

## Zinc controls RyR2 activity during excitation-contraction coupling

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Cardiac excitation-contraction (EC) coupling is a process which governs contractility of the heart through the controlled release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR). The type-2 ryanodine receptor (RyR2) is the route through which  $\text{Ca}^{2+}$  is released from the SR providing the necessary driving force for cellular contraction. In heart failure, RyR2-channels become abnormally active, or 'leaky', and are unable to remain closed during diastole resulting in unwanted irregular contractile and electrical activity.<sup>1</sup> Defective  $\text{Zn}^{2+}$  handling has been shown to contribute to the cellular pathology of certain cardiomyopathies which give rise to impaired contractility including heart failure.<sup>2</sup> This is likely a consequence of altered EC coupling as a result of modified RyR2 function. How zinc impacts upon the contractile force and the release of calcium from intracellular stores in heart is not fully understood.

In the recent study by Woodier and co-workers<sup>3</sup> it was shown that cytosolic  $\text{Zn}^{2+}$  can act as a high affinity activator of RyR2. In the aforementioned study, single RyR2 channels were incorporated into phospholipid bilayers under voltage-clamp conditions and the direct action of  $\text{Zn}^{2+}$  at the cytosolic face of the channel studied. This approach enabled the study of RyR2 function under tight control of the chemical environment. Concentrations of free  $\text{Zn}^{2+} \leq 1$  nM potentiated RyR2 activity but the presence of activating levels of cytosolic  $\text{Ca}^{2+}$  was a requirement for channel activation. At concentrations of free  $\text{Zn}^{2+} > 1$  nM, the main activating ligand became  $\text{Zn}^{2+}$  and the requirement of  $\text{Ca}^{2+}$  for channel activation was removed. Under these conditions channel gating was altered and RyR2 gated in exceptionally long-lived open states. The ability of  $\text{Zn}^{2+}$  at a

concentration of 1 nM to directly activate RyR2 reveals that RyR2 has a much higher affinity for  $\text{Zn}^{2+}$  than  $\text{Ca}^{2+}$  (by  $\sim 3$ -orders of magnitude). These data suggest that RyR2-mediated  $\text{Ca}^{2+}$ -homeostasis is intimately related to intracellular  $\text{Zn}^{2+}$  levels. Woodier *et al.* also showed that  $\text{Zn}^{2+}$  modulated both the frequency and amplitude of  $\text{Ca}^{2+}$ -waves in cardiomyocytes in a concentration-dependent manner.<sup>3</sup> Reduction of the concentration of intracellular  $\text{Ca}^{2+}$  to sub-activating concentrations did not abolish  $\text{Ca}^{2+}$ -waves in the presence of 1 nM  $\text{Zn}^{2+}$ . This suggests that RyR2 gating is altered under these conditions whereby RyR2 gates in a  $\text{Ca}^{2+}$ -independent manner with  $\text{Zn}^{2+}$  the sole activating ligand. These data indicate that channel dysregulation, through aberrant  $\text{Zn}^{2+}$  homeostasis, may play a fundamental role in the generation of heart failure and other arrhythmic diseases.

Cardiomyocytes contain a small but measurable pool of free  $\text{Zn}^{2+}$  in the cytosol reported to be  $\sim 100$  pM.<sup>4</sup> Since small changes in the  $\text{Zn}^{2+}$  level will have a marked effect on RyR2 activity, this becomes highly relevant when we consider that concentrations of  $\text{Zn}^{2+}$  have recently been reported to be transiently elevated to  $\sim 50$  nM during  $\text{Zn}^{2+}$ -signaling events.<sup>5</sup> Live-cell detection of intracellular  $\text{Zn}^{2+}$  and  $\text{Ca}^{2+}$  using selective fluorophores reveal that intracellular  $\text{Zn}^{2+}$  concentrations are altered during cardiac EC coupling and that spatio-temporal fluctuations in free  $\text{Zn}^{2+}$  levels are comparable to those of  $\text{Ca}^{2+}$ .<sup>6</sup> Extracellular  $\text{Zn}^{2+}$  can also enter cardiomyocytes through the L-type  $\text{Ca}^{2+}$  channel in a similar manner to  $\text{Ca}^{2+}$ .<sup>7</sup> These  $\text{Zn}^{2+}$  fluctuations may serve to modulate RyR2 activity highlighting a potential role for  $\text{Zn}^{2+}$  in fine-tuning graded  $\text{Ca}^{2+}$ -release events to control the force and duration of

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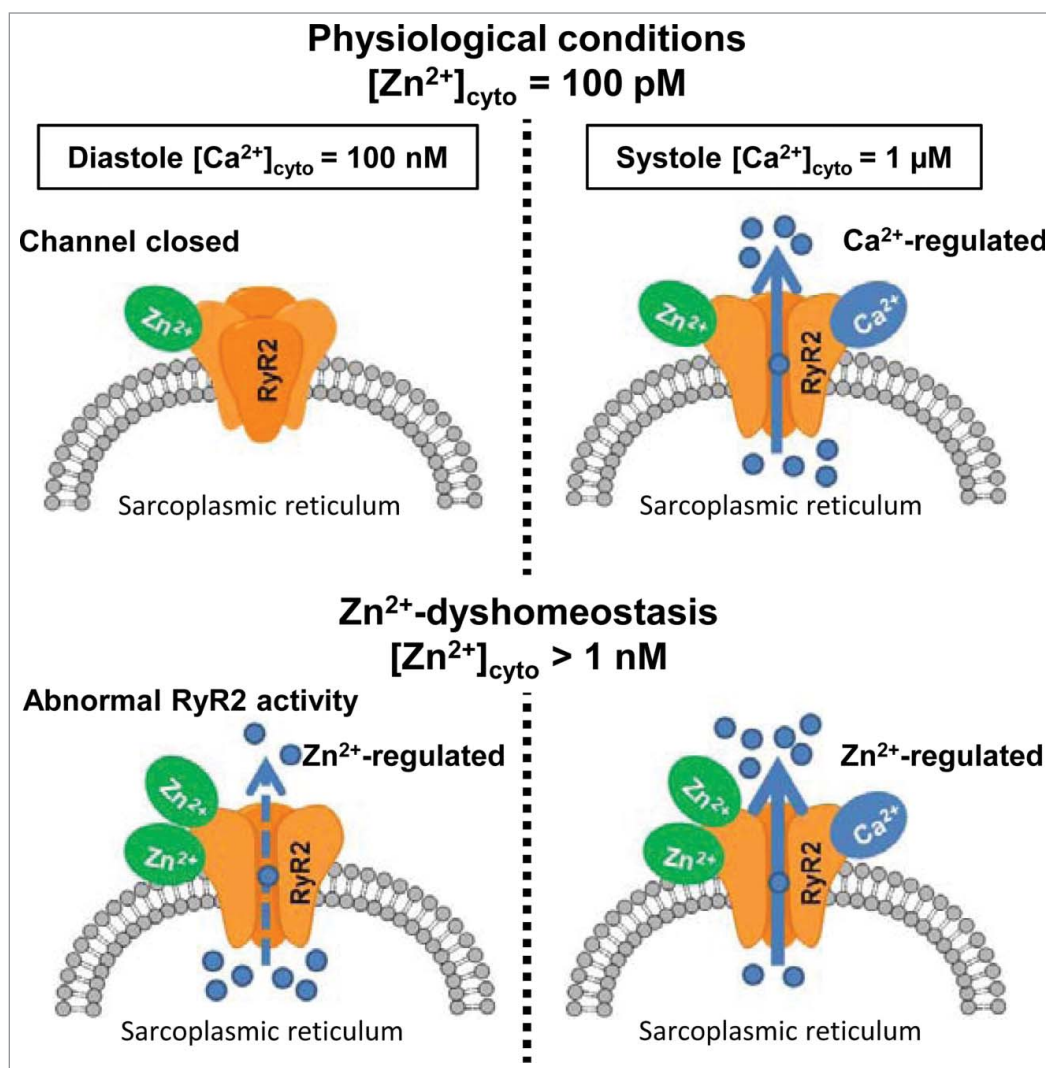
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**Figure 1.** A model to show how zinc may regulate RyR2-mediated sarcoplasmic reticulum calcium release. Physiological conditions: During the resting phase of the cardiac cycle RyR2 is closed as the cytosolic  $[Ca^{2+}]$  is sub-activating (100 nM). The normal trigger for RyR2 activation during systole is a transient rise in  $[Ca^{2+}]$  ( $\geq 1 \text{ } \mu\text{M}$ ).  $Zn^{2+}$ -dyshomeostasis: If the intracellular  $[Zn^{2+}]$  rises  $> 1 \text{ nM}$ , the dependency on  $Ca^{2+}$  for RyR2 openings is removed and  $Zn^{2+}$  becomes the activating ligand. This leads to abnormally active channels.

cardiac contractions. Under certain pathophysiological conditions including heart failure, diabetes and ischemia, concentrations of intracellular  $Zn^{2+}$  are chronically elevated and are reported to be in the high nanomolar range (*c.a.* 30 nM).<sup>2,8</sup> Under such conditions RyR2 will decouple from the regulatory effects of cytosolic  $Ca^{2+}$  and be under control of  $Zn^{2+}$ . This may contribute toward abnormally high RyR2 channel activity which is associated with heart failure and fatal arrhythmias.

The role of  $Zn^{2+}$  as a high affinity activator of RyR2 able to modulate channel function in the absence of  $Ca^{2+}$  represents a paradigm shift in our understanding of how RyR2 is activated during

EC coupling. These new data provide a plausible mechanistic explanation linking  $Zn^{2+}$  dyshomeostasis to certain cardiomyopathies characterized by defective contractility and dysregulated  $Ca^{2+}$ -responses (Fig. 1). However, in order to substantiate a model for integrated  $Zn^{2+}$ -signaling in the heart, a more detailed understanding of the molecular mechanism by which  $Zn^{2+}$  modulates RyR2 function is required and the further impact this has on cardiac function needs to be examined. Determining the origin of  $Zn^{2+}$  fluxes during the cardiac contraction-relaxation cycle in both health and disease states will also be crucial in advancing our understanding

of how cellular  $Zn^{2+}$  shapes EC coupling. Understanding  $Zn^{2+}$  signaling in the heart and unveiling new mechanisms involved in regulating intracellular  $Ca^{2+}$  dynamics in cardiac tissue may highlight potential new drug targets in the fight against heart failure and fatal arrhythmias.

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No potential conflicts of interest were disclosed.

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