movement. This is provided in the schematic model of a fish cardiac myocyte in **Fig. 2**B. The key components involved in cellular Ca^{2+} flux are labeled and briefly described in the legend, but will be discussed in detail later. The actual morphology and fine structural details to which this schematic corresponds are covered in a separate article.

Fig. 2B shows the sarcolemma (SL) which is the outer membrane of the myocyte and the interface between the extracellular and intracellular space (cytosol). Embedded in the SL are ion channels that allow ions to pass from outside to inside or inside to outside. The primary SL membrane proteins involved in the Ca^{2+} transient are the L-type Ca^{2+} channels (LTCC) and the Na^{+}-Ca^{2+} exchanger (NCX). The key intracellular structure involved in generating the Ca^{2+} transient is the sarcoplasmic reticulum (SR). This organelle acts as a site for Ca^{2+} storage, Ca^{2+} uptake, and Ca^{2+} release. Ca^{2+} release from the SR occurs primarily through proteins called ryanodine receptors (RyR). Ca^{2+} uptake into the SR occurs via a Ca^{2+}-pump called SERCA. There are a number of other intracellular organelles like the mitochondria and the nucleus that can accumulate Ca^{2+}, but this Ca^{2+} is not directly involved in e-c coupling so it is not dealt with in this article. The myofilaments are also illustrated in **Fig. 2**B as well as being evident from the striations in **Fig. 2**A. Ca^{2+} must bind to the myofilaments for contraction to occur and be removed from the myofilaments for relaxation to proceed, as discussed in detail later.

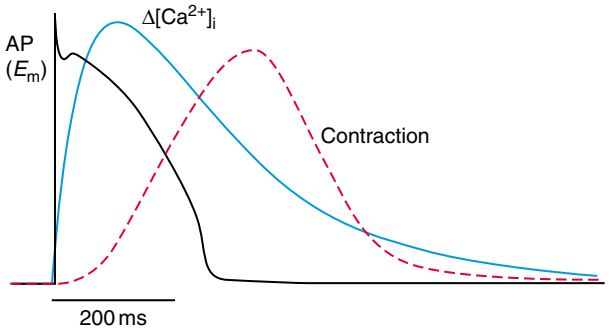


Fig. 1 The 3 key players in the process of excitation-contraction coupling. The figure shows the time course of an action potential (black line), Ca^{2+} transient (blue line) and myocyte contraction (red dashed line). Data are from a rabbit ventricular myocyte at 37 °C. Adapted with permission from Fig. 1 inset in Bers (2002).

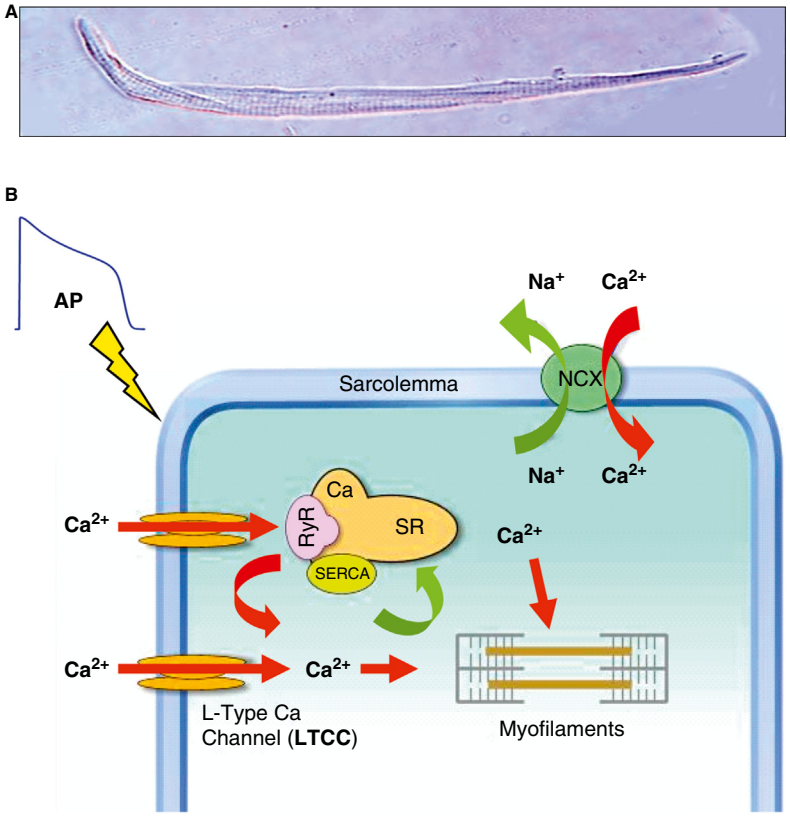


Fig. 2 (A) A light micrograph image of a living Pacific bluefin tuna (*Thunnus orientalis*) ventricular myocyte. Notice the striations formed by sarcomeres. The myocyte is 10 μm in diameter at its widest point. (B) Schema for excitation-contraction coupling in the fish cardiac myocyte. The figure shows a portion of a fish cardiac myocyte. The sarcolemma (SL) is the cell membrane separating the intra- and extracellular space. The figure shows the SL being excited by an action potential (AP) which opens L-type Ca^{2+} channels (LTCC) in the cell membrane allowing Ca^{2+} influx (red arrows) down its concentration gradient into the cell. Ca^{2+} can also enter the cell via reverse-mode Na^{+}—Ca^{2+} exchange (NCX). Ca^{2+} influx can trigger Ca^{2+} release from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). Together these Ca^{2+} influxes cause a transient rise in Ca^{2+} that initiates contraction at the myofilaments. Relaxation occurs when Ca^{2+} is removed from the cytosol (green arrows) either back across the SL via forward-mode NCX or back into the SR via the SR Ca^{2+}-pump (SERCA). (A) The image is adapted from Shiels et al. (2004).

The rising phase of the Ca^{2+} transient

The rising phase of the Ca^{2+} transient in Fig. 1 is due to Ca^{2+} influx into the cytosol. This Ca^{2+} can be of extracellular origin (coming in across the SL) or of intracellular origin (released from the SR). In fish in general, the extracellular Ca^{2+} route is by far the most important. However, in certain species like trout and tuna, and under certain conditions like stress or exercise, Ca^{2+} release from the SR can also play a role (Shiels and Galli, 2014).

Extracellular Ca^{2+} influx—The L-type Ca^{2+} channel

The main Ca^{2+} influx pathway across the SL is the L-type Ca^{2+} channel (LTCC). This is a voltage gated ion channel that opens during depolarization (e.g., during an AP) allowing Ca^{2+} to flow into the myocyte. There is a large influx of Ca^{2+} into the myocyte when these channels open because the concentration of extracellular Ca^{2+} (mM) is more than 100-fold greater than the concentration of intracellular Ca^{2+} (nM) when the cell is relaxed. This large driving force, the high number of LTCC in the SL membrane, and the large surface area to volume ratio of the myocyte are responsible for the rapid rising phase of the Ca^{2+} transient (Vornanen et al., 2002). This rapid rising phase is clearly seen as the steep ascending limb of the Ca^{2+} transients from a bluefin tuna (*Thunnus orientalis*) cardiac myocyte (**Fig. 3**) (Shiels et al., 2011). One can measure the amount of Ca^{2+} entering the cell via LTCCs during an AP in fish myocytes using electrophysiological techniques. Studies show that between 20 and 80 $\mu molL^{-1}$ Ca^{2+} can enter the fish cardiac myocyte (range depending on fish species and method of investigation) through LTCCs (Vornanen, 1997). This influx generates a Ca^{2+} transient that is sufficient on its own to initiate contraction of the myocyte.

It is important to notice that the rising phase of the Ca^{2+} transient, though rapid, is quite short. This is because LTCC's stop conducting Ca^{2+} soon after opening. This inactivation depends on 2 features: the repolarization of the membrane during the AP (voltage-dependent inactivation), and the fact that Ca^{2+} itself can stop the channel from conducting more Ca^{2+} (Ca^{2+}-dependent

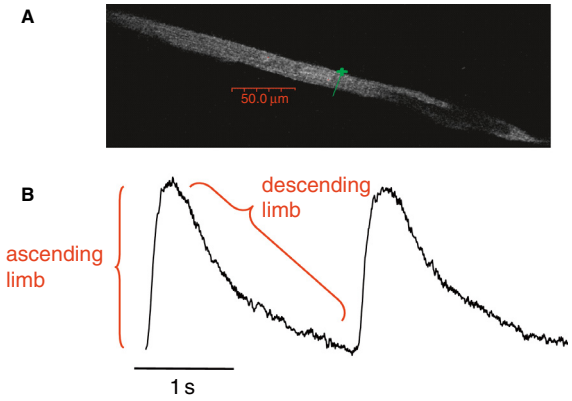


Fig. 3 Cellular Ca^{2+} transient in a ventricular myocyte of the Pacific bluefin tuna (*Thunnus orientalis*). (A) A tuna myocyte filled with a fluorescent dye (white color) that images Ca^{2+}. The green line shows where in the myocyte the Ca^{2+} transient (B) is measured from. The scale bar is 50 µm. (B) Time course of two consecutive Ca^{2+} transients from the above myocyte. Note the fast rising phase and the slow decaying phase of the transient (ascending and descending limbs, respectively). The units of Ca^{2+} are arbitrary. Data is from Shiels et al. (2011).

inactivation). The cessation of Ca^{2+} conductance is very important for the heart. Without it, cytosolic Ca^{2+} would stay elevated, and the heart would not be able to relax to fill with blood between beats.

Extracellular Ca^{2+} influx — the Na^{+}—Ca^{2+} exchanger

The NCX can also bring Ca^{2+} across the SL membrane and can thus contribute to the rising phase of the Ca^{2+} transient. The NCX transfers 3 Na^{+} and 1 Ca^{2+} in opposite directions across the SL membrane. Because of the unequal charge transfer (total Na^{+} movement carries a charge of 3 and Ca^{2+} carries a charge of 2), there is a net movement of positive charge in the direction of Na^{+} transport. When the myocyte is relaxed, the exchanger moves Ca^{2+} out of the cell and brings Na^{+} in. This is called forward-mode exchange. During the upstroke of the AP when the membrane is depolarized, the exchanger reverses direction and brings Ca^{2+} into the cell and moves Na^{+} out. This is called reverse-mode exchange. The direction of the exchanger is also dependent on the concentrations of Ca^{2+} and Na^{+} on either side of the membrane. Thus, the activity of the exchanger is complex with the direction of ion transport depending on both membrane potential and ion concentrations.

There is only a small range of membrane voltages (~0−10 mV during a normal AP) which favor Ca^{2+} influx on the NCX (reverse-mode). In most mammalian myocytes, this Ca^{2+} influx pathway is not very important to the rising phase of the Ca^{2+} transient. However, fish cardiac myocytes are spindle shaped, and therefore have a higher surface area to volume ratio relative to mammalian cardiomyocytes. This means that this pathway should be more effective in fish cardiac myocytes (Vornanen et al., 2002). Also, fish cardiac myocytes tend to have a higher intracellular Na^{+} concentration than those from mammals (Birkedal and Shiels, 2007). Together, this increases the efficacy of reverse-mode NCX and allows Ca^{2+} influx via this pathway to be a significant player in the rise of the Ca^{2+} transient in fish compared with mammals. Indeed, the Ca^{2+} transient resulting from Ca^{2+} influx on the NCX is alone sufficient to cause partial myofilament contraction in most fish species studied to date (e.g. trout, carp) (Hove-Madsen and Tort, 2001; Vornanen, 1999). If Ca^{2+} influx on the NCX is inhibited, it reduces the contractility of trout myocytes by 30%−50%. In carp (*Carassius carassius*), ~70 µmolL^{-1} Ca^{2+} can be brought into the cell during the upsweep of an AP via reverse-mode NCX (Vornanen, 1999). Thus, almost 50% of the total SL Ca^{2+} influx occurs via reverse-mode NCX in this species. Together, the NCX and the LTCC can bring in ~150 µmolL^{-1} Ca^{2+} from the extracellular space which generates a Ca^{2+} transient of sufficient magnitude to activate a full-scale contraction (Hove-Madsen and Tort, 2001; Vornanen, 1999) without contribution from intracellular Ca^{2+} stores.

Similar to the LTCC, there are processes which stop Ca^{2+} influx on the NCX thereby limiting the size and duration of the rising phase of the Ca^{2+} transient. The first is repolarization of the membrane potential during phase 3 of the AP which removes the permissive membrane voltage window for Ca^{2+} influx. Secondly, as the Ca^{2+} transient rises, the concentration of Ca^{2+} in the cytosol also rises, reducing the favourable concentration gradient for Ca^{2+} influx via NCX. In fact, together, the repolarization of the membrane and the rising phase of the Ca^{2+} transient itself cause the NCX to change direction and start moving Ca^{2+} out of the cell. As discussed more in the following sections, this switch to forward-mode NCX activity (Ca^{2+} transported out of the cell) is important for the decay of the Ca^{2+} transient and myocyte relaxation. Together, the inactivation of the LTCCs and the change in direction of the NCX brings the contribution of *extracellular* Ca^{2+} influx to the rising phase of the Ca^{2+} transient to an end (Hove-Madsen et al., 2000).

Intracellular Ca^{2+} influx—Sarcoplasmic reticulum

The sarcoplasmic reticulum (SR) is a large intracellular membranous network-like organelle that forms intimate contacts with the SL and the myofilaments. In cardiac myocytes the primary role of the SR is to act as a storage and release site for Ca^{2+}. The amount of SR in the fish myocyte varies between species. Although there are exceptions to this rule, it appears that active fish species like salmonids (e.g., salmon, trout) and scombrids (e.g., tunas, mackerels) have a greater amount of SR than less active species (e.g., carp) (Haverinen and Vornanen, 2009). A basal fish species, the river lamprey (*Lampetra fluviatilis*), also has a significant complement of SR and relies heavily on the SR during e-c coupling (Vornanen and Haverinen, 2012). Cold-active species (like the burbot and the salmon shark) also have a large complement of SR in their myocytes which appears to be greatly involved in cellular Ca^{2+} flux during e-c coupling (Shiels and Galli, 2014). Furthermore, when fish are chronically exposed to the cold (for example during winter) some fish build more SR in their myocytes, supporting a greater reliance on the SR in delivering and removing Ca^{2+} during the Ca^{2+} transient in cold-acclimated fish (Haverinen and Vornanen, 2009). Interestingly, cold-tolerant mammals, like hibernators, also increase their SR content during the cold (Li et al., 2011). Thus, intracellular Ca^{2+} flux through the SR may be an evolutionarily conserved adaptation for cold-tolerance.

In general, the fish SR plays a minor role (between 0 and 40%) in contributing Ca^{2+} to the rising phases of the transient when compared with adult mammals (between 60 and 80%) (Shiels and Galli, 2014). Indeed, e-c coupling in fish differs from mammals primarily with respect to the role of the SR. In mammals, extracellular Ca^{2+} influx is small and contributes only a small amount of Ca^{2+} to the rising Ca^{2+} transient. However, this small influx is vital as it acts as an ionic trigger to cause the ryanodine receptors to open and the SR to release its Ca^{2+} into the cytosol. This SR Ca^{2+} release is far larger than the Ca^{2+} influx across the SL and thus the rate and magnitude of SR Ca^{2+} release is the key determinant of the rising phase of the Ca^{2+} transient in mammals.

Ca^{2+}-induced Ca^{2+}-release

The process by which Ca^{2+} influx across the SL causes Ca^{2+} release from the SR is called Ca^{2+}-induced Ca^{2+}-release (abbreviated CICR). CICR is completely absent in some fish species, usually (but not always) slow and sluggish fish species like carp (Shiels and Galli, 2014). Athletic fish with fast heart rates and high blood pressures (like salmon and tuna) often show more CICR and thus Ca^{2+} release from the SR plays a larger role in the rise of the Ca^{2+} transient in these species. This is illustrated for bluefin tuna myocytes in **Fig. 4**A (Shiels et al., 2011). As indicated by the green trace, inhibiting the SR (and thus CICR) reduces the overall Ca^{2+}

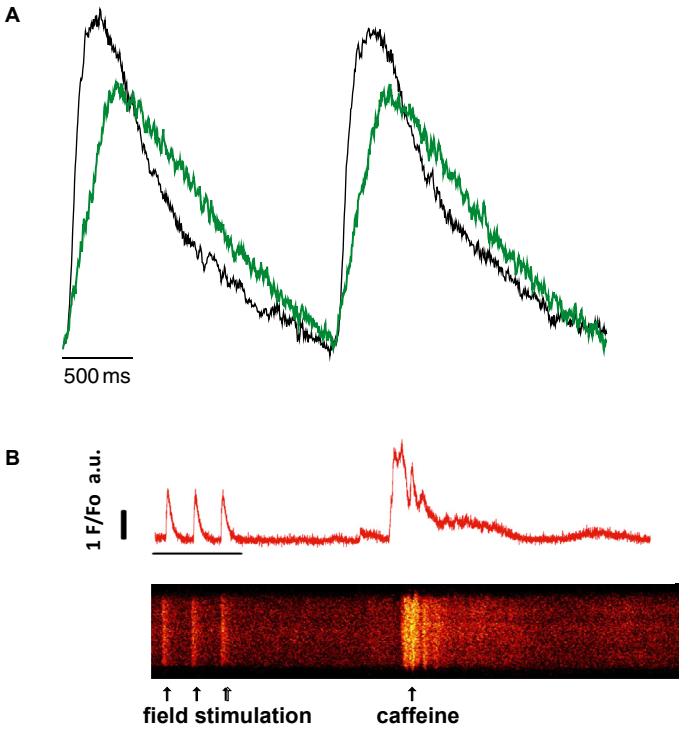


Fig. 4 Role of the SR in the fish cellular Ca^{2+} transient. (A) The role of the SR in the intracellular Ca^{2+} transient in a bluefin tuna (*Thunnus orientalis*) ventricular myocyte. The black line shows the time course of the Ca^{2+} transient under normal conditions. The green line shows the effect of inhibiting SR Ca^{2+} release and SR Ca^{2+} uptake on the shape of the Ca^{2+} transient. In this species, inhibiting SR Ca^{2+} release decreases Ca^{2+} transient amplitude and slows the rising phase of the Ca^{2+} transient. Inhibiting SR Ca^{2+} uptake slows the decay of the Ca^{2+} transient. (B) Time course of intracellular Ca^{2+} transients (upper) measured from confocal line scan images (lower) during normal contractions elicited by electrical field stimulation for a rainbow trout (*Oncorhynchus mykiss*) ventricular myocyte. It also shows the large release of Ca^{2+} from the SR elicited by the rapid application of caffeine. (A) Data is from Shiels et al. (2011). (B) Data is from Shiels and White (2005).

transient amplitude and slows the rising phase of the Ca^{2+} transient in this species. Also apparent is how inhibiting the SR slows the falling phase of the Ca^{2+} transient. This is discussed below.

Physiological stimuli like rapid changes in temperature and *in vivo* levels of adrenergic stimulation can increase the importance of CICR in trout cardiac myocytes (Cros et al., 2014). Nevertheless, only a small percentage (<10%) of total SR stores appears to be released via CICR in fish hearts under normal conditions. For example, the application of caffeine demonstrates that the Ca^{2+} transient of a trout cardiomyocyte can be substantially increased relative to what is seen via typical field stimulation (**Fig. 4**B). Theoretically, the Ca^{2+} influx across the SL that causes CICR can be through either or both the LTCC and the NCX. In mammals it is almost exclusively via the LTCC. However, due to the larger role for Ca^{2+} influx on the NCX in fishes, studies show that NCX can induce CICR in the fish myocyte (Hove-Madsen et al., 2000). The fact that large levels of adrenergic stimulation can induce greater CICR may suggest that under periods of stress or intense activity the SR stores are released to augment SL Ca^{2+} fluxes and generate a larger and faster Ca^{2+} transient (Cros et al., 2014).

Cardiac myocyte contraction and the sarcomere

Contraction of fish, and indeed all vertebrate hearts, is powered by the generation of cross-bridges between two very abundant proteins that are found in muscle cells, termed thick and thin filaments (Gordon et al., 2000). The thick filaments are composed of the protein myosin and the thin filaments are primarily composed of the protein actin. Actin monomers are aligned in a chain, like beads on a string (**Fig. 5**). The myosin molecule contains a head and a tail and the thick filament is structured with the tails of the myosin molecules aligned in series (Gordon et al., 2000). The myosin heads protrude out from this structure. Within the cardiac myocytes, the myosin thick filaments and actin thin filaments are arranged in a parallel overlapping pattern within structures called sarcomeres (**Fig. 2**A).

When a muscle is relaxed, actin and myosin are not interacting, and active force cannot be generated. This is because the sites where myosin binds to the actin are blocked by a rod-shaped protein called tropomyosin (TM) (Solaro and Rarick, 1998). This protein lies along the actin filament (**Fig. 5**) and is locked in place by a group of proteins called the cardiac troponin (*c*Tn) complex (Li et al., 2004). The *c*Tn complex is made up of three different proteins: cardiac troponin I (*c*TnI) (I for inhibitory), which binds directly to actin; cardiac troponin T (*c*TnT) (T for tropomyosin), which binds the *c*Tn complex to TM; and cardiac troponin C (*c*TnC) (C for Ca^{2+}) which binds Ca^{2+} to trigger cardiac myocyte contraction (Li et al., 2004). Thus, the *c*Tn complex is bound directly to the thin filament but acts as a latch to initiate the binding of actin and myosin in the presence of Ca^{2+}.

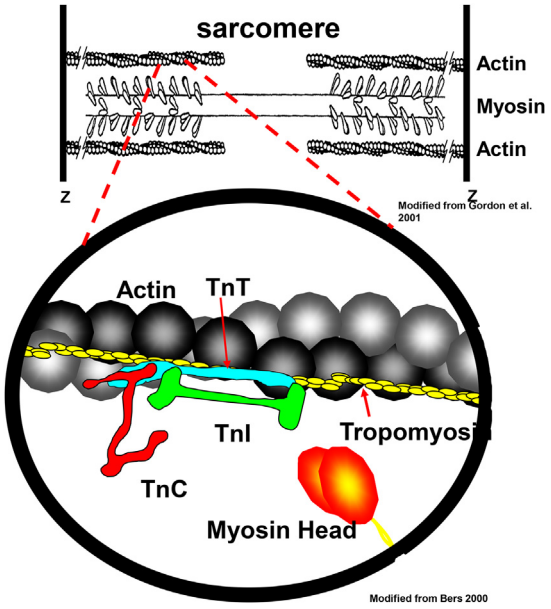


Fig. 5 Schematic diagram of the cardiac sarcomere (top) and the regulatory troponin (Tn) complex (bottom) associated with the actin filament. The Tn complex (TnT, TnI, and TnC), when activated by Ca^{2+}, triggers the contractile reaction leading to the formation of cross-bridges between actin and myosin. Myosin heads extend out from the myosin thick filament toward the actin thin filament. At low intracellular Ca^{2+} concentrations, tropomyosin prevents interaction between myosin and actin. The Ca^{2+} activation of the Tn complex following an action potential reveals the myosin binding sites on the actin thin filament. This then allows for the myosin heads to bind to the actin thin filament forming a cross-bridge. Upon ATP hydrolysis by actin-myosin ATPase, the head of the cross-bridge flexes causing the generation of force by the cross-bridge. This causes the actin thin filament to move toward the center of the thick filament and as a result the sarcomeres shorten and the associated myocyte contracts. The regulatory complex as drawn is composed of the troponin complex and tropomyosin.

Muscle contraction is initiated following an AP when intracellular Ca^{2+} rises and binds to cTnC (Gordon et al., 2000). This causes cTnC to change its shape (a conformational change), which exposes charged amino acids within the core of cTnC. These amino acids then attract and pull cTnI away from the actin thin filament, allowing TM to move freely across the surface of actin. This movement of TM uncovers the myosin binding sites on the actin molecules. Myosin heads can now rotate out from the thick filament and form temporary connections with the actin thin filament (Gordon et al., 2000). Each connection is termed a cross-bridge (Video 2). Flexing of the attached myosin heads, powered by ATP hydrolysis, acts to pull the actin thin filament toward the center of the thick filament (Gordon et al., 2000). This movement of the thin filament causes the distance between adjacent z lines to decrease by about 10%, thereby shortening the length of the sarcomere. The actin filaments are anchored at the z line (**Fig. 5**) and demark the boundaries of each adjacent sarcomere. So when myosin pulls the actin filament, the z lines are pulled closer together. This translates into a shortening of the myocyte and is termed a contraction.

During relaxation the cross-bridges release and the myosin and actin filaments return to their original positions (Gordon et al., 2000). The cross-bridge releases when intracellular Ca^{2+} decreases (see the falling phase of the Ca^{2+} transient discussed below) causing Ca^{2+} to be released from cTnC. As a result, cTnC returns to its original conformation and releases cTnI, which reattaches to the actin filament. Thus, TM returns to its blocking position over the myosin binding sites. As no new cross-bridges can form, the myocyte relaxes. This mechanism of muscle contraction is described by the sliding filament model. Thus, each time the heart contracts every cardiac myocyte undergoes this contractile reaction almost in unison for each cardiac chamber. For a heart that is beating 20 times per minute, it repeats every 3 s.

The cTnC present in the heart of temperate fishes, like the rainbow trout, can be activated at a lower concentration than that found in the mammalian heart (Gillis et al., 2000). This indicates that more cross-bridges, and therefore more force, could be generated for the same amount of Ca^{2+} in the trout heart than in a mammalian heart (Gillis and Tibbits, 2002). This higher sensitivity is thought to enable cardiac function in trout at comparatively low physiological temperatures (Gillis et al., 2000; Keen et al., 2016). The effects of temperature on cardiac performance are covered in a separate article.

The falling phase of the Ca^{2+} transient

Ca^{2+} removal from the cytosol leads to the falling (decay) phase of the Ca^{2+} transient as seen for both the mammalian (**Fig. 1**) and the fish cardiac myocyte (**Fig. 3**). This Ca^{2+} removal can occur via either of the two main efflux pathways illustrated in **Fig. 2**B: Ca^{2+} can either be pumped back across SL membrane via the NCX, or into the Ca^{2+} stores of the SR via the SERCA Ca^{2+}-pump. Because the majority of Ca^{2+} enters the fish cardiac myocyte across the SL membrane, the majority of Ca^{2+} must also leave the cell via this route for Ca^{2+} content of the cell to be in a steady-state (Eisner et al., 1998). Similar to Ca^{2+} influx, the large surface area to volume ratio of the fish myocyte will aid in the efficacy of SL Ca^{2+} efflux (Vornanen et al., 2002). However, the SR may also play a role in the decay of the Ca^{2+} transient. Indeed, the SR of active fish species can sequester large quantities of Ca^{2+} (Haverinen and Vornanen, 2009). This is illustrated in **Fig. 4** by the significant slowing of the Ca^{2+} transient when SR uptake is inhibited in the bluefin tuna ventricular myocyte (Shiels et al., 2011).

Extracellular Ca^{2+} efflux

The NCX operating in forward-mode transports Ca^{2+} out of the cell. This is the primary Ca^{2+} removal pathway and is the main cause of the decay in the Ca^{2+} transient in fish cardiac myocytes. Forward-mode NCX is favourable during the onset of relaxation because of the initially high intracellular Ca^{2+} concentration and the repolarization of the membrane potential. The prominent role of the NCX in relaxation is evidenced by the fact that NCX activity alone (i.e., SR function inhibited) can clear the cytosol of Ca^{2+} and allow relaxation in trout myocytes, whereas inhibiting the NCX severely slows relaxation (Hove-Madsen et al., 2000). Inhibiting both the NCX and the SR abolishes relaxation (Hove-Madsen et al., 2000). This is because Ca^{2+} levels remain elevated inside of the cytosol, maintaining cross-bridges and the contracted state of the myofilaments.

Intracellular Ca^{2+} storage in the SR

The SR contributes to the falling phase of the Ca^{2+} transient by pumping Ca^{2+} out of the cytosol and into the SR. This pumping action is achieved through the efforts of the SR Ca^{2+}-pump, SERCA. Pumping Ca^{2+} into the SR takes energy which is generated by breaking down ATP. The activity of SERCA is temperature-dependent and adrenergically regulated in fish (Castilho et al., 2007; Landeira-Fernandez et al., 2004).

A surprising finding is that despite its limited role in e-c coupling, the fish SR can store very large quantities of Ca^{2+}. The steady-state SR Ca^{2+} content of the trout atrial myocyte is $>1000\ \mu molL^{-1}$ (measured via application of caffeine which opens ryanodine receptors) (Haverinen and Vornanen, 2009). Indeed, the large release of SR Ca^{2+} following caffeine application can be seen in **Fig. 4**B for a trout myocyte. Mammalian SR Ca^{2+} content is in the range of ~50 to 250 $\mu molL^{-1}$ (Bers, 2002). Furthermore, the mammalian SR spontaneously releases Ca^{2+} when the content gets much above 150 $\mu molL^{-1}$. Thus, fish SR can hold much greater amounts of Ca^{2+} without spontaneously releasing it (Shiels and Galli, 2014). How the fish SR is able to store this level of Ca^{2+} is unclear. Differences in intra-SR Ca^{2+} buffering via calsequestrin do not seem to be involved (Korajoki and Vornanen, 2009). Other

Ca^{2+} buffers inside the SR or accessory proteins (e.g., triadin, junctin) that regulate ryanodine receptor function may be involved, but this awaits future studies.

Summary

Excitation-contraction coupling is the progression from membrane excitation with an AP to the rise of intracellular Ca^{2+} to the cell contraction and is the cellular corelate of the heartbeat. The rate and amplitude of the rise and fall of the Ca^{2+} transient determines the rate and number of cross-bridges that cycle, which in turn determines the rate and strength of cardiac contraction. In fish, environmental factors such as temperature, or autonomic stimulation such as adrenaline, influence many steps in this process, driving changes in heart function. These regulatory pathways are described in subsequent articles.

Supplementary material

Supplementary data related to this article can be found online at https://doi.org/10.1016/B978-0-323-90801-6.00120-8.

See Also: Cellular ultrastructure of cardiac cells in fishes; Control of cardiovascular function; Electrical excitation, action potential and impulse conduction; Energy metabolism of cardiac pumping; Integrated response of the cardiovascular system to hypoxia; Cardiac thermal acclimation and adaption of the heart to extreme temperatures; Integrated responses of the heart to acute changes in temperature; Physiology of cardiac pumping.

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