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WHITE PAPER

Mechano-electric and mechano-chemo-transduction in cardiomyocytes

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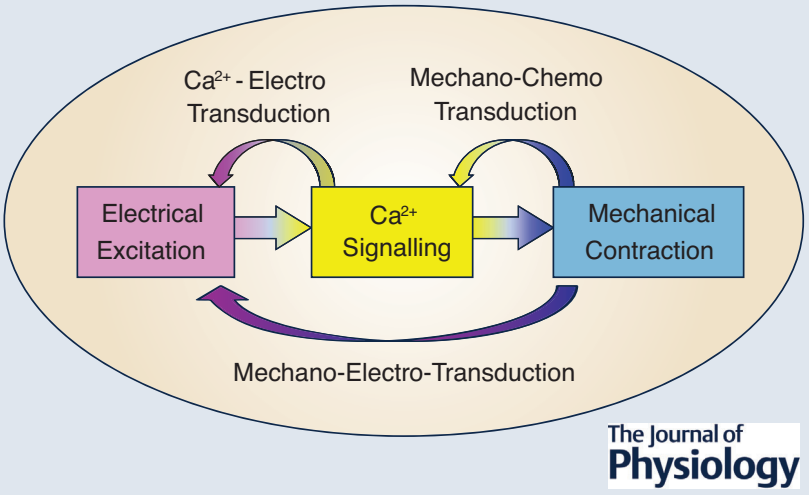

Abstract Cardiac excitation-contraction (E-C) coupling is influenced by (at least) three dynamic systems that couple and feedback to one another (see Abstract Figure). Here we review the mechanical effects on cardiomyocytes that include *mechano-electro-transduction* (commonly referred to as *mechano-electric coupling*, MEC) and *mechano-chemo-transduction* (MCT) mechanisms at cell and molecular levels which couple to Ca^{2+}-electro and E-C coupling

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reviewed elsewhere. These feedback loops from muscle contraction and mechano-transduction to the Ca^{2+} homeodynamics and to the electrical excitation are essential for understanding the E-C coupling dynamic system and arrhythmogenesis in mechanically loaded hearts. This white paper comprises two parts, each reflecting key aspects from the 2018 UC Davis symposium: *MEC* (how mechanical load influences electrical dynamics) and *MCT* (how mechanical load alters cell signalling and Ca^{2+} dynamics). Of course, such separation is artificial since Ca^{2+} dynamics profoundly affect ion channels and electrogenic transporters and vice versa. In time, these dynamic systems and their interactions must become fully integrated, and that should be a goal for a comprehensive understanding of how mechanical load influences cell signalling, Ca^{2+} homeodynamics and electrical dynamics. In this white paper we emphasize current understanding, consensus, controversies and the pressing issues for future investigations. Space constraints make it impossible to cover all relevant articles in the field, so we will focus on the topics discussed at the symposium.

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Abstract figure legend Cardiac excitation-contraction coupling and feedback pathways by mechano-electro- and mechano-chemo-transduction.

Preamble

Recognizing the critical need to combine experimental methods and mathematical models in studying complex heart disease mechanisms, the UC Davis Cardiovascular Symposia series provides an on-going forum for experimentalists, modellers, physicians and scientists to exchange ideas and build collaborations. The goal of this meeting series is to develop an in-depth quantitative understanding of the interplay of cardiac electrical, Ca^{2+} and mechanical signalling, and how each of these systems feed back on each other dynamically (Abstract Figure). Such feedback systems can both stabilize physiological function and cause pathological dysfunction. Understanding these feedback systems is critically important for identification of causes of cardiac arrhythmias and contractile dysfunction (e.g. in heart failure) and the development of effective therapies.

The UC Davis Cardiovascular Symposium series has been organized to integrate such experimental and modelling studies in a series of meetings. The first symposium (2010) took an integrative view of E-C coupling and arrhythmia mechanisms that set the stage for a series of more focused topics: on Ca^{+} channels and Ca^{2+} signalling system (2012), Na^{+} channels and Na^{+} regulation (2014), and K^{+} channels and K^{+} regulation (2016) and mechanics and energetics (2018). Each of these are essential to our integrated understanding and benefit greatly from combining experimental investigations with mathematical modelling studies and clinical perspectives. We shall come full circle from the first 2010 meeting in 2020, when we return to focus on integration among these areas. (See https://basicscience.ucdmc.ucdavis.edu)

Mechano-electric coupling (MEC)

Consensus, controversies and open issues

There is consensus about the following:

(1) Many ion channels (including sarcolemmal and non-sarcolemmal) are mechanically modulated.
   - Ion channels that change their open probability primarily in response to mechanical stimulation (mechano-sensitive channels) can be divided into cell-stretch and cell-volume activated ion channels (SAC, VAC, respectively).
   - VAC increase their open probability in response to cell swelling; they are constitutively active in hypertrophic myocardium – but they are not believed to be major determinant of beat-by-beat mechano-sensory behaviour or drivers of responses to acute mechanical stimulation.
   - SAC can be divided into cation-non-selective (SAC_{NS}) and potassium-selective channels (SAC_{K}).
   - Non-myocytes (fibroblasts, macrophages) are mechano-sensitive; they express SAC and can contribute to cardiac electrophysiology.

(2) Mechano-sensitive channels affect cardiac electrophysiology
   - SAC_{NS} appear to contribute to acute electrical responses to stretch, so much so that

electrophysiological responses to mechanical stimuli can generally be accounted for in quantitative models by simply invoking SAC_{NS}.
- SAC_K cause re/hyperpolarization; they may contribute to action potential (AP) shortening, in particular in pathological settings such as ischaemia.
- SAC_{NS} activation can increase intracellular Ca^{2+}, either directly or indirectly (via Na^+/Ca^{2+} exchange, NCX). The resulting intracellular Ca^{2+} signals can be highly compartmentalized.
- Drug actions, including drug effects on ion channels, can be mechano-modulated.

Controversies and open issues are:
- Amplitudes and kinetics of SAC and VAC in physiological and pathological states, and how they affect the delicate balance of currents during the cardiac AP, are ill-explored.
- We can track strain, but not stress, at the cell level in native cardiac tissue. As a result, for many mechano-electric coupling processes we do not know key biophysical input parameters.
- The explicit in vivo molecular mechanisms of activation, and how best to mimic them experimentally, are unclear.
- Our ability to interpret dynamic mechanical effects on electrophysiology is equally limited by difficulties in identifying 'differential' and 'proportional' signalling pathways.
- The dynamics of 3-D deformation of sub-cellular nano-structures, and their relevance for micro-to-macro cardiac function are ill-explored and form a key frontier for further research.
- Mechanistic links between acute (beat-by-beat time scale or faster), intermediate (second to minutes) and chronic effects (e.g. involving cell and tissue remodelling) of stretch-induced changes in cardiac electrophysiology are unclear.
- In general, we know more about MEC effects in pathological settings (arrhythmogenic effects) than about the role of VAC and SAC in normal homeostasis.

MEC conceptual orgins

Clinical awareness of mechanical modulation of heart rate and rhythm can be traced back by at least a century in the Western medical literature, with examples both for mechanical induction (Meola, 1879) and mechanical termination (Schott, 1920) of heart rhythm disturbances. Following early experimental work that identified the intra-cardiac nature of mechanically induced changes in cardiac electrophysiology (Schlomka & Hinrichs, 1932), mechano-electric coupling (MEC) was conceptualized as part of cardiac electro-mechanical auto-regulation by Ursula Ravens (née Thophile) in 1967 (Kaufmann & Theophile, 1967). Since then, pioneering work by Max Lab, Frederick Sachs, Michael Franz and others has moved MEC from a footnote in cardiac electrophysiology to its appreciation as a fundamental part of cardiac functional integration (for a comprehensive collection of international research on the topic, see the multi-author textbook, edited by Kohl, Franz and Sachs (Kohl *et al.* 2011)).

MEC is based on at least two interacting molecular mechanisms: mechano-sensitive ion channels (see next section) and mechanical modulation of intracellular calcium handling (see section on MEC effects via changes in intracellular Ca^{2+} handling).

MEC effects via mechano-sensitive ion channels

General aspects. Mechano-sensitive ion channels can be split into channels that require a change in cell volume (cell volume-activated ion channels: VAC) and those that activate directly in response to membrane deformation (stretch-activated ion channels: SAC). Since acute effects of mechanical stimulation on cardiac electrophysiology, including beat-by-beat responses, are thought not to involve cell volume changes, SAC are usually considered a primary target for MEC research as it pertains to acute electrophysiological responses to mechanical stimulation.

It is important, however, to note that VAC contribute to cardiac MEC responses in cells subjected to swelling, for example during ischaemia or upon reperfusion. Interestingly, VAC are constitutively activated in the enlarged cells of failing hearts (Clemo *et al.* 1999), suggesting the presence of a 'set point' for cardiomyocyte cell volume by an as-yet-unidentified mechanism. Like many other changes in cardiac structure and function, causal chains of events occurring during chronic tissue remodelling are difficult to explore. This represents an important area for further study.

SAC can be sub-divided by their ion selectivity into cation non-selective (SAC_{NS}) and potassium-selective (SAC_K) channels. While somewhat arbitrary, this division is helpful when considering effects on cell electrophysiology, as SAC_{NS} will depolarize resting cardiomyocytes, while SAC_K will have a re- or hyperpolarizing effect. Given that resting cardiomyocytes respond to mechanical stimulation (if they show any response at all) by depolarization, it is thought that SAC_{NS} dominate MEC effects in healthy cells under normal conditions (Kohl *et al.* 2006).

In addition to ion channels whose open probability is primarily graded by mechanical events, many ligand- and voltage-gated ion channels are mechanically modulated – both in terms of their open probability (Morris, 2011) and in terms of their functional integration into the surface sarcolemma (Boycott *et al.* 2013). Figure 1 provides a recent overview of mechano-sensitive ion channels

that highlights both their ubiquitous presence across living kingdoms and the breadth of proteins identified (from Peyronnet *et al.* 2016). This listing is in constant flux, and the relatively recently identified Piezo channels (Coste *et al.* 2012), which appear to be gated by bilayer tension (Cox *et al.* 2016), have now been found in the heart as well (Liang *et al.* 2017). In addition, we now appreciate that there are individual mutations that may convey increased mechano-sensitivity to ion channels such as TREK-1, which may be associated with an increased risk for arrhythmogenesis in patients (Decher *et al.* 2017). These findings could open up pathways to 'mechano-transduction targeting' for personalized therapeutic strategies.

That said, in general it has remained challenging to identify biologically relevant biophysical input signals for mechano-sensitive ion channel gating. Part of the challenge is that, while we are getting better at tracking cell strain in native tissue (Botcherby *et al.* 2013), we are still far from true readouts of cell stress in the myocardium. While suitable fluorescent probes for measuring cytoskeletal stress have been developed (Rahimzadeh *et al.* 2011), challenges remain in terms of their calibration and use within native tissue, such as the heart.

Given our current inability to accurately identify biologically relevant input parameters, can we at least unequivocally identify the respondents to mechanical stimulation? Well, to some extent yes. As underlying molecular targets are increasingly being identified, both up- and down-regulation approaches have been used to substantiate the players involved in stretch effects on cardiac electrophysiology. Of course, genetic modification is burdened by the possibility (probability) of compensatory changes in the presence and/or activity of other pathway elements.

This can, in part, be compensated using acute pharmacological interventions. A problem here is that we are lacking target-specific blockers and activators (as reviewed in detail elsewhere: Peyronnet *et al.* 2016). To assess SAC_{NS} contributions, GsMTx-4, a peptide from the venom of the Chilean tarantula *Grammostola spatulata* (Suchyna *et al.* 2000), is the intervention tool of choice. GsMTx-4 shows good selectivity for SAC_{NS} and is useful not only in cardiac cells, but also in native tissue (Bode *et al.* 2001). This is not 'a given' and has led to false-negative observations with other, less suitable pharmacological agents such as gadolinium and streptomycin (Cooper & Kohl, 2005; Zhang *et al.* 2018). A note of caution is warranted here for all cell culture studies: streptomycin, an obligatory component of most commercial culture media, is a formidable blocker of SAC_{NS} and stretch-induced changes in Ca^{2+} handling (Gannier *et al.* 1994; Belus & White, 2003).

The experimental difficulties in linking biophysical inputs to molecular players, and cellular responses to organ-level mechano-sensitivity, have contributed to the high relevance of computational models in the quantitative assessment of potential pathways of cardiac MEC (see section on Modelling MEC at the organ level).

Healthy myocardium. The physiological response of cardiac myocytes to acute mechanical stimulation

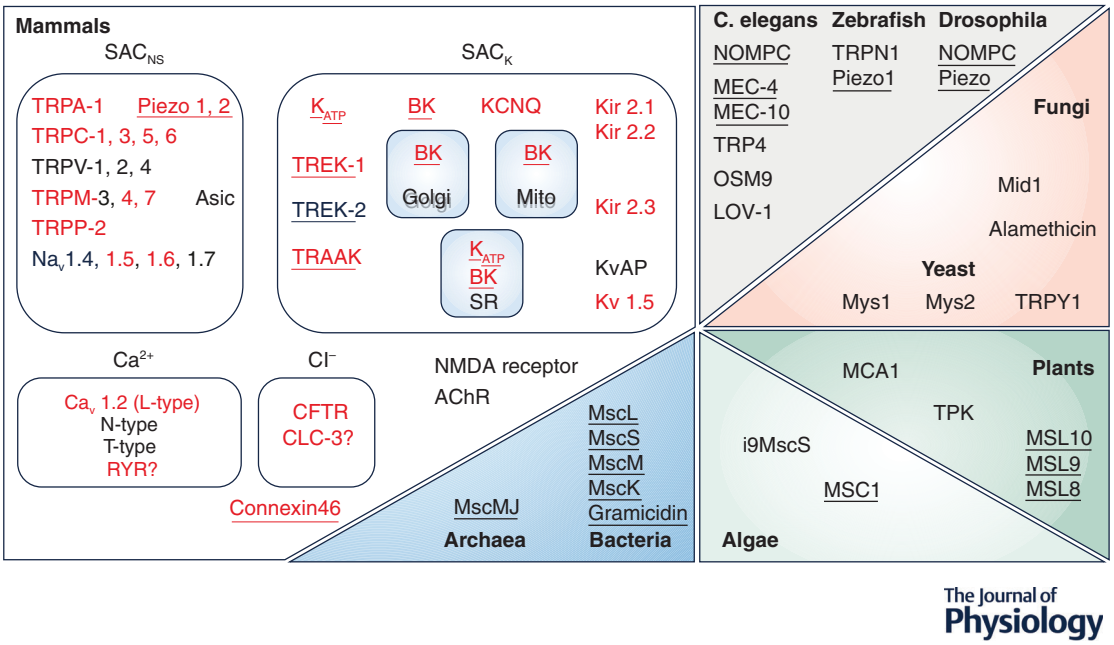

Figure 1. Mechanically gated and modulated ion channels across living organisms
Proteins highlighted in red have been identified in the mammalian heart, channels confirmed as stretch-activated are underlined. From Peyronnet *et al.* (2016).

is diastolic depolarization, which facilitates diastolic depolarization in pacemaker (Cooper *et al.* 2000) and conduction cells (Kaufmann & Theophile, 1967). This can trigger excitation in resting atrial (Bode *et al.* 2001) and ventricular myocardium (Franz *et al.* 1992). This is in line with a major contribution by SAC_{NS}, whose pharmacological block can abolish ectopy. During the action potential (AP), acute stretch responses depend on the difference between current cellular membrane potential and reversal potential of stretch-activated channels (for SAC_{NS} between 0 and −15 mV), causing abbreviation of early and prolongation of late AP time courses, including early and delayed afterdepolarization-like potential changes (EAD, DAD; see Fig. 2 (Kohl *et al.* 2001)).

It has been difficult to identify an evolutionary advantage for cardiac MEC. One line of thought is that mechano-regulation of the ionic milieu would have been an early requirement during the emergence of cellular life forms, whose presence/preservation did not exert a negative evolutionary pressure – so mechano-modulation of ion channels is conserved in all cell types that have been assessed. Alternatively, the electrophysiological consequences of mechano-sensitive ion channel activation could be a side-effect of a different, more important role in the heart: the local matching of cellular Ca^{2+} load (and, hence, contractile performance) to global circulatory demand (Markhasin *et al.* 2003). This would be relevant for an organ that relies on synchronized and force-balanced contraction of millions of muscle cells that lack targeted external control mechanisms (such as individual neuro-muscular junctions), in particular as each of these cells experiences slightly different stress-strain dynamics during normal cardiac activity. Relevant mechanisms would include both (i) mechanical modulation of trans-sarcolemmal Ca^{2+} flux (whether directly by permeation of Ca^{2+} through SAC, or indirectly, e.g. by SAC-medicated Na^{+} influx that alters NCX (Gannier *et al.* 1996; Jin *et al.* 2017; Zhao *et al.* 2017)) and (ii) mechanical modulation of Ca^{2+} flux between intracellular stores and cytosol (for example via a stretch-induced increase in ryanodine receptor (RyR) open probability (Iribe *et al.* 2009*a*) or mitochondrial Ca^{2+} release (Belmonte & Morad, 2008)). Whatever the explanation, the physiological benefit of cardiac mechano-electric cross-talk – beyond potential contributions to preload-adjusted sino-atrial node pacemaking (Quinn & Kohl, 2012) – remains a matter of debate.

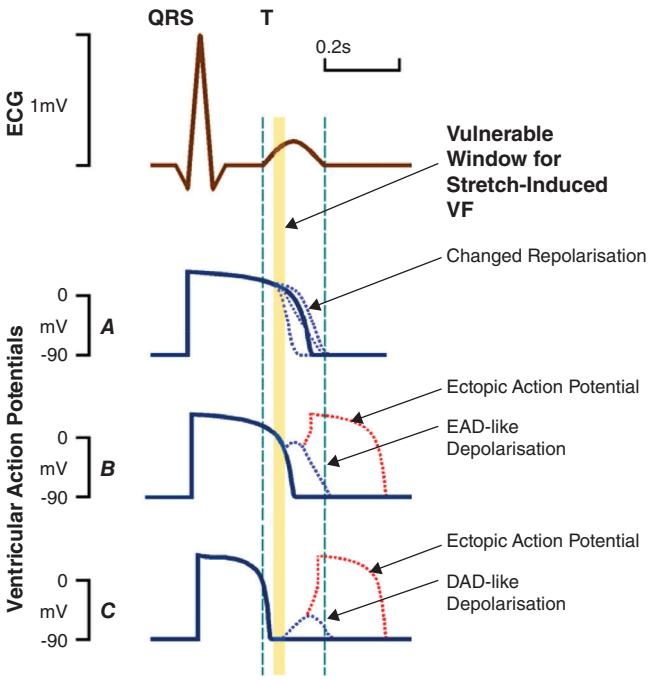

Figure 2. Schematic representation of cardiomyocyte action potential (AP) responses to stretch-activated ion channel (SAC) opening at different timings during the AP
Within a short critical time-window during early ventricular repolarization (just prior to the peak of the T wave; see schematic ECG trace), these can combine to render both trigger (red) and sustaining mechanisms (blue) for ventricular rhythm disturbances (Commotio cordis). Reproduced from Kohl *et al.* (2001).

What is well-established is that the arrhythmogenic potential of mechanical stimulation, if ill-timed, may trigger sustained ventricular tachyarrhythmias (Schlomka & Hinrichs, 1932; Kohl *et al.* 2001; Link, 2012). Interestingly, this is an example of cardiac MEC research where conceptual (Kohl *et al.* 2001) and computational prediction of quantitatively plausible mechanisms in 2-D (Garny & Kohl, 2004) and 3-D (Li *et al.* 2004) computational models of ventricular myocardium preceded experimental confirmation by many years (Quinn *et al.* 2017). We now know that focal electrical excitation, for example triggered by a precordial impact in myocardium just underneath the impact site (Link, 2012), may give rise to reentrant excitation if this ectopic focus occurs just on the trailing edge of the preceding repolarization wave, which acts as a functional block zone preventing uniform spread of the ectopic wave (Quinn *et al.* 2017). While this may give rise to sustained tachyarrhythmia, the vulnerable window for this is impact location-dependent and short (Fig. 2), so that most mechanically induced ectopic beats are benign in healthy myocardium.

Diseased myocardium. Overall, mechanistic insight into MEC in diseased tissue is limited. Reasons include the aforementioned inability to determine local stress levels in native tissue and the often significant structural and functional remodelling in chronic disease settings.

Functional remodelling includes alterations in ion channel presence and activity, such as an upregulation of VAC in hypertrophied cells (Clemo *et al.* 1999),

or metabolic and mechanical co-activation of ATP_K channels (Van Wagoner, 1993) in ischaemic tissue (Huang *et al.* 2013), with demonstrable knock-on effects on local intra- and extracellular ion concentrations and arrhythmogenesis (Bollensdorff & Lab, 2011). In addition, mutations that alter ion channel mechano-sensitivity are starting to emerge as clinically relevant contributors to arrhythmogenesis (Decher *et al.* 2017).

Structural remodelling gives rise to significant heterogeneity in active and passive mechanical properties, which will affect MEC responses. Examples include inhomogeneous stress-strain behaviour in and around ischaemic foci (where paradoxical segment lengthening can be observed), scar border zones (BZ; where stiff scar tissue gives rise to varying stress gradients as the surrounding tissue changes from resting to contracted and back), or asynchronous activation of different cardiac tissue regions (for example as a result of bundle branch block or during cardiac resynchronization therapy). Some of the scenarios in which non-uniform contraction contributes to arrhythmogenesis are explained the next two sections: MEC effects via changes in intracellular Ca^{2+} handling and Modelling MEC at the organ level.

MEC effects via changes in intracellular Ca^{2+} handling

General aspects. E-C coupling is traditionally seen as a sequence of events, where electrical activity (e.g. an AP) results in the rise and decay of an intracellular Ca^{2+} transient, with the consequential rise and fall of contraction. Yet there is feedback from mechanics to Ca^{2+} and electrophysiology, as well as from Ca^{2+} to electrophysiology. This could be called reverse mode E-C coupling. A key signalling molecule involved is intracellular Ca^{2+}. A typical example of Ca^{2+}-related arrhythmogenesis is when Ca^{2+} is released from the sarcoplasmic reticulum (SR) stores by 'leaky' RyR during diastole, or during AP repolarization. This Ca^{2+} can initiate a Ca^{2+} wave, followed by DAD or EAD (Bers, 2014). Below we will focus on an example that depends on Ca^{2+} release from myofilaments, rather than the SR.

Ectopy secondary to non-uniform EC coupling. In failing hearts, wall stress increases as a result of dilatation of the left ventricle (LV). This increased wall stress aggravates regional differences in contractile strength around the impaired muscle. Regional differences in contractile strength may cause paradoxical stretching of impaired muscle by contractions of neighbouring more viable muscle. This forms a BZ between regions of stretch and shortening.

In 2005, Wakayama *et al.* (2005) described spatially non-uniform muscle contractions that lead to DAD-induced arrhythmias. Quite different from Ca^{2+} leak-induced Ca^{2+} waves (Ter Keurs & Boyden, 2007), the arrhythmogenic Ca^{2+} waves initiating these triggered propagated contractions in this multicellular cardiac preparation arise from acute reduction of myofilament Ca^{2+} binding in regions near damaged areas. In this case, Ca^{2+} waves are dependent on mechanical stretch and release at the border between the damaged and intact regions.

For these experiments, ter Keurs *et al.* created a controlled local reduction of myofilament force development in a length of normal multicellular rat trabecula. This was accomplished by exposing the preparation to a small jet of a solution that contained either 2,3-butanedione monoxime (BDM), or low external Ca^{2+} concentration ($[Ca^{2+}]_o$; see Fig. 3*B*, inset). They measured sarcomere length (SL) and with the jet off there was SL shortening in this region. However, when the jet was on, the weakened region near the centre of the jet experienced stretch by the strong regions on either side of the jet (increased SL, Fig. 3*A*, right, and *B*).

Here the initial Ca^{2+} rise during the wave initiation was shown to be caused by Ca^{2+} dissociation from myofilaments as a result of quick release and shortening of active sarcomeres (Wakayama *et al.* 2001). This is because the Ca^{2+} binding affinity is positively correlated with the number of Ca^{2+}-activated force-bearing cross-bridges (Allen & Kentish, 1985). Rapid shortening of sarcomeres in the BZ during relaxation causes Ca^{2+} release from troponin C on the thin filaments. This release can initiate the Ca^{2+} waves. Elimination of SL shortening will inhibit the initiation of Ca^{2+} waves, while SR Ca^{2+} load and release will enhance such waves. Thus the initiation is not caused by *Ca^{2+} leak* from SR, but by an *intracellular Ca^{2+} surge* from myofilaments that are a major site of Ca^{2+} buffering in myocytes (Bers, 2001). Such a Ca^{2+} surge can then induce SR Ca^{2+} release waves which lead to DAD that, under certain conditions, trigger electrical activity (Fig. 4). Indeed, the amount of Ca^{2+} dissociated from myofilaments affects the frequency of the arrhythmias. For example, the maximum rate of muscle relaxation was inversely correlated with the cycle length of the arrhythmia. An unresolved issue is that these waves propagate in multicellular preparations at 0.2–2.8 mm s^{-1}, too fast for SR-mediated Ca^{2+} waves travelling via Ca-induced Ca-release ($\sim$0.1 mm s^{-1}; Brette *et al.* 2005), which normally do not cross gap junctions between myocytes, but is much slower than expected for full-blown AP propagation in tissue ($>$200 mm s^{-1}; Myles *et al.* 2012). While the mechanism may be a hybrid, some mechanistic details about this potentially important MEC mechanism in arrhythmogenesis need further testing.

EAD generation secondary to myofilament protein dynamics: computational model. Recent computational modelling has concluded that myofilament protein

dynamics in a hypertropic cardiomyopathy model could affect EAD formation and possibly arrhythmias in this heritable substrate (Zile & Trayanova, 2017). The paper concluded that incorporation of troponin C buffering of cytosolic Ca^{2+} alters the AP repolarization reserve, which leads to EAD emergence and triggers for arrhythmia. The utility of computational modelling at the organ level is discussed in more detail in the next section.

Modelling MEC at the organ level

We focus on organ-level electro-mechanical modelling studies, as these have advanced over recent years, progressing through different scopes of mechanistic research and corresponding model complexity. MEC effects at the level of individual cells have, in contrast, been modelled before the turn of the Millennium (Landesberg & Sideman, 1994; Noble *et al.* 1998; Kohl *et al.* 1998; Rice *et al.* 1999) and they are thus not discussed in detail here. In short, experimentally observed responses of single cardiac cells to stretch have generally lent themselves to being reproduced using computational models (for reviews, see Kohl *et al.* 1999; Trayanova & Rice, 2011). Below we present the main areas of MEC exploration by computational modelling at the whole-heart level.

Role of mechanical impact in arrhythmogenesis and arrhythmia termination in the heart. In early whole-heart studies, mechanical stimulation was incorporated through its impact on the electrophysiology of the heart, namely through uniform regional SAC activation. In the modelling study by Li *et al.* (2004), both SAC_{NS} and SAC_K are recruited upon mechanical impact. The impact is administered at various coupling intervals following pacing at the apex. The results demonstrated that the impact induces sustained reentrant excitation only when (1) a new activation is elicited by mechanical stimulation (caused by activation of SAC_{NS}), and (2) upon return to the original region of impact, this activation does not encounter an extension of AP duration (prevented by activation of SAC_K, such as K_{ATP}). Furthermore, a subsequent study by Li *et al.* (2006) elucidated the mechanisms involved in termination of ventricular tachycardia by precordial thump and its decreased rate of success in ischaemia. Results demonstrate that precordial thump-induced SAC_{NS} opening in normoxia reduces heterogeneity in transmembrane potential by partially repolarizing excited tissue and depolarizing resting myocardium, causing foci of excitation that eradicate the excitable gap, thus facilitating tachycardia termination. Decreased precordial thump efficacy in ischaemia was caused by recruitment of K_{ATP}, which diminishes the depolarizing effect of SAC_{NS} on resting tissue and caused pronounced AP shortening, thus facilitating establishment of reentry.

Electromechanical transduction and arrhythmogenesis in the diseased heart. In these studies, model complexity has increased, with representation of both electrical and mechanical function and their coupling. Past research explored the role of acute stretch as a trigger of arrhythmia

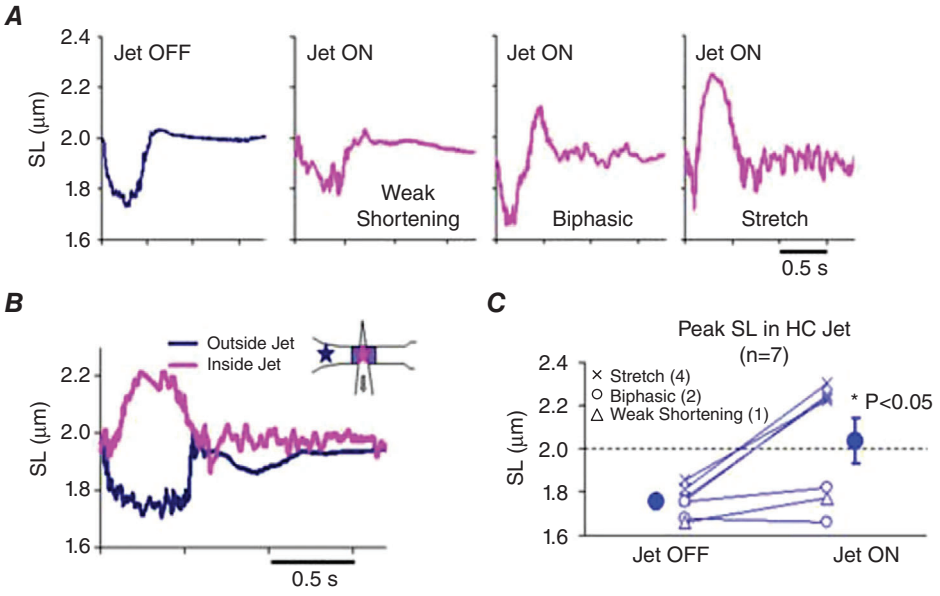

Figure 3. Experimental model to mimic for non-uniform EC coupling in rat trabeculae
Sarcomere length (SL) changes measured from different regions during experiments in a single trabecula when jet of butane BDM was ON or OFF. Note: in some regions there was weak shortening while in others stretch was present. This set up conditions of non-uniform EC coupling. From ter Keurs *et al.* (2006).

under the conditions of acute ischaemia, as well as the effect of acute stretch on the substrate for ventricular fibrillation (VF; Fig. 5; Jie *et al.* 2010). Jie *et al.* showed that the ventricles had a central ischaemic zone (CIZ) and an intermediate BZ, and this was used to represent the electrophysiological and mechanical milieu in the heart at several stages post occlusion. The study found that large muscle strain developed in the ischaemic region during contraction. In both BZ and CIZ, cell membranes underwent mechanically induced subthreshold depolarizations, or DAD-like events, whereas such depolarizations were absent in the normal zone. The DAD-like events resulted in mechanically induced ventricular premature beats (VPB), originating from the ischaemic border (particularly in the LV endocardium where muscle strain and strain rate were largest), but not from CIZ, since in the latter ischaemic injury suppressed excitability. VPB beats then travelled intramurally until emerging from the ischaemic border on the epicardium, initiating reentry.

A subsequent study by Hu *et al.* (2013) examined the role of SAC recruitment on scroll wave stability in VF using an even more complex model, and image-based human ventricular electromechanics model. In VF, SAC recruitment is heterogeneous, and this affects electrophysiology and VF complexity. The study used a wide variety of SAC reversal potentials and channel conductances. Opening of SAC with a reversal potential of −60 mV diminished scroll wave breakup for all values of conductances by flattening heterogeneously the AP duration restitution curve. Opening of SAC with a reversal potential of −10 mV inhibited partially scroll wave break-up at low conductance values by flattening heterogeneously the conduction velocity restitution relation. For large conductance values recruitment of SAC with a reversal potential of −10 mV did not reduce the likelihood of scroll wave break-up, because Na^+ channel inactivation in regions of large stretch led to conduction block, which counteracted the increased scroll wave stability due to an overall flatter conduction velocity restitution.

The role of mechano-electric coupling in defibrillation. Finally, mechano-electric transduction effects in defibrillation were explored through a bidomain electromechanics model suitable for the delivery of external electrical shocks (Li *et al.* 2008). This is a computationally complex model of the heart, particularly its electrophysiology component. The results of these

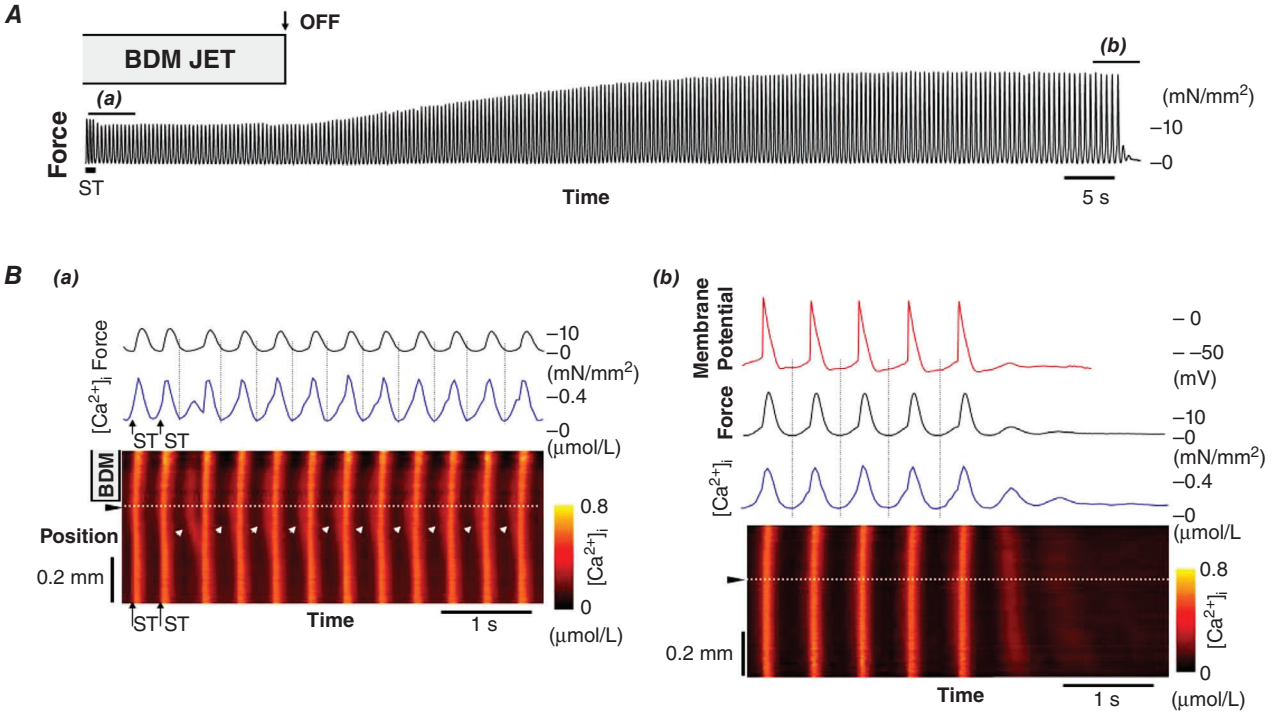

Figure 4. Effect of regional application of BDM via a jet on a sustained arrhythmia
A, continuous chart recording of force during a sustained arrhythmia induced by electric stimulation (ST). Arrow with OFF indicates when the BDM jet was turned off. After the jet was turned off, the contractions became larger and slower. *B*, expanded recordings of force (black lines), membrane potential (red line), $[Ca^{2+}]_i$ at the position indicated by the dotted white lines on the bottom (blue lines), and regional changes in $[Ca^{2+}]_i$ (bottom) during the periods indicated by lines *a* and *b* in *A*. Note initiation of arrhythmia by Ca^{2+} wave seen in *Ba*. This Ca^{2+} wave is dependent on the amount of Ca^{2+} released from troponin C upon quick release. From Miura *et al.* (2010).

whole-heart studies demonstrated that volume overload in the ventricles could lead to an up to 20% increase in the upper limit of vulnerability, a surrogate for a defibrillation threshold.

The theoretical studies discussed above demonstrate very nicely the quantitative feasibility that SAC and MEC effects can contribute significantly to cardiac arrhythmias (for rationally designed models and properties of myofilaments and SAC). What would be especially nice to see going forward is direct experimental validation that these mechanisms suffice to mediate arrhythmogenesis in the functioning mammalian heart, and for example can be prevented by the inhibition of these implicated MEC perpetrators.

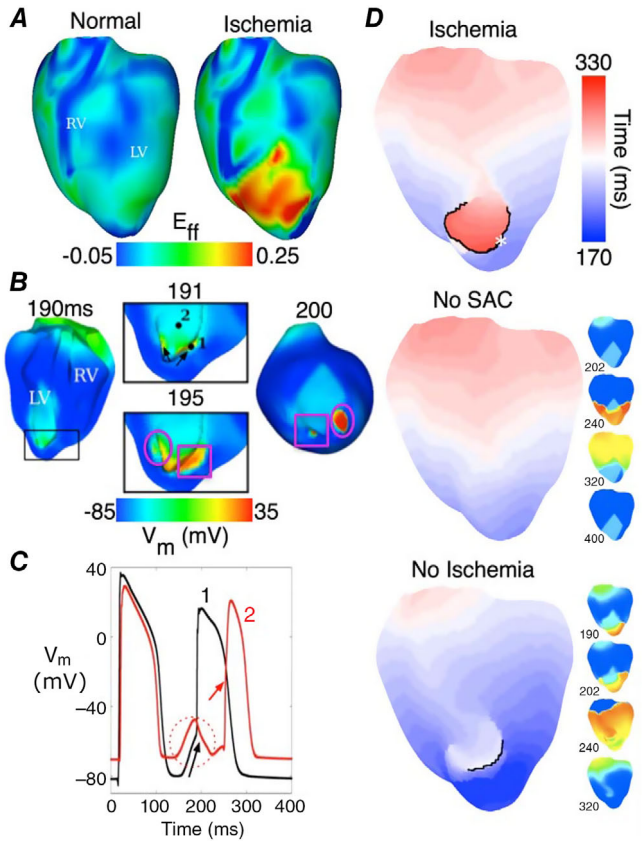

Figure 5. Mechanically induced arrhythmia in the regionally ischaemic rabbit heart
A, distribution of muscle strain (E_{ff}) in the normal and ischaemic heart during systole. *B*, evolution of a mechanically induced ventricular premature beats (VPB). *C*, V_m traces at sites 1 and 2 marked in the 191-ms inset in *B*. Black arrow and dashed circle denote mechanically induced depolarization at these sites. Red arrow indicates activation at site 2 by propagation of the mechanically induced VPB. *D*, activation maps for the full model, model 'No SAC', and model 'No Ischemia' during VPB. No SAC incorporates all the electrophysiological changes in ischaemia, but without involvement of SAC, and, thus, without mechanically induced DAD; external stimuli were applied at the same time and locations where the mechanically induced VPB originated in the full model (arrows in 191-ms inset in *B*). No Ischemia included SAC and thus the spatial distribution of mechanically induced DAD, but without ischaemia-induced electrophysiological changes. Black lines denote conduction block. Asterisk: reentry exit site. Snapshots of transmembrane potentials are also presented for models No SAC and No Ischaemia. Modified from Jie *et al.* (2010).

Mechano-chemo-transduction (MCT)

Consensus, controversies and open issues

There is consensus on the following:

(1) The Frank-Starling (F-S) mechanism and Anrep effect.

- The heart has the intrinsic ability to adapt to changes in preload and afterload. Intrinsic adaptation has two components: the Frank-Starling mechanism (heterometric) and the Anrep effect (homeometric or inotropic).
- The Frank-Starling mechanism is activated by elevated end-diastolic volume (and sarcomere length) and is thus under heterometric regulation. The F-S mechanism is instantaneous (beat to beat) without requiring Ca^{2+} changes.
- The Anrep effect occurs gradually over many beats (minutes timescale) and increasing contractility by increasing the cytosolic Ca^{2+} transients and/or myofilament Ca sensitivity. Multiple biochemical signalling pathways have been implicated. Anrep can restore EDV and sarcomere length towards the initial state (and is thus homeometric).

(2) Mechano-chemo-transduction (MCT) pathways.

- MCT activates various chemo-transducers that lead to modulation of the Ca^{2+} handling system in the cardiomyocyte.
- MCT pathways involve cardiac mechanosensors, chemotransducers, and effectors;
- The mechanical sensors may include myofilament proteins (e.g. titin and crossbridges) and cytoskeletal and transmembrane protein complexes that interact with extracellular matrix and neighbouring cells (e.g. at focal adhesions, dystrophin, integrins and desmosomes).

Controversies and open issues are:

- Different labs have found different roles for nitric oxide (NO) signalling in mechano-chemo-transduction: some found that NO plays no role in stretch-induced Ca^{2+} sparks, but others found that NO is critically important for MCT.
- The apparent controversy above might be explained by hypothesizing that different 'dimensionality' of the mechanical strain or stress imposed on surface *versus*

internal mechanosensors may activate different MCT pathways.
- How do cardiomyocytes distinguish mechanical strain (e.g. stretch) and mechanical stress (e.g. active force or passive stiffness)?
- What are the mechanosensors activated by cell-longitudinal strain and stress?
- What are the mechanosensors activated by cell-transverse compression?
- What are the mechanosensors activated by surface traction and shear stress?
- What are the mechano-chemo-transduction pathways downstream from each mechanosensor to chemo-transducers and to effectors: which, where and how?
- How does mechano-chemo-transduction orchestrate concerted modulations of multiple Ca^{2+} handling molecules to autoregulate the Ca^{2+} transient and contractility?

Frank-Starling mechanism and Anrep effect

The heart has the remarkable ability to adapt its output to tremendous variations in the haemodynamic demand of the body, with the cardiac output ranging from 3 L min^{-1} at resting level to 20 L min^{-1} during vigorous exercise. The heart must not only be able to increase the output but also do so in the face of afterloads (arterial pressure) that range from < 100 to > 300 mmHg (MacDougall *et al.* 1985). As Ernest Starling writes in his Linacre Lecture, this adaptation to changing demands "occurs equally well after total destruction of the nerves connecting the heart with the central nervous system" (p.5, Starling, 1915). In other words, the adaptive ability of the heart lies largely within the heart itself. Otto Frank (Frank, 1895) is equally credited for what is usually called the F-S mechanism. The quest for molecular understanding has occupied physiologists since that time.

In this section we will not recapitulate the excellent recent review on MCT (Schonleitner *et al.* 2017). Instead, we will recount some old and new research that brings into focus fundamental questions that remain unanswered and suggest modern experiments that may cast new light onto the intrinsic mechanisms that underlie the adaptation of the heart to changing mechanical loads.

Starling's promulgation of the 'law of the heart' (Patterson *et al.* 1914) and Anrep's work on the heart's adaptation to afterload (von Anrep, 1912) mark the modern period of investigating the heart's adaptive response to mechanical load. Starling (Patterson *et al.* 1914, Starling, 1915) described the law of the heart as "the energy of contraction ... is a function of the length of the muscle fibre." The Frank-Starling mechanism and Anrep effect are both illustrated by the pressure-volume (*P-V*) loops obtained from the LV in a mouse in Fig. 6*A*. Curve *a* is the *P-V* loop before the outflow resistance was increased by transaortic constriction (TAC). Immediately after TAC, the LV end-diastolic volume (EDV) expands (note the rightward shift of *P-V* loop *b*). This expansion engages the F-S mechanism resulting in more forceful contraction that manifests itself as an increase in developed pressure. The relationship between increasing contraction force and increasing muscle length stands as one of the great foundation stones of cardiac physiology. This fundamental relationship at the sarcomeric level is shown by the monotonically increasing F-S curve (F-S) in Fig. 6*B*, based on experiments using trabeculae (Allen & Kurihara, 1982), which shows developed force (F) as a function of sarcomere length (L).

The F-S law of the heart has stood the test of time, but even as it was being established, evidence emerged that muscle length was not the sole determinant of contractile force. Gleb von Anrep (von Anrep, 1912) found that increasing the outflow resistance caused an initial increase in LV EDV (the rightward shift in the *P-V* loop *a* to *b* in Fig. 6*A*) but after 10–30 min EDV recovered almost completely (*P-V* loop shift from *b* to *c*), exhibiting the normal stroke volume at much higher pressure and stroke work (SW). Indeed, compared to *P-V* loop *a*, the LV was producing much higher pressure at the same EDV (or SL). This is the eponymous Anrep effect.

Figure 6*C* and *D* shows the time course of *P-V* loop changes (from panel *A*) in finer detail for EDV, ESV, ejection fraction (EF) and dP/dt_{max} upon TAC at time 0. These data show that immediately following TAC there is an expansion of the EDV and ESV (panel *C*), a decrease in ejection fraction, and an increase in contraction force as indexed by a greater dP/dt (panel *D*). The increase in contraction force is expected from Starling's law as a consequence of the increased EDV. After 30 min, both the EDV and ESV have returned to their pre-TAC values. Despite the heart's return to the initial LV chamber volume, the contractile force remains sufficiently large (measured by dP/dt) for the ejection fraction – and therefore the stroke volume (indicated by the equal widths of the *P-V* loops before and 30 min after TAC in panel *A*) – to recover completely. At the 30 min time point the increased force cannot be due to the F-S mechanism because the EDV is at its initial value so the muscle lengths are the same.

Load-tuning property of cardiac muscle: generation of different forces under different mechanical loads at the same muscle length

We can use Figure 6*B* to interpret the results shown in panels *A* and *C*. Let L_o be the muscle length at end-diastole ($\sim EDV^{1/3}$) before outflow resistance increase. At this time the muscle develops force $F(a)$. Let F^* be the force needed

to eject the blood at the higher outflow resistance. The initial increase in EDV engages the F-S mechanism and after a few beats the LV expands to the point where the force of contraction is sufficient to eject the volume of blood equal to the venous return. This chamber volume corresponds to length L^* and the muscle generates force $F(b)$. During this phase, the muscle length travels along path A of the Allen-Kurihara F-S curve. As the EDV decreases (Fig. 6*A* and *C*) the force of contraction must be maintained to eject blood against the higher output resistance, so the trajectory of the muscle length is horizontal along path B. After about 15 min the EDV stops decreasing to a value equal to its initial value L_o. At the steady state the muscle length equals L_o but must generate force $F(c) = F(b)$. Therefore, the muscle of length L_o can generate different forces, as seen in $F(a)$ and $F(c)$. The difference between these two forces, $\Delta F = F(c) - F(a)$ reflects the increase in contractility. We call this ability of the muscle to generate different forces under different loads at a given muscle length the *load-tuning property*.

In fact, such load tuning is an inherent property of the heart, whose contractions have to be 'internally heterogeneous' to achieve 'externally homogeneous' pump function (Katz & Katz, 1989). The auto-regulatory ability of the heart to adjust its regional contractility to spatio-temporally varying alterations that are driven by haemodynamic demand has been studied extensively, including 'duplex models' of isolated interacting muscle segments. These are kept in defined mechanical and biochemical conditions (interacting mechanically, while positioned in two separate baths), including more recent approaches where biological muscle segments

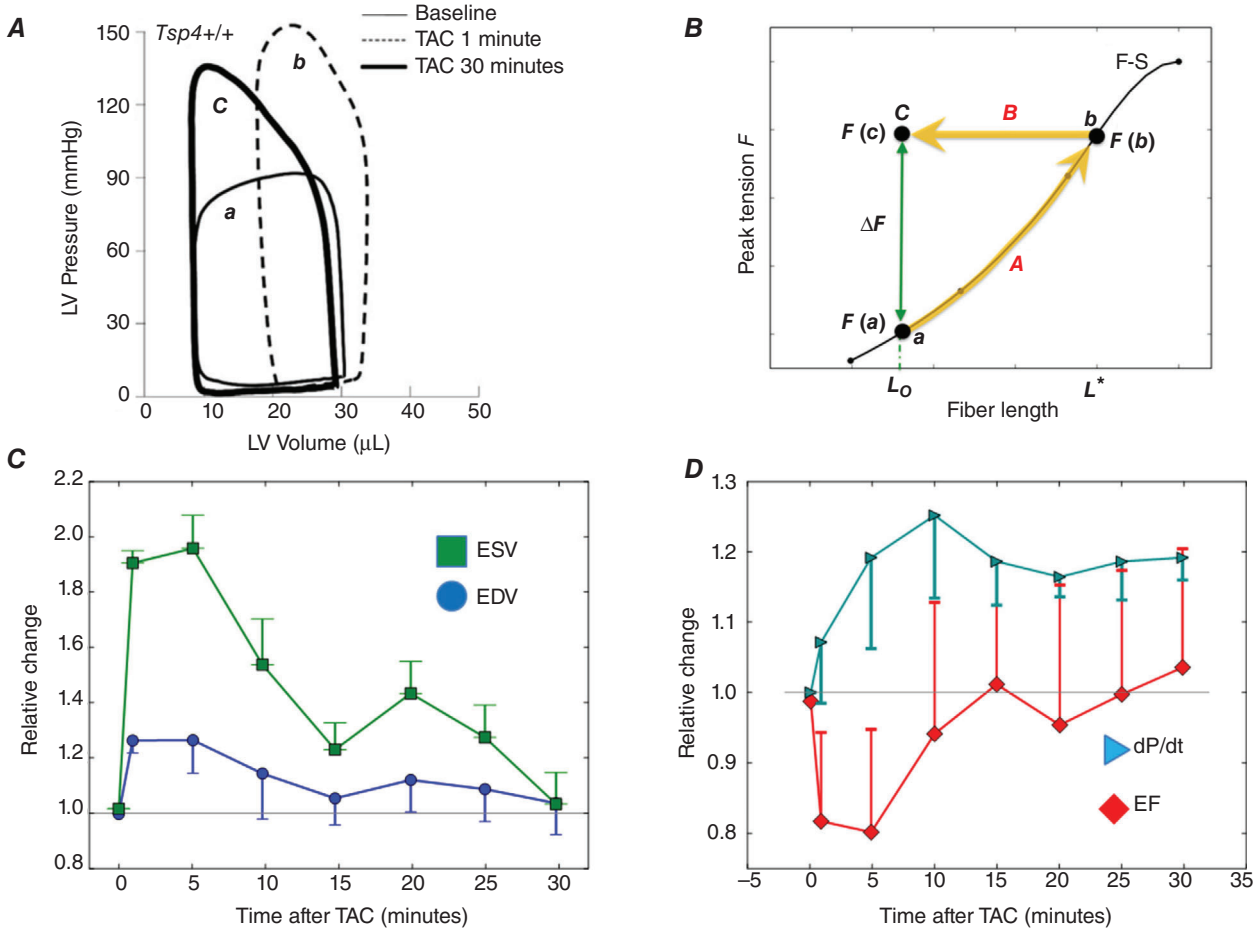

Figure 6. Frank-Starling law and Anrep effect
A, pressure-volume (*P-V*) loops before TAC (light black), 1 min after TAC (dashed curve), and 30 min after TAC (heavy black). From Cingolani *et al.* (2011). *B*, black curve shows the relationship between *fibre* length and force generated by Frank-Starling (F-S) mechanism. L_o is the initial muscle length and steady state muscle length after the chamber volume has decreased due to stress-stimulated contractility (SSC) increase. ΔF (green arrow) is the force SSC needs to generate. F-S curve redrawn from Allen & Kurihara (1982). *C* and *D*, time evolution of left ventricular (LV) haemodynamic parameters following transaortic constriction (TAC). Values are normalized to initial values, when TAC imposed. EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; d*P*/d*t*, maximum rate of LV pressure change. Data redrawn from Cingolani *et al.* (2011).

'interacted' mechanically and in real time with actuators that were controlled by computational models of cardiac electro-mechanical activity (Tyberg *et al.* 1969; Wiegner *et al.* 1978; Markhasin *et al.* 2003). These studies highlight that MCT is the basis on which individual myocardial segments match their contractility to actual demand – in the absence of neuro-muscular junctions that grade skeletal muscle performance (for review, see Solovyova *et al.* 2014).

Given the muscle's load-tuning force output, it is clear that muscle length cannot be the sole determinant of contractile force. Other than *strain* (fractional length change), what mechanical stimulus could also be involved? A logical candidate is the mechanical *stress* (force/area). Laplace's law tells us that the wall stress is proportional to the product of the chamber pressure and chamber radius. Anrep's observation was the first indication that the heart responded not only to mechanical strain as described by Starling's law but also to mechanical stress. The response of the heart to an increase in the outflow resistance shown in Fig. 6 actually has four names: (1) the *Anrep effect*, which honors its discoverer; (2) *homeometric autoregulation (Sarnoff et al. 196*0), which emphasizes the increase of contractility at the same muscle length; (3) *slow force response* (Parmley & Chuck, 1973; Donald *et al.* 1976; Allen & Kurihara, 1982; Kentish & Wrzosek, 1998), which emphasizes the slow time-dependent signalling (up to tens of minutes) to increase inotropy; and (4) *stress-stimulated contractility* (Seo *et al.* 2014), which highlights the role of stress instead of strain in increasing contraction force. We think that the attachment of four different names to the same phenomenon attests to the long history of trying to understand this puzzling phenomenon. The Anrep effect presents two fundamental challenges that were addressed in this symposium: (1) How does the cardiomyocyte distinguish mechanical stress from strain? (2) What are the cellular and molecular mechanisms underlying the stress-stimulated contractility?

Stress-stimulated contractility is caused by increased Ca^{2+} transient

The ability of cardiac muscle to generate two different forces at the same muscle length suggests that the cardiomyocyte can distinguish stress from strain. Around the 1980s, researchers used multicellular preparations such as trabeculae and papillary muscle to measure strain and stress, as well as cytosolic Ca^{2+} level. In a typical experiment, the muscle was stretched to a fixed length and isometric tension was measured. The consensus emerging from these studies is that stretch of the muscle caused an immediate increase in the contractile force (Frank-Starling), followed by a gradual further increase of force over minutes, called the slow force or Anrep response (Parmley & Chuck, 1973; Donald *et al.* 1976; Allen & Kurihara, 1982; Kentish & Wrzosek, 1998) Importantly, the immediate force increase did not involve any change in the Ca^{2+} transient, which is caused by the strain-dependent myofilament-based F-S mechanism (Moss & Fitzsimons, 2002; Solaro, 2007), whereas the slow force response involves increased cytosolic Ca^{2+} transients and in some cases enhanced myofilament Ca^{2+} sensitivity to enhance contractility (Allen & Kurihara, 1982; Kentish & Wrzosek, 1998).

Since the 1990s, researchers further developed techniques to study the strain and stress effects on single cardiomyocytes that allow in-depth exploration of the cellular and molecular mechanisms. A classical technique is to attach a pair of micro-cantilevers (i.e. carbon fibres, glass rods) to the surface of cardiomyocyte and then stretch the cell along the longitudinal direction (Gannier, *et al.* 1994).

This one-dimensional (1-D) stretch technique applies longitudinal strain and stress on the cell but not transverse compression, because the cell is free to expand transversely in bath solution. The Kass group (Seo *et al.* 2014) found that myocyte stretch during auxotonic contraction caused an immediate increase of the force, and then a gradual increase of contractility accompanied by increased Ca^{2+} transient; this single cell response recapitulates the F-S and Anrep effects. Kohl's group (Iribe *et al.* 2009*b*) and Lederer's group (Prosser *et al.* 2011) found that stretch of the resting cardiomyocyte caused a transient rise of Ca^{2+} sparks and waves, and repetitive stretches caused sustained rise of Ca^{2+} sparks. (Prosser *et al.* 2013).

The Chen-Izu group developed a 'cell-in-gel' system allowing them to embed single cardiomyocytes in a 3-D hydrogel made of polymer matrix in solution (Jian *et al.* 2014). When the cell is paced to contract in the gel, the viscoelastic gel resists both the cell shortening and broadening to exert 3-D stress (i.e. longitudinal tension, transverse compression, and surface traction with normal and shear stresses; Shaw *et al.* 2013) The stiffness of the gel reduces shortening, but increases Ca^{2+} transient amplitude and Ca^{2+} spark frequency (Fig. 7). A distinct novelty of the 'cell-in-gel' approach is to apply stress on the myocyte during auxotonic contraction against a 3-D viscoelastic matrix, without pre-stretching the cell, and thereby separating the stress from strain. How mechanical stresses affect the electrical dynamics in the cell-in-gel system is yet to be studied.

Mechano-chemo-transduction pathways linking mechanical forces to Ca^{2+} dynamics

Research from the 1990s to the present has focused on delineating the molecular pathways, or MCT, that link cellular mechanics to changing Ca^{2+} dynamics. These

pathways have been the subject of excellent reviews by Cingolani *et al.* (2013) and Schonleitner *et al.* (2017). Here we briefly catalogue the known pathways and highlight some differences that might reveal different mechano-sensing modalities. We also consider that different experimental settings involve different strains and/or stresses on the cell (Chen-Izu & Izu, 2017), which need to be taken into account in order to understand and compare the data obtained using different experimental settings.

Integrin-angiotensin-NHE pathway. Mechanical stretch of β_1 integrin at the cardiomyocyte surface initiates a number of different signalling pathways by causing release of angiotensin II (Ang II) from myocytes (Browe & Baumgarten, 2004). The Ang II then activates angiotensin II type 1 receptor (AT_1 receptor), which begins a cascade that ultimately activates chloride-permeant stretch-activated channels (Browe & Baumgarten, 2004) or the Na^+-H^+ exchanger (NHE) and NCX, leading to a secondary increase in the Ca^{2+} transient (Alvarez *et al.* 1999; Calaghan & White, 2004; Cingolani *et al.* 2013).

Thrombospondin pathway. Thrombospondin-4 (TSP4) is an matricellular protein that increases in hypertension and heart failure. Cingolani *et al.* (2011) found that in mice lacking TSP4 the *P-V* loops did not have the evolutionary hallmark characteristics of the Anrep effect shown in Fig. 6*A* following acute TAC. Instead, 30 min post TAC the *P-V* loop remained right-shifted and the stroke volume was not restored. This paper shows the interaction of the extracellular matrix and the myocyte in mechano-sensing.

TRPC6/3-PKG pathway. Using the 1-D stretch system with compliant carbon fibres attached on the cardiomyocyte, Seo *et al.* (2014) tested the hypothesis that the transient receptor potential canonical channel 6 (TRPC6) participates in stress-stimulated contractility. Inhibiting TRPC6 using cGMP to activate protein kinase G (PKG) or using a TRPC6 knockout eliminated the increase in Ca^{2+} transient amplitude and slow force response of papillary muscle to stretch. In whole-animal studies, where outflow resistance was increased by TAC, the pressure-volume loops in control animals exhibited the kind of response shown in Fig. 6*A*. Immediately following TAC, the *P-V* loop shifted to the right and narrowed but after 15 min the end-diastolic volume of the *P-V* loop shifted back to the left (in fact below the control volume in their figure) and the stroke volume increased, indicating an increase of stress-stimulated contractility. The TRPC6 knockout lacked the decrease in the end-diastolic volume after 15 min. These elegant studies reveal the importance of TRPC6 in stress-stimulated contractility increase but leaves open the identity of the mechanosensor, a possible G-coupled protein receptor.

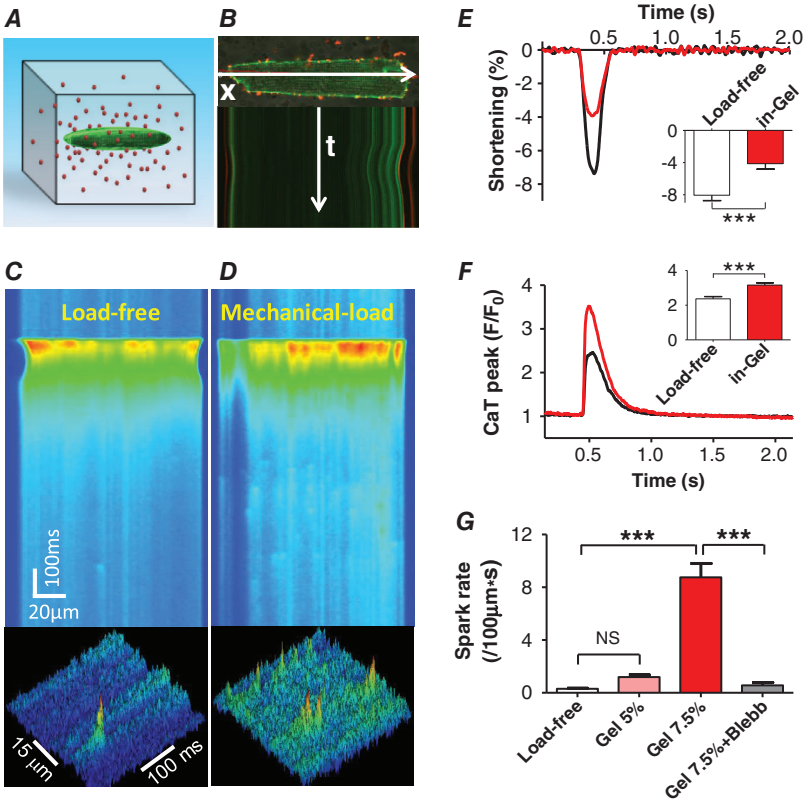

Figure 7. Stress-stimulated Ca^{2+} transient and Ca^{2+} sparks
A, schematic diagram of a myocyte embedded in 3-D hydrogel matrix containing red fluorescence beads. *B*, confocal imaging of the myocyte and beads demonstrating cell contraction and gel deformation as seen in the movement of the cell's edge. *C*, cell contracting in normal Tyrode's solution provides load-free control. *D*, cell contracting in gel under load. *E*, fractional shortening of cell contraction in gel ($n = 17$ cells) compared with load-free control ($n = 17$). *F*, systolic Ca^{2+} transient (CaT) peak in cell-in-gel ($n = 17$) compared with load-free control ($n = 17$). *G*, diastolic Ca^{2+} spark frequency in the cells for load-free control ($n = 18$), in soft gel made of 5% crosslinker (Gel5%, $n = 9$), in gel with 7.5% crosslinker (Gel7.5%, $n = 18$), and after blebbistatin treatment ($n = 5$). One-way ANOVA with Bonferroni *post hoc* test was used for pair-wise comparison: $^{***}P < 0.001$. Reproduced from Jian *et al.* (2014).

NOX-ROS pathway. Using the 1-D stretch system with carbon fibres attached to the cardiomyocyte, Iribe *et al.* (2009*b*) showed that stretch of resting cardiomyocytes elicited a transient increase in the Ca^{2+} spark rate. Subsequently, Prosser *et al.* (2011) showed that reactive oxygen species (ROS) production linked to isoform 2 of NADPH oxidase (NOX2), which they call X-ROS signalling, was responsible for the stretch-induced Ca^{2+} spark rate increase. The link between cellular mechanics and X-ROS signalling is compelling because an intact microtubular network is essential for the stretch-induced Ca^{2+} spark rate increase (Iribe *et al.* 2009*b*; Prosser *et al.* 2011; Khairallah *et al.* 2012; Robison *et al.* 2016). In support of this possibility, modelling studies by Limbu *et al.* (2015) show that X-ROS activation during the diastolic filling phase of the heart could increase the magnitude of the Ca^{2+} transient.

NOS-NO pathway. Using a cell-in-agar technique, Petroff *et al.* (2001) found that stretching the tubing encasing the cell-in-agar increased Ca^{2+} transient amplitude and the Ca^{2+} spark rate in cardiomyocytes. This MCT pathway involves nitric oxide (NO) signalling because both the Ca^{2+} sparks and the Ca^{2+} transient increase were abolished by inhibiting nitric oxide synthases (NOSs) using L-NAME (N^G-nitro-L-arginine methyl ester) or by genetic knockout of eNOS ($eNOS^{-/-}$). Using the 3-D cell-in-gel system, Jian *et al.* (2014) found that stress-stimulated Ca^{2+} sparks were abolished by pharmacological inhibition of nNOS or by genetic knockout of nNOS ($nNOS^{-/-}$). Furthermore, both constitutive nNOS and eNOS are involved in a stress-stimulated increase in the Ca^{2+} transient, and the stress effect on the Ca^{2+} transient was abolished by inhibiting NOS (Jian *et al.* 2014). These data strongly support the hypothesis that NOSs and NO signalling serve as a critically important chemo-transducer in the stress-stimulated MCT pathway.

The above studies show that mechanical strain and stress on cardiomyocytes activate various chemotransducers that lead to modulation of the intracellular Na^+ and Ca^{2+} handling system. The effects of mechanical strain and stress on the Ca^{2+} homeodynamics have several functional consequences. For example, a stress-induced increase of systolic Ca^{2+} transient through the TRPC6/3 and NOS-NO pathways is expected to enhance the cardiomyocyte contractility. Strain- and stress-induced spontaneous Ca^{2+} sparks and waves are expected to promote arrhythmogenic activities. However, the electrophysiological and Ca^{2+} handling systems in rodent myocytes differ significantly from that of larger mammalian (e.g. rabbit, dog, pig, human; Bassani & Bers, 1994; Bers, 2001). Rodent (mouse and rat) ventricular myocytes exhibit very brief APD driven by robust transient outward current (I_{to}) and a late low plateau level dominated by inward NCX current. In contrast, larger mammals have long APDs with positive plateaus, little I_{to} and repolarization that is driven by delayed rectifier K^+ currents (I_{Kr} and I_{Ks}), which are absent in rodents. Furthermore, in rodents, Ca^{2+} handling is overwhelmingly dominated by SR Ca^{2+} release and reuptake, with little Ca^{2+} influx via Ca^{2+} current, and SR Ca^{2+} stores are higher at baseline and are maintained in resting cardiomyocytes. In larger mammals, a greater fraction of E-C coupling-related Ca^{2+} enters with each beat via Ca^{2+} current and leaves via NCX, and SR Ca^{2+} content increases with heart rate are critical to inotropic reserve. Another complicating factor is that experiments are often performed at room temperature, which can alter electrophysiological, Ca^{2+} handling and contractile properties. Hence, caution needs to be taken in translating the experimental findings in rodent models to human pathophysiology.

Different mechano-sensing modalities

Interestingly, the involvement of NOS-NO signalling in the MCT pathway differs drastically in different experimental settings. As mentioned before, the NOS-NO signalling has been found to be critically involved in stress-stimulated MCT in the 3-D cell-in-gel system (Jian *et al.* 2014) and in the 3-D cell-in-agar experiment (Petroff *et al.* 2001). In contrast, inhibiting NOS with L-NAME did not affect strain-stimulated MCT in the 1-D stretch system, as found in the studies by Calaghan & White (2004), Seo *et al.* (2014), Iribe *et al.* (2009*b*) and Prosser *et al.* (2011) To resolve this apparent controversy, Chen-Izu & Izu (2017) proposed a hypothesis on different mechano-sensing modalities based on the 'dimensionality' of the mechano-sensors (i.e. 1-D longitudinal strain *vs.* 3-D stresses).

The apparent controversy on the NOS-NO involvement in MCT pathways opens a window into the different mechano-sensing modalities. One critical difference between the 1-D stretch experiments and the 3-D cell-in-gel experiments is the engagement of cell-surface mechanosensors (Chen-Izu & Izu, 2017). Figure 8 illustrates a conceptual model of how different mechano-sensors may respond to different strains and stresses. The surface mechanosensors are illustrated as the yellow circles (or ellipses if under strain/stress), and the internal mechanosensors are seen as the red or green circles (or ellipses if under strain/stress). When the myocyte lengthens (or contracts) along the longitudinal axis, the cell also narrows (or widens) in the transverse direction because cell volume is conserved. The internal mechanosensors experience the same strain (relative length change) for a given cell strain during stretch or contraction, regardless of the external environment. By contrast, the surface mechanosensors are profoundly

affected by the stiffness of the external environment. Specifically, in the 1-D stretch experiments, myocytes were immersed in bath solution and stretched along the long axis, so the myocyte is largely under longitudinal strain and stress. The bath solution imposes little transverse compression or shear stress on the cardiomyocyte; hence, the cell-surface mechanosensors are not under stress during stretch or contraction. However, in the cell-in-gel system, the myocyte embedded in a 3-D viscoelastic gel must pull the gel along the long axis as it contracts and push the gel along the transverse axis as it widens, and the cell surface pulls against shear force (Shaw *et al.* 2013); hence, the surface mechanosensors are subject to transverse and shear stresses during stretch or contraction (as in the myocardium, extracellular matrix, or hydrogel polymer matrix). Therefore, we propose a simple hypothesis, according to Occam's Razor, that the cell-surface mechanosensors activate the NOS-NO signalling pathway.

Perspectives

Linking modelling and experiments

The study of the heart has enjoyed a stronger interaction between mathematical modelling and experimental work than many other areas of physiology. Niederer *et al.* (2019) give an excellent history of the development of mathematical models of cardiac mechanics. A special issue in the JMCC (introduced by Smith and Niederer (Smith & Niederer, 2016)) focuses on cardiac modelling at both the cellular and tissue and whole-organ levels. An important theme from this special issue is the increased specificity of the models, which enables a closer comparison between experimental data and simulation results.

Here we focus first on what we consider success stories and later on challenges that arise in studying mechano-electrical coupling and mechano-chemo-transduction. Our understanding of the mechanisms underlying the Anrep effect in the whole heart and its correlates, slow force response and stress-stimulated contractility in cardiac muscle and single cardiomyocytes, remains incomplete. Because experimental work in the 1980s showed that the SFR required Ca^{2+} entry, a stretch-sensitive L-type Ca^{2+} channel would seem to be important for the SFR. The importance of modelling is highlighted by Bluhm *et al.* (1998), who showed that a stretch-sensitive Ca^{2+} channel would not account for the slow increase of force characteristic of the Anrep effect. Rather they were able to model both the magnitude and timing of the SFR by assuming that the Na^{+}-K^{+} pump or the Na^{+} leak current were stretch dependent. Later Niederer & Smith (2007) were able to model the SFR using a non-specific stretch-activated current that allowed Na^{+} entry in conjunction with NHE. A surprising and important finding was that the stretch-sensitive NHE could not, by itself, generate the SFR, contrary to the view arising from extensive experimental studies. These studies illustrate some of the important contributions modelling can make to understanding cardiac physiology.

The Niederer & Smith (2007) study showed how the interaction of SAC and NHE generates the SFR. Importantly, they explained how the experimental protocol of preincubation with NHE blockers might have led to a misinterpretation of the primary role of the NHE in SFR. The explanation is subtle; SFR depends on the

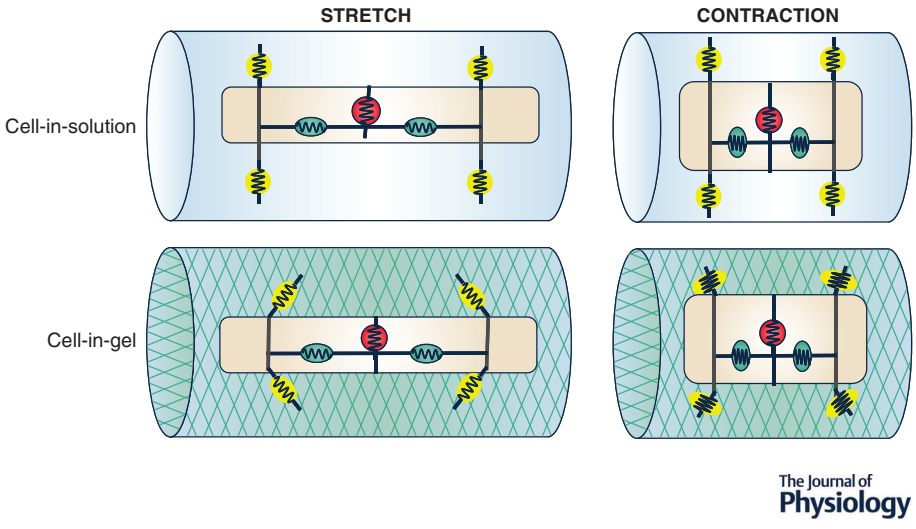

Figure 8. Conceptual model of how different mechanosensors may sense different strain and stress
Upper row: when the cell is in solution, the surface mechanosensors (yellow circle with spring) are not under transverse stress and surface traction. Lower row: when the cell is contracting in 3-D viscoelastic environment, the surface mechanosensors are under transverse compression and shear stresses. The internal mechanosensors (green and red circles with spring) are under longitudinal tension during stretch or compression during contraction.

balance between Na^+ influx and efflux, which can shift with the resting Na^+ level because of the cubic $[Na^+]_I$ dependence of the NCX flux. Long incubation with an NHE blocker reduced $[Na^+]_i$ to a point that limits the ability of NCX to raise $[Ca^{2+}]_i$ to support the development of the SFR. It would probably be difficult to disentangle these interactions by experiments alone.

Iribe *et al.* (2009*a*) and Prosser *et al.* (2011, 2013) showed that stretch immediately increases Ca^{2+} spark rate, possibly through X-ROS signalling. In an editorial Mark Cannell (Cannell, 2009) suggested that the stretch-induced Ca^{2+} spark rate increase might contribute to Starling's law of the heart and in particular the SFR. Limbu *et al.* (2015) modelled X-ROS signalling under cyclic stretching in a single myocyte and calculated spark rates and Ca^{2+} transients. The model faithfully reproduced the Ca^{2+} spark data from experiments but the Ca^{2+} transient results diverged from experimental data. In their simulations the initial stretch caused an immediate increase in the Ca^{2+} transient magnitude but its peak magnitude declined on subsequent stretches. These results are inconsistent with the body of experimental results showing no increase in the Ca^{2+} transient in the initial stretch and slowly increasing Ca^{2+} transient amplitude. Such trenchant differences between model results and experiments should be viewed positively because they open opportunities for modellers and experimentalists to focus on how the model got some things right and where the model needs modification.

We think the current computational models of cardiac E-C coupling need to be further improved. Many parameters in the current models were derived from experimental data obtained in different species and under various artificial conditions. Hence the models need to be improved based on new data obtained under more physiological conditions, such as using mechanically loaded cardiomyocytes (instead of load-free cells). Furthermore, adding mechanical load and mechano-chemo-electro-transduction feedbacks into the E-C coupling model will be a necessary step towards developing more realistic models. Recognizing the importance of the back-and-forth dialogue between modelling and bench experiments, it is critically important to bring together modellers and experimentalists to have this dialogue, to learn from each other, and to develop interdisciplinary collaborations.

Plurality of mechano-chemo-electro-transduction pathways

The plurality of mechano-chemo-electro-transduction pathways discovered so far suggest that mechanical strain and stress on the cardiomyocyte can be sensed and transduced by various mechano-sensors located in different sub-cellular structures. In this white paper we focus only on a few mechano-electro-transduction and mechano-chemo-transduction pathways discussed in the symposium and depicted in Figure 9. We recognize the existence of many other mechano-sensors and their mechano-electro-transduction and mechano-chemo-transduction pathways and recommend excellent reviews on the Anrep effect (Cingolani *et al.* 2013), SAC (Baumgarten, 2007; Peyronnet *et al.* 2016), and mechano-sensitivity of Ca^{2+} signalling (Schonleitner *et al.* 2017). Here we wish to emphasize that different experimental settings involve different mechanical strains and stresses, which would affect different mechano-sensors depending on their sub-cellular localization and dimensionality. It is plausible that the internal mechano-sensors are poised to sense longitudinal strain and tension, whereas cell-surface mechanosensors are poised to sense surface traction and transverse compression. Therefore, caution is needed in clarifying the precise mechanical stimuli for analysing molecular mechanisms and comparing the data from different experimental settings. It is also important to use different mechanical stimuli to interrogate different mechanosensors in order to piece together a more precise and comprehensive map of the mechano-chemo-electro-transduction pathways.

Why so many pathways? The heart has a plethora of ion channels, electrogenic exchangers and pumps that are needed to adapt the action potential to the changing

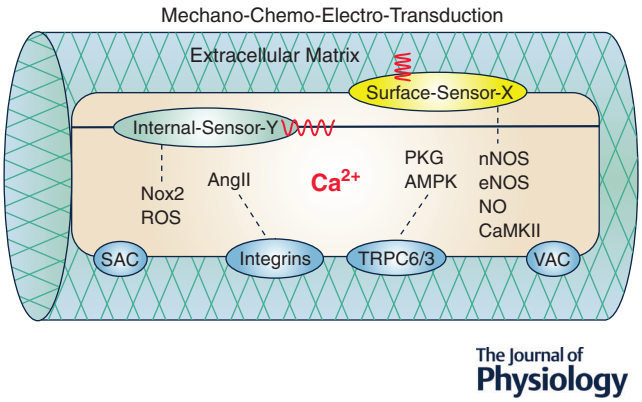

Figure 9. Schematic diagram of the mechano-chemo-electro-transduction (MCET) pathways in the cardiomyocyte embedded in extracellular matrix (ECM)
Stretching the cell applies mechanical strain that can be sensed by internal mechanosensors (green). Cell contraction against ECM applies mechanical stress that can be sensed by surface-mechanosensors (yellow). The molecular identities of many mechanosensors are yet to be discovered (labelled X and Y). Depicted are several known MCET pathways: Nox2-ROS, integrin-AngII, TRPC6/3 and nNOS-eNOS-NO-CaMKII pathways; all these pathways lead to modulation of intracellular Ca^{2+} homeodynamics (see MCT section); all these pathways lead to modulation of intracellular Ca^{2+} homeodynamics (see MCT section). Stretch-activated channels (SAC) and volume activated channels (VAC) have also been linked to Ca^{2+} permeability that may affect the Ca^{2+} entry and intracellular Ca^{2+} homeodynamics (see MEC section).

demands of the body. One wonders whether the plurality of mechanosensors and MCT pathways might be needed to adapt the force generation to different stressors and demands. Different mechanosensors and MCT pathways might stratify into different time and force hierarchies.

Time hierarchy. The F-S mechanism and the Anrep effect have distinct time domains. The F-S mechanism is fully engaged within one or two heartbeats while the Anrep effect develops over many minutes. Teleologically such a separation into rapid and slow time domains makes sense. Changes in venous return due to postural changes or left-right ventricular flow mismatch are transient disturbances that the F-S mechanism rapidly corrects without the expense of increased Ca^{2+} signalling. Adjusting to sustained increases in afterload caused by activities with a large static component such as boxing (Mitchell *et al.* 2005) or shovelling snow (Franklin *et al.* 1995), on the other hand, requires a different strategy. The heart might be trading off the cost of Ca^{2+} signalling to reduce the energetic cost of contracting against higher wall tension resulting from wall stretch. Research discussed here focused on acute changes in mechanical loading where the response time of the heart is on the order of a few seconds to a few tens of minutes. How chronic hypertension, operating on time scales of months to years, affects MEC and MEC remains largely unexplored.

Force hierarchy. Systolic blood pressure is around 120 mmHg but can reach 350 mmHg while doing double leg presses (MacDougall *et al.* 1985). Just as different tools are used to measure distance and force it seems reasonable that different mechanosensors and MCT pathways might respond to different levels of strain and stress.

To go beyond teleological speculation, future experiments must pay careful attention to the characteristics of mechanical loading (i.e. strain, stress, location, magnitude) imposed on the myocyte and determine which MCET pathway is operative by pharmacological or genetic dissection. Sequential application of specific channel blockers enabled electrophysiologists to 'peel back' the layers of ionic currents that underlie the action potential (Banyasz *et al.* 2011; Hegyi *et al.* 2018). Perhaps a similar approach might be useful to determine how different MCET pathways stratify in time and force hierarchies.

MCT and MEC feedbacks in cardiac excitation-contraction coupling

The heart pumps blood against an ever-changing mechanical load. The heart also has intrinsic mechanisms to sense the strain and stress and autoregulate contractile force to maintain a constant cardiac output. Although the heart's response to mechanical loading was described by Frank, Starling and Anrep more than a century ago, the cellular and molecular mechanisms that transduce mechanical load to influence the Ca^{2+} handling and electrical activities in the cardiomyocyte are far from fully understood. The classic description of excitation-contraction coupling has information flowing from the electrical system to the Ca^{2+} control system and then to the contractile system. MCT and MEC reverse the flow of information enabling a continuously bidirectionally information feedforward and feedback between these three dynamic systems. According to control theory, such closed-loop feedback is necessary for autoregulation of the system. Continuing research on MCT and MEC is essential for understanding the heart as a smart pump that senses mechanical strain and stress and autoregulates contractile force to maintain cardiac output. But as with any control system operating out of its design space, Ca^{2+} dysregulation and arrhythmias can emerge.

References

Allen DG & Kentish JC (1985). The cellular basis of the length-tension relation in cardiac muscle. *J Mol Cell Cardiol* **17**, 821–840.

Allen DG & Kurihara S (1982). The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *J Physiol* **327**, 79–94.

Alvarez BV, Perez NG, Ennis IL, Camilion de Hurtado MC & Cingolani HE (1999). Mechanisms underlying the increase in force and Ca^{2+} transient that follow stretch of cardiac muscle: a possible explanation of the Anrep effect. *Circ Res* **85**, 716–722.

Banyasz T, Horvath B, Jian Z, Izu LT & Chen-Izu Y (2011). Sequential dissection of multiple ionic currents in single cardiac myocytes under action potential-clamp. *J Mol Cell Cardiol* **50**, 578–581.

Bassani RA & Bers DM (1994). Na-Ca exchange is required for rest-decay but not for rest-potentiation of twitches in rabbit and rat ventricular myocytes. *J Mol Cell Cardiol* **26**, 1335–1347.

Baumgarten CM (2007). Cardiac Mechanotransduction, pp. 8–27. Landes Bioscence.

Belmonte S & Morad M (2008). 'Pressure-flow'-triggered intracellular Ca^{2+} transients in rat cardiac myocytes: possible mechanisms and role of mitochondria. *J Physiol* **586**, 1379–1397.

Belus A & White E (2003). Streptomycin and intracellular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. *J Physiol* **546**, 501–509.

Bers DM (2001). *Excitation-Contraction Coupling and Cardiac Contractile Force*. Kluwer Academic Press, Dordrecht, Netherlands.

Bers DM (2014). Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol* **76**, 107–127.

Bluhm WF, Lew WY, Garfinkel A & McCulloch AD (1998). Mechanisms of length history-dependent tension in an ionic model of the cardiac myocyte. *Am J Physiol Heart Circ Physiol* **274**, H1023–H1040.

Bode F, Sachs F & Franz MR (2001). Tarantula peptide inhibits atrial fibrillation. *Nature* **409**, 35–36.

Bollensdorff C & Lab MJ (2011). Stretch effects on potassium accumulation and alternans in pathological myocardium. In *Cardiac Mechano-Electric Coupling and Arrhythmias*, 2nd edn, eds Kohl P, Sachs F & Franz MR. Oxford University Press, Oxford.

Botcherby EJ, Corbett A, Burton RA, Smith CW, Bollensdorff C, Booth MJ, Kohl P, Wilson T & Bub G (2013). Fast measurement of sarcomere length and cell orientation in Langendorff-perfused hearts using remote focusing microscopy. *Circ Res* **113**, 863–870.

Boycott HE, Barbier CSM, Eichel CA, Costa KD, Martins RP, Louault F, Dilanian G, Coulombe A, Hatem SN & Balse E (2013). Shear stress triggers insertion of voltage-gated potassium channels from intracellular compartments in atrial myocytes. *Proc Natl Acad Sci U S A* **110**, E3955–E3964.

Brette F, Despa S, Bers DM & Orchard CH (2005). Spatiotemporal characteristics of SR Ca^{2+} uptake and release in detubulated rat ventricular myocytes. *J Mol Cell Cardiol* **39**, 804–812.

Browe DM & Baumgarten CM (2004). Angiotensin II (AT1) receptors and NADPH oxidase regulate Cl^- current elicited by beta1 integrin stretch in rabbit ventricular myocytes. *J Gen Physiol* **124**, 273–287.

Calaghan S & White E (2004). Activation of Na^+-H^+ exchange and stretch-activated channels underlies the slow inotropic response to stretch in myocytes and muscle from the rat heart. *J Physiol* **559**, 205–214.

Cannell MB (2009). Pulling on the heart strings: a new mechanism within Starling's Law of the Heart? *Circ Res* **104**, 715–716.

Chen-Izu Y & Izu LT (2017). Mechano-chemo-transduction in cardiac myocytes. *J Physiol* **595**, 3949–3958.

Cingolani HE, Pérez NG, Cingolani OH & Ennis IL (2013). The Anrep effect: 100 years later. *Am J Physiol Heart Circ Physiol* **304**, H175–H182.

Cingolani OH, Kirk JA, Seo K, Koitabashi N, Lee DI, Ramirez-Correa G, Bedja D, Barth AS, Moens AL & Kass DA (2011). Thrombospondin-4 is required for stretch-mediated contractility augmentation in cardiac muscle. *Circ Res* **109**, 1410–1414.

Clemo HF, Stambler BS & Baumgarten CM (1999). Swelling-activated chloride current is persistently activated in ventricular myocytes from dogs with tachycardia-induced congestive heart failure. *Circ Res* **84**, 157–165.

Cooper PJ & Kohl P (2005). Species- and preparation-dependence of stretch effects on sino-atrial node pacemaking. *Ann N Y Acad Sci* **1047**, 324–335.

Cooper PJ, Lei M, Cheng LX & Kohl P (2000). Selected contribution: axial stretch increases spontaneous pacemaker activity in rabbit isolated sinoatrial node cells. *J Appl Physiol (1985)* **89**, 2099–2104.

Coste B, Xiao BL, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M & Patapoutian A (2012). Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* **483**, 176–181.

Cox CD, Bae C, Ziegler L, Hartley S, Nikolova-Krstevski V, Rohde PR, Ng CA, Sachs F, Gottlieb PA & Martinac B (2016). Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. *Nat Commun* **7**, 10366.

Decher N, Ortiz-Bonnin B, Friedrich C, Schewe M, Kiper AK, Rinne S, Seemann G, Peyronnet R, Zumhagen S, Bustos D, Kockskamper J, Kohl P, Just S, Gonzalez W, Baukrowitz T, Stallmeyer B & Schulze-Bahr E (2017). Sodium permeable and "hypersensitive" TREK-1 channels cause ventricular tachycardia. *EMBO Mol Med* **9**, 403–414.

Donald TC, Peterson DM, Walker AA & Hefner LL (1976). Afterload-induced homeometric autoregulation in isolated cardiac muscle. *Am J Physiol* **231**, 545–550.

Frank O (1895). Zur Dynamik des Herzmuskels. *Z Biol* **32**, 370–447.

Franklin BA, Hogan P, Bonzheim K, Bakalyar D, Terrien E, Gordon S & Timmis GC (1995). Cardiac demands of heavy snow shoveling. *JAMA* **273**, 880–882.

Franz MR, Cima R, Wang D, Profitt D & Kurz R (1992). Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. *Circulation* **86**, 968–978.

Gannier F, White E, Garnier & Le Guennec JY (1996). A possible mechanism for large stretch-induced increase in $[Ca^{2+}]_i$ in isolated guinea-pig ventricular myocytes. *Cardiovasc Res* **32**, 158–167.

Gannier F, White E, Lacampagne A, Garnier D & Le Guennec JY (1994). Streptomycin reverses a large stretch induced increases in $[Ca^{2+}]_i$ in isolated guinea pig ventricular myocytes. *Cardiovasc Res* **28**, 1193–1198.

Garny A & Kohl P (2004). Mechanical induction of arrhythmias during ventricular repolarization: modeling cellular mechanisms and their interaction in two dimensions. *Ann N Y Acad Sci* **1015**, 133–143.

Hegyi B, Bossuyt J, Griffiths LG, Shimkunas R, Coulibaly Z, Jian Z, Grimsrud KN, Sondergaard CS, Ginsburg KS, Chiamvimonvat N, Belardinelli L, Varro A, Papp JG, Pollesello P, Levijoki J, Izu LT, Boyd WD, Banyasz T, Bers DM & Chen-Izu Y (2018). Complex electrophysiological remodeling in postinfarction ischemic heart failure. *Proc Natl Acad Sci U S A* **115**, E3036–E3044.

Hu Y, Gurev V, Constantino J, Bayer JD & Trayanova NA (2013). Effects of mechano-electric feedback on scroll wave stability in human ventricular fibrillation. *PLoS One* **8**, e60287.

Huang H, Liang L, Liu P, Wei H, Sachs F, Niu W & Wang W (2013). Mechanical effects on K_{ATP} channel gating in rat ventricular myocytes. *PLoS One* **8**, e63337.

Iribe G, Ward CW, Camelliti P, Bollensdorff C, Mason F, Burton RA, Garny A, Morphew MK, Hoenger A, Lederer WJ & Kohl P (2009*a*). Axial stretch of rat single ventricular cardiomyocytes causes an acute and transient increase in Ca^{2+} spark rate. *Circ Res* **104**, 787–795.

Iribe G, Ward CW, Camelliti P, Bollensdorff C, Mason F, Burton RAB, Garny A, Morphew MK, Hoenger A, Lederer WJ & Kohl P (2009*b*). Axial stretch of rat single ventricular cardiomyocytes causes an acute and transient increase in Ca^{2+} spark rate. *Circ Res* **104**, 787–795.

Jian Z, Han H, Zhang T, Puglisi J, Izu LT, Shaw JA, Onofiok E, Erickson JR, Chen Y-J, Horvath B, Shimkunas R, Xiao W, Li Y, Pan T, Chan J, Banyasz T, Tardiff JC, Chiamvimonvat N, Bers DM, Lam KS & Chen-Izu Y (2014). Mechanochemotransduction during cardiomyocyte contraction is mediated by localized nitric oxide signaling. *Sci Signal* **7**, ra27.

Jie X, Gurev V & Trayanova N (2010). Mechanisms of mechanically induced spontaneous arrhythmias in acute regional ischemia. *Circ Res* **106**, 185–192.

Jin H, Iribe G & Naruse K (2017). Effects of bepridil on stretch-activated BKca channels and stretch-induced extrasystoles in isolated chick hearts. *Physiol Res* **66**, 459–465.

Kaufmann R & Theophile U (1967). Autonomously promoted extension effect in Purkinje fibers, papillary muscles and trabeculae carneae of rhesus monkeys. *Pflügers Archiv* **297**, 174.

Katz AM & Katz PB (1989). Homogeneity out of heterogeneity. *Circulation* **79**, 712-717.

Kentish JC & Wrzosek A (1998). Changes in force and cytosolic Ca^{2+} concentration after length changes in isolated rat ventricular trabeculae. *J Physiol* **506**, 431–444.

Khairallah RJ, Shi G, Sbrana F, Prosser BL, Borroto C, Mazaitis MJ, Hoffman EP, Mahurkar A, Sachs F, Sun Y, Chen Y-W, Raiteri R, Lederer WJ, Dorsey SG & Ward CW (2012). Microtubules underlie dysfunction in Duchenne muscular dystrophy. *Sci Signal* **5**, ra56.

Kohl P, Bollensdorff C & Garny A (2006). Effects of mechanosensitive ion channels on ventricular electrophysiology: experimental and theoretical models. *Exp Physiol* **91**, 307–321.

Kohl P, Day K & Noble D (1998) Cellular mechanisms of cardiac mechano-electric feedback in a mathematical model. *Can J Cardiol* **14**, 111–119.

Kohl P, Hunter P & Noble D (1999) Stretch-induced changes in heart rate and rhythm: clinical observations, experiments and mathematical models. *Prog Biophys Mol Biol* **71**, 91–138

Kohl P, Nesbitt AD, Cooper PJ & Lei M (2001). Sudden cardiac death by Commotio cordis: role of mechano-electric feedback. *Cardiovasc Res* **50**, 280–289.

Kohl P, Sachs F & Franz MR (2011). *Cardiac Mechano-Electric Coupling and Arrhythmias.* Oxford University Press.

Landesberg A & Sideman S (1994). Coupling calcium binding to troponin C and cross-bridge cycling in skinned cardiac cells. *Am J Physiol Heart Circ Physiol* **266**, H1260–H1271

Li W, Gurev V, McCulloch AD & Trayanova NA (2008). The role of mechanoelectric feedback in vulnerability to electric shock. *Prog Biophys Mol Biol* **97**, 461–478.

Li W, Kohl P & Trayanova N (2004). Induction of ventricular arrhythmias following mechanical impact: a simulation study in 3D. *J Mol Histol* **35**, 679–686.

Li W, Kohl P & Trayanova N (2006). Myocardial ischemia lowers precordial thump efficacy: an inquiry into mechanisms using three-dimensional simulations. *Heart Rhythm* **3**, 179–186.

Liang JL, Huang BS, Yuan GY, Chen Y, Liang FS, Zeng HY, Zheng SX, Cao L, Geng DF & Zhou SX (2017). Stretch-activated channel Piezo1 is up-regulated in failure heart and cardiomyocyte stimulated by AngII. *Am J Transl Res* **9**, 2945–2955.

Limbu S, Hoang-Trong TM, Prosser BL, Lederer WJ & Jafri MS (2015). Modeling local X-ROS and calcium signaling in the heart. *Biophys J* **109**, 2037–2050.

Link MS (2012). Commotio cordis: ventricular fibrillation triggered by chest impact-induced abnormalities in repolarization. *Circ Arrhythm Electrophysiol* **5**, 425–432.

MacDougall JD, Tuxen D, Sale DG, Moroz JR & Sutton JR (1985). Arterial blood pressure response to heavy resistance exercise. *J Appl Physiol* **58**, 785–790.

Markhasin VS, Solovyova O, Katsnelson LB, Protsenko Y, Kohl P & Noble D (2003). Mechano-electric interactions in heterogeneous myocardium: development of fundamental experimental and theoretical models. *Prog Biophys Mol Biol* **82**, 207–220.

Meola F (1879). La commozione toracica. *Gior Internaz Sci Med* **1**, 923–937.

Mitchell JH, Haskell W, Snell P & Van Camp SP (2005). Task Force 8: classification of sports. *J Am Coll Cardiol* **45**, 1364–1367.

Miura M, Nishio T, Hattori T, Murai N, Stuyvers BD, Shindoh C & Boyden PA (2010). Effect of nonuniform muscle contraction on sustainability and frequency of triggered arrhythmias in rat cardiac muscle. *Circulation* **121**, 2711–2717.

Morris CE (2011). Voltage-gated channel mechanosensitivity: fact or friction? *Front Physiol* **2**, 25.

Moss RL & Fitzsimons DP (2002). Frank-Starling relationship: long on importance, short on mechanism. *Circ Res* **90**, 11–13.

Myles RC, Wang L, Kang C, Bers DM & Ripplinger CM (2012). Local beta-adrenergic stimulation overcomes source-sink mismatch to generate focal arrhythmia. *Circ Res* **110**, 1454–1464.

Niederer SA, Campbell KS & Campbell SG (2019). A short history of the development of mathematical models of cardiac mechanics. *J Mol Cell Cardiol* **127**, 11–19.

Niederer SA & Smith NP (2007). A mathematical model of the slow force response to stretch in rat ventricular myocytes. *Biophys J* **92**, 4030–4044.

Noble D, Varghese A, Kohl P & Noble P (1998). Improved guinea-pig ventricular cell model incorporating a diadic space, I_{Kr} and I_{Ks}, and length- and tension-dependent processes. *Can J Cardiol* **14**, 123–134.

Parmley WW & Chuck L (1973). Length-dependent changes in myocardial contractile state. *Am J Physiol* **224**, 1195–1199.

Patterson SW, Piper H & Starling EH (1914). The regulation of the heart beat. *J Physiol* **48**, 465–513.

Petroff MG, Kim SH, Pepe S, Dessy C, Marban E, Balligand JL & Sollott SJ (2001). Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca^{2+} release in cardiomyocytes. *Nat Cell Biol* **3**, 867–873.

Peyronnet R, Nerbonne JM & Kohl P (2016). Cardiac mechano-gated ion channels and arrhythmias. *Circ Res* **118**, 311–329.

Prosser BL, Ward CW & Lederer WJ (2011). X-ROS signaling: rapid mechano-chemo transduction in heart. *Science* **333**, 1440–1445.

Prosser BL, Ward CW & Lederer WJ (2013). X-ROS signalling is enhanced and graded by cyclic cardiomyocyte stretch. *Cardiovasc Res* **98**, 307–314.

Quinn TA, Jin H, Lee P & Kohl P (2017). Mechanically induced ectopy via stretch-activated cation-nonselective channels is caused by local tissue deformation and results in ventricular fibrillation if triggered on the repolarization wave edge (commotio cordis). *Circ Arrhythm Electrophysiol* **10**, e004777.

Quinn TA & Kohl P (2012). Mechano-sensitivity of cardiac pacemaker function: pathophysiological relevance, experimental implications, and conceptual integration with other mechanisms of rhythmicity. *Prog Biophys Mol Biol* **110**, 257–268.

Rahimzadeh J, Meng FJ, Sachs F, Wang JB, Verma D & Hua SZ (2011). Real-time observation of flow-induced cytoskeletal stress in living cells. *Am J Physiol-Cell Physiol* **301**, C646–C652.

Rice JJ, Winslow RL & Hunter WC (1999) Comparison of putative cooperative mechanisms in cardiac muscle: length dependence and dynamic responses. *Am J Physiol Heart Circ Physiol* **276**, H1734–H1754.

Robison P, Caporizzo MA, Ahmadzadeh H, Bogush AI, Chen CY, Margulies KB, Shenoy VB & Prosser BL (2016). Detyrosinated microtubules buckle and bear load in contracting cardiomyocytes. *Science* **352**, aaf0659.

Sarnoff SJ, Mitchell JH, Gilmore JP & Remenssnyder JP (1960). Homeometric autoregulation in the heart. *Circ Res* **8**, 1077–1091.

Schlomka G & Hinrichs A (1932). Experimentelle Untersuchungen über den Einfluß stumpfer Brustkorbverletzungen auf das Elektrokardiogramm. *Zeitschrift für die Gesamte Experimentelle Medizin* **81**, 43–61.

Schonleitner P, Schotten U & Antoons G (2017). Mechanosensitivity of microdomain calcium signalling in the heart. *Prog Biophys Mol Biol* **130**, 288–301.

Schott E.(1920). Über Ventrikelstillstand (Adam-Stokes'sche Anfälle) nebst Bemerkungen über andersartige Arrhythmien passagerer Natur. (On ventricular standstill [Stokes-Adams attacks] and other arrhythmias of temporary nature.). *Dt Arch klin Med*, 211–229.

Seo K, Rainer PP, Lee DI, Hao S, Bedja D, Birnbaumer L, Cingolani OH & Kass DA (2014). Hyperactive adverse mechanical-stress responses in dystrophic heart are coupled to TRPC6 and blocked by cGMP-PKG modulation. *Circ Res* **114**, 823–832.

Shaw J, Izu L & Chen-Izu Y (2013). Mechanical analysis of single myocyte contraction in a 3-D elastic matrix. *PLoS One* **10**, e75492.

Smith N & Niederer S (2016). Is computational modeling adding value for understanding the heart? *J Mol Cell Cardiol* **96**, 1.

Solaro RJ (2007). Mechanisms of the Frank-Starling law of the heart: the beat goes on. *Biophys J* **93**, 4095–4096.

Solovyova O, Katsnelson LB, Konovalov PV, Kursanov AG, Vikulova NA, Kohl P & Markhasina VS (2014). The cardiac muscle duplex as a method to study myocardial heterogeneity. *Prog Biophys Mol Biol* **115**, 115–128.

Starling EH (1915). *The Linacre Lecture on the Law of the Heart.* Longmans, Green and Co., London.

Suchyna TM, Johnson JH, Hamer K, Leykam JF, Gage DA, Clemo HF, Baumgarten CM & Sachs F (2000). Identification of a peptide toxin from *Grammostola spatulata* spider venom that blocks cation-selective stretch-activated channels. *J Gen Physiol* **115**, 583–598.

ter Keurs HE & Boyden PA (2007). Calcium and arrhythmogenesis. *Physiol Rev* **87**, 457–506.

ter Keurs HE, Wakayama Y, Sugai Y, Price G, Kagaya Y, Boyden PA, Miura M & Stuyvers BD (2006). Role of sarcomere mechanics and Ca^{2+} overload in Ca^{2+} waves and arrhythmias in rat cardiac muscle. *Ann N Y Acad Sci* **1080**, 248–267.

Trayanova NA & Rice JJ (2011) Cardiac electromechanical models: from cell to organ. *Front Physiol* **2**, 43.

Tyberg JV, Parmley WW & Sonnenblick EH (1969). In-vitro studies of myocardial asynchrony and regional hypoxia. *Circ Res* **25**, 569–579.

Van Wagoner DR (1993). Mechanosensitive gating of atrial ATP-sensitive potassium channels. *Circ Res* **72**, 973–983.

von Anrep G (1912). On the part played by the suprarenals in the normal vascular reactions of the body. *J Physiol* **45**, 307–317.

Wakayama Y, Miura M, Stuyvers BD, Boyden PA & ter Keurs HE (2005). Spatial nonuniformity of excitation-contraction coupling causes arrhythmogenic Ca^{2+} waves in rat cardiac muscle. *Circ Res* **96**, 1266–1273.

Wakayama Y, Miura M, Sugai Y, Kagaya Y, Watanabe J, ter Keurs HE & Shirato K (2001). Stretch and quick release of rat cardiac trabeculae accelerates Ca^{2+} waves and triggered propagated contractions. *Am J Physiol Heart Circ Physiol* **281**, H2133–H2142.

Wiegner AW, Allen GJ & Bing OH (1978). Weak and strong myocardium in series: implications for segmental dysfunction. *Am J Physiol* **235**, H776–H783.

Zhang H, Walcott GP & Rogers JM (2018). Effects of gadolinium on cardiac mechanosensitivity in whole isolated swine hearts. *Sci Rep* **8**, 10506.

Zhao H, Yu Y, Wu X, Liu S, Liu B, Du J, Li B, Jiang L & Feng X (2017). A role of BK channel in regulation of Ca^{2+} channel in ventricular myocytes by substrate stiffness. *Biophys J* **112**, 1406–1416.

Zile MA & Trayanova NA (2017). Myofilament protein dynamics modulate EAD formation in human hypertrophic cardiomyopathy. *Prog Biophys Mol Biol* **130**, 418–428.

Additional information

Competing interests

All authors have no conflict of interest.

Author contributions

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containing published data. Conception or design of the work: L.T.I., P.K., D.M.B. and Y.C.I.; drafting the work or revising it critically for important intellectual content: L.T.I., P.K., P.A.B., M.M., T.B., N.C., N.T., D.M.B. and Y.C.I. All authors have approved the final version of the manuscript. Lead authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

Anrep effect, cardiac muscle, excitation-contraction coupling, Frank-Starling, mechano-chemo-transduction, mechano-electric coupling, myocardial contractility