A network-oriented perspective on cardiac calcium signaling

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George CH, Parthimos D, Silvester NC. A network-oriented perspective on cardiac calcium signaling. Am J Physiol Cell Physiol 303: C897–C910, 2012. First published July 25, 2012; doi:10.1152/ajpcell.00388.2011—The normal contractile, electrical, and energetic function of the heart depends on the synchronization of biological oscillators and signal integrators that make up cellular signaling networks. In this review we interpret experimental data from molecular, cellular, and transgenic models of cardiac signaling behavior in the context of established concepts in cell network architecture and organization. Focusing on the cellular Ca\(^{2+}\) handling machinery, we describe how the plasticity and adaptability of normal Ca\(^{2+}\) signaling is dependent on dynamic network configurations that operate across a wide range of functional states. We consider how (mal)adaptive changes in signaling pathways restrict the dynamic range of the network such that it cannot respond appropriately to physiologic stimuli or perturbation. Based on these concepts, a model is proposed in which pathologic abnormalities in cardiac rhythm and contractility (e.g., arrhythmias and heart failure) arise as a consequence of progressive desynchronization and reduction in the dynamic range of the Ca\(^{2+}\) signaling network. We discuss how a systems-level understanding of the network organization, cellular noise, and chaotic behavior may inform the design of new therapeutic modalities that prevent or reverse the disease-linked unraveling of the Ca\(^{2+}\) signaling network.

Cardiac cell signaling is underpinned by the coupling of biological oscillators, multitasking signal integrators, and parallel-processing pathways and thus lends itself to a systems-level interrogation. In this review, we interpret findings from experimental models of cardiac (dys)function in the context of established principles of cell networks to illustrate the intricate organization of molecular and cellular events that control normal cardiac cell behavior. We extend these concepts into pathological scenarios and propose a model of cardiac dysfunction that is linked to the progressive desynchronization of cellular processes and the reduced plasticity\(^1\) of the signaling network. Finally, we consider how our emerging understanding of Ca\(^{2+}\) signaling from a network perspective may be useful in guiding new therapeutic approaches for heart disease.

Dynamic Control of Signal Transmission in Cell Signaling Networks

Cell signaling networks are constructed from the hierarchical organization of molecular components, termed nodes, into modules that can perform higher-order functions such as switching, information storage, and amplification (58, 67, 88, 89, 101, 108, 152). In a cardiac cell context, nodes [that include cAMP- and Ca\(^{2+}\)-dependent protein kinases (PKA and PKC), Ca\(^{2+}\)/calmodulin-dependent kinase (CaMKII), and ryanodine receptor type 2 (RyR2)] are assembled into modules [e.g., the macromolecular complex that regulates RyR2-dependent Ca\(^{2+}\)]
release (13, 28, 32, 94)) via protein-protein interaction and compartmentalization in specialized environments (11, 29, 171). From the precise configuration of these nodes and modules emerge “small world” networks that are characterized by delay, robustness, plasticity, synchronization, and enhanced signal-speed propagation (3, 145, 162) (Table 1).

Tools such as topological mapping and graph theory have enabled the architecture of cell networks to be visualized as biological circuit diagrams in which signaling information is transmitted through densely clustered, highly interconnected pathways (39, 42, 157, 160, 174). However, such biological circuitry is not “hardwired” but rather is distinguished by the plasticity of molecular interaction that can be reconfigured on an activity-dependent basis in response to defined signal inputs (2, 68, 96). Thus cellular networks evolve under the influence of local (e.g., intracellular) and long-distance (e.g., intercellular) factors that include electrical and metabolic cues, hormonal exposure, hemostatic environment, and their functional coupling to neighboring cells.

The proper routing of signals through cell networks depends on the exquisite interactivity of nodes and modules yet paradoxically these signaling hubs must operate relatively independently of each other. Recent work has shown that the relative abundance of proteins within a network is an important factor that determines the functional independence of modules despite them being embedded in a “globally connected topology” (96, 97, 175).

Protein abundance is the product of synthetic and degradative pathways and is synchronized with other aspects of cardiac signaling network via mechanisms such as excitation-transcription coupling (ETC), in which gene transcription is regulated by the cellular contractile machinery (7, 33, 170), transcription-translation feedback looping (TTFL) (128), and transcriptional reinforcement (180). These mechanisms effectively act as cellular surveillance systems that ensure the responsive tuning of protein abundance, such that proteins are not overexpressed (and thus damp signal transmission), nor are they underrepresented thereby compromising their biological utility. For example, the expression levels of proteins that act as scaffolds for signal transduction complexes are typically kept low to prevent promiscuous nonspecific interactions with cytoplasmic proteins (62).

Consequently, altered protein abundance or the derangement of protein localization within cells would be anticipated to profoundly reroute signal flow in the signaling network. In a cardiac setting, there is substantial evidence supporting the role of altered cellular abundance and/or distribution of Ca2+ signaling proteins in both chronic and acute heart disease. In end-stage heart failure, severe contractile abnormalities are associated with grossly perturbed Ca2+-handling dysfunction resulting from changes in the cellular abundances of L-type Ca2+ channels (LTCC), phospholamban (PLB), RyR2, sarcoplasmic reticulum Ca2+-ATPase (SERCA), and Na+/Ca2+ exchanger (NCX) (65). Elsewhere, the altered interaction of regulatory co-proteins in the RyR2 macromolecular complex [e.g., FKBP12.6, phosphodiesterase 4D (PDE4D), and protein phosphatase 1 (PP1)] is controversially proposed as an early causative event in atrial fibrillation, heart failure, and stress-induced ventricular tachycardia (VT) (14, 44, 64, 79, 95, 121, 156, 163, 164). In the context of monogenic arrhythmia syndromes, long-QT syndrome type 4 (LQT4) is caused by mutations in the cytoplasmic adaptor protein ankyrin B that result in the sarcolemmal disordering of otherwise functional Na-K-ATPase and NCX (102).

These examples of perturbed signaling serve to highlight the inherent limitations of any experimental system that involves the alteration of protein levels or the modification of their biological activities (e.g., transgenic overexpression or knock-
down/knockout strategies) (26). In these scenarios, the alteration of protein abundance or activity may fundamentally "re-wire" the physical (spatial aspect of a component) and logical (route of signal transmission) topological status of the network to a configuration very different to that normally occurring in situ. Consequently, when devising experimental approaches to interroge cell signaling networks, the utility and appropriateness of animal models in which network structure may have been reconfigured (for example, following the targeted ablation of a phosphorylation motif) should be borne in mind. We expand upon this concept in the section Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State.

Synchronization of Cellular Processes and Uncoupling as a Pathogenic Event

In the section Dynamic Control of Signal Transmission in Cell Signaling Networks, we briefly described some of the mechanisms controlling protein expression to illustrate synchronization at a horizontal level i.e., synchronization of mechanisms that converge on a common process. However, network complexity emerges from the layering of multiple horizontal levels (e.g., protein expression, fluxes through ion channels) into vertical network structures that coordinate more diverse processes (e.g., ion fluxes feeding into intercellular communication or protein expression governing multicellular susceptibility to apoptosis). In the biological context, in principle each layer of connectivity can be associated with a specific set of pathologies, e.g., defects in contractile, electrical, or energetic behavior in the diseased heart.

One of the best known examples of horizontal network structure in cardiac signaling is the synchronization of membrane and intracellular Ca^{2+} oscillations (75). The sarcoplasmic reticulum (SR), the main intracellular Ca^{2+} reservoir, is inherently predisposed to spontaneous RyR2-dependent Ca^{2+} release and functions as an internal Ca^{2+} oscillator (termed the Ca^{2+} clock) (78, 158, 172). In normal ventricular myocytes the Ca^{2+} clock is suppressed by entrainment with sarcolemmal ion fluxes (membrane clock) mediated by the hyperpolarization-activated cyclic nucleotide (HCN) channels ("funny" current, I_f) (76, 77). Interestingly, in sinoatrial node cells (SANC) the heightened activities of PKA and CaMKII increase RyR2 phosphorylation (among other downstream targets) and partially uncouple the Ca^{2+} and membrane clocks thus driving spontaneous oscillatory behavior (which manifests as pacemaker activity) (76, 77).

The horizontal coupling of SR and sarcolemmal oscillators is embedded in a network organization with mitochondrial oscillation, the synchronization of which is dependent on both the physical coupling of mitochondria (71, 124) and the Ca^{2+} microenvironment at the SR/mitochondrial interface (34, 122, 126, 147). In turn, synchronized SR, sarcolemmal, and mitochondrial oscillations are connected to hubs of cellular metabolism such as the mammalian target of rapamycin resistance (mTOR)/AMP-activated protein kinase (AMPK) axis that integrates nutrient availability, gene expression, and protein synthesis (5, 41, 87, 91, 139). In this example of vertical network structure, energy-dependent processes at the SR (e.g., SERCA-dependent Ca^{2+} uptake into the SR) can be matched to energy production (e.g., mitochondrial ATP synthesis) with both processes being responsive to the cellular metabolic environment (e.g., via mTOR/AMPK "surveillance").

Consequently, the desynchronization of horizontal or vertical network structures is considered a pivotal event in cardiac disease (18). With reference to the SR/sarcolemmal entrainment of Ca^{2+} oscillation, failing ventricular cardiomyocytes exhibit abnormally high levels of RyR2 phosphorylation (a pathogenic mimic of the situation in SANC) that uncouples the SR and surface membrane clocks (i.e., results in a "de-repressed" Ca^{2+} clock) and predisposes the system to electrical instabilities (12). This idea of pathogenic desynchronization is reinforced by the demonstration that the physical and functional uncoupling of mitochondria is arrhythmogenic (21). Alternatively, it is plausible that arrhythmias may arise as a consequence of a loss of entrainment between SR, mitochondrial, and cytoplasmic events.

The negative consequences of uncoupling vertical network structure is also illustrated by the loss of cardiomyocytes by apoptosis, now recognized as a causal driver of cardiac dysfunction (130, 140). It has recently been shown that the susceptibility of a multicellular population to apoptosis is linked to molecular and cellular divergence within the population (16, 22, 104, 120, 137). Consequently, cellular uncoupling, which is also accelerated by the desynchronization of dynamic Ca^{2+}-modulated processes (59), disrupts both the electrical connectivity in the myocardium (31, 161) and the intercellular synchronization of signal pathways. Thus in the context of cardiac disease, apoptosis may be triggered in response to an irrecoverable loss of synchronization between multiple cellular processes and/or between populations of cells. In another example of the pathogenic unraveling of vertical network structure, the uncoupling of cellular metabolism from sarcolemmal Na, K^{+}, and Ca^{2+} ionic fluxes exacerbates the rhythm and contractile perturbations in heart failure (66, 106, 144, 155).

To further explore such disease-linked network "unraveling," experimental approaches to untangle the complex web of nonlinear interactions that exist within and between cellular processes should not consider events in terms of linear "cause-and-effect"-type scenarios (23). Indeed, network complexity emerges from enmeshing discrete processes in a global topological network and thus the ability to experimentally track causal events that trigger successive network dysfunction requires a deep knowledge as to how each process is functionally integrated in the network structure (see section Dynamic Control of Signal Transmission in Cell Signaling Networks). To this end, we have begun investigating the functional behavior of individual cardiac cells within multicellular syncytia (135). In the next section, we expand on these concepts and describe how the unraveling of network synchronization and organization may fundamentally drive the onset and progression of cellular dysfunction and cardiac disease.

The Pathogenic Consequences of Reduced Network Complexity

So far, we have described cardiac Ca^{2+} signaling in terms of complexity, plasticity, dynamic range, oscillatory behavior, and synchronization. These descriptive features are recognized hallmarks of a system with an intrinsic propensity for chaotic behavior (51, 133). Indeed cardiac function is dependent on
Inherently chaotic processes underpinning a homeostatic state that is characterized by a very high level of complexity and the perpetual approximation of stable equilibria over a wide dynamic range (43, 51, 53). Thus although it might be presumed that such a dynamic homeostatic state capable of adopting numerous functional configurations would be easy to perturb, the hierarchical organization of normal cell signaling networks in-builds a redundancy and robustness to create a scenario that is paradoxically both highly plastic and inherently stable.

However, if there were chronic alterations within the network, for example the long-term reduction in the abundances or activities of key signaling proteins (see section Dynamic Control of Signal Transmission in Cell Signaling Networks), the network would be forced to operate across a diminished number of functional states (i.e., restricted dynamic range). Thus it has been proposed that cardiac dysfunction is the product of a tractable loss of synchronization and a reduction in chaotic flexibility (51, 52, 113, 133, 166).

Over 30 years ago Mackey and Glass showed that destabilization of homeostatic physiological systems with an intrinsic propensity for chaotic behavior can generate sustained desynchronized oscillations (52, 90). They coined the term “dynamical diseases,” which are characterized by “the operation of a basically normal control system in a region of physiological parameters that produces pathological behavior” (90). This concept was advanced by Chialvo and colleagues (24), who described a chaotic disease-linked trajectory in which the speed of functional decline was predicted by the initial conditions. These studies beautifully describe the “out-of-gamut” consequences of normal physiological adaptation and galvanize the view that a system’s susceptibility to perturbation (e.g., the response to a proarrhythmicogen trigger) is dependent on its basal characteristics and stability. These concepts also raise the issue of predictability and this is discussed in more detail in the section Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State.

In Fig. 1A we describe a schematic model in which the progressive decline in cardiac function is linked to successive reductions in cellular network dynamic range. What are the factors that likely contribute to the progressive nature of diminished plasticity and complexity at the cellular level? Earlier in this section we considered the potential role of imbalanced protein levels or abundances in signaling pathways, and, intuitively, the gradual diminution of nodal protein abundance would be consistent with the progressive reduction in the dynamic range of the network.

It is also plausible that a reduction in complexity of a graded output would restrict functional plasticity. Here we consider the example of β-adrenoceptor (β-AR) signaling that modulates cardiac contractility and rhythmicity through the synchronized activation of multiple downstream targets including RyR2, PLB, and LTCC (123) via modulation by PKA and CaMKII-dependent phosphorylation (159, 178, 179). If we borrow Lim’s concept of protein kinases (on) and phosphatases (off) as digital “writers” and “erasers” (81) that modulate the approximately thirty phosphorylation substrates in the cascade, then the β-AR pathway could, in principle, support a complexity of approximately 1 billion permutations of phosphorylation states (230). So, even if we consider it very unlikely that every possible phosphorylation state could be achieved in situ, or that there would be a distinct functional outcome for each eventual phosphorylation configuration, it is easy to appreciate the enormous functional plasticity inherent in “normal” β-AR-dependent modulation of heart rate, rhythm, and contractility.

In contrast, chronic cardiac diseases are frequently associated with derangement in β-AR signaling that renders the modulation of heart rate and contractile function much less flexible. There is substantial evidence to support the idea that the graded output (or operational range) of the β-AR cascade becomes diminished by virtue of disease-linked alterations in the patterns of phosphorylation/dephosphorylation of downstream effectors. However, given the possible number of configurations that could be established in the β-AR cascade it is very unlikely that β-AR-linked phosphorylation goes wrong in an “all-or-none” manner. Rather, it is much more likely that successive (small) defects in phosphorylation, which arise as a consequence of altered expression or subcellular mislocalization of phosphatases or kinases or the accessibility of phosphorylation substrates, are linked to a progressive, incremental reduction in the dynamic range of the β-AR pathway. It is also conceivable that the graded loss of output could be the result of less directed (more randomly uniform) phosphorylation. Such scenarios could be experimentally tested through phosphoproteomic analysis using models of progressive cardiac dysfunction [e.g., transverse aortic constriction (TAC) induced heart failure].

In Fig. 1A, we describe a disease-linked trajectory involving the transition through discrete dysfunctional states (I–IV), with each state characterized by an incremental reduction in dynamic range and shifted further away from the initial “homeostatic” state (N). This model is consistent with the concepts drawn from chaotic behavior suggesting that dysfunctional phenotypic configurations arise via the inability of the network to appropriately reestablish stable steady states in response to (mal)adaptive changes that lie within the normal (physiologic) range (43, 52, 90). There is precedent for diminished physiologic function being attributed to the loss of complexity (54, 83). The depiction of four dysfunctional states (I–IV) in Fig. 1 represents an oversimplification of the process, and the progression from normal state to phenotypic dysfunction would likely occur via numerous discrete transitions, some of which (at least in the earlier stages) may be associated with an apparently normal phenotype. In the section Towards an Understanding of Reduced Dynamic Range, Maladaptation and the Disease-Linked Network State, we discuss how phenotypic deterioration may arise from an altered basal Ca2+ network “state” with reference to the pathogenic trajectory of cardiac dysfunction in the SERCA-knockout mouse.

In the section Synchronization of Cellular Processes and Uncoupling as a Pathogenic Event, we considered that attempting to describe the pathogenic unraveling of the cellular Ca2+ signaling network in terms of linear cause-and-effect-type thinking is of little use. Consequently, it is more useful to define the events that underpin these transitional reconfigurations between “healthy” and “diseased” states from a dynamical perspective. Specifically, transitions in a multidimensional parametric space can result in reductions in phase-space volume and/or system dimension. Pathological situations may arise owing to either of these transitions, and subtle experimental investigation on causality is required to distinguish between the two scenarios. Ultimately, however, the two properties are interrelated and sustained reduction in phase-space...
Fig. 1. Progressive and incremental reduction in system dynamic range is associated with dysfunction in coupled systems. A: incremental destabilization of a state defined as normal (N) through successive pseudo-stable states (I–V, solid black line) arises as a consequence of persistent (mal)adaptation that attempts to rebalance the network following perturbation. Intuitively, progressive network destabilization is expected to be associated with successively greater dysfunction (i.e., increasing step size) owing to the residual effect of successive malfunctions. In this scheme, the first two pseudo-stable states (I, II) are associated with small changes in interlinked systems [e.g., arrhythmia susceptibility (blue), cell death (green), and metabolic dysfunction (orange)], but a normal phenotype is maintained [cardiac function (red)]. However, the crossing of a threshold of system dysfunction, possibly as a consequence of failure to attenuate perturbation, accelerates these abnormalities and causes a steep decline in cardiac function. This scheme also illustrates that arrhythmia-linked genetic mutations may profoundly reduce basal network complexity (gray line) and introduce a heightened propensity to arrhythmia (dashed blue line) that is exacerbated by progression through successive pseudo-stable states (I–IV). B: the progressive instability and dysfunction described in A is reproduced in bifurcation diagrams generated by a model of the third-order system of differential equations describing cardiovascular dynamics developed by Parthimos and colleagues (111). This mathematical model of Ca$^{2+}$ cycling incorporates terms that describe the activities of voltage- and receptor-operated Ca$^{2+}$ channels (VOCC and ROC), Na$^+$/Ca$^{2+}$ exchanger (NCX), Ca$^{2+}$ extrusion via plasma membrane ATPase (PMCA), sarcoplasmic (SR) reticulum Ca$^{2+}$-ATPase (SERCA), and ryanodine receptor type 2 (RyR2) (111). Here we plotted the loci of maxima and minima of Ca$^{2+}$ oscillatory activity for values of RyR2 activity (an index of the open state probability of RyRs or alternatively, proportional to the number of RyRs on the SR membrane) in a single cell (red lines) and two Ca$^{2+}$-coupled cardiac cells (blue lines/points). In each scenario, continuous lines correspond to periodic solutions, whereas widely distributed points represent chaotic solutions or other hallmark types of nonlinear dynamics. Modeling of Ca$^{2+}$ dynamics in single cells, where there is zero potential for intercellular desynchronization, results in entirely periodic solutions (red lines). Specific patterns of oscillatory behavior at various values of RyR2 activity (indicated by arrows) are shown in the series of panels N and I–IV. Inset, periodic windows of profoundly reduced complexity and low periodicity (gray shading) are superimposed over the bifurcation diagrams.
volume will result in lower dimension by the suppression of underlying physiological processes. From a practical or diagnostic point of view, quantification of a system’s dimension is a potent way to establish a lower limit for the number of dominant elementary processes involved in a biological mechanism (148). Put more simply, the properties of maladapted transitional states are predicated on preexistent scenarios and depend on both a reduction in phase-space volume and a system dimension that ultimately results in a state of reduced complexity.

Importantly, our concept of a pathologic trajectory characterized by the progressive reduction in complexity illustrated in Fig. 1A is recapitulated by the mathematical modeling of cellular Ca$^{2+}$ oscillations in response to the isolated perturbation of a single molecular component (RyR2) (Fig. 1B). The profile of the dynamic range associated with altered RyR2 activity in a single cell (red) and in two coupled cells (blue) is essentially the same, reflecting the fundamental similarities in Ca$^{2+}$ handling dynamics in these two scenarios. However, intercellular coupling unmask a new level of dynamic complexity and promotes the emergence of chaotic behavior (illustrated as the fracture of continuous blue lines into discrete points at RyR2 activity $>1.2$). Importantly, the same dynamical profile is maintained in numerical simulations of multicellular arrays. Although such configurations can exhibit emergent types of behavior, such as wave formation, the overall complexity of the system is ultimately limited by entrainment of its components.

In this coupled-cell scenario, panel N presents an example of apparently “regular” oscillatory behavior, which nevertheless resides on the border of chaotic dynamics (Fig. 1B). This positioning [akin to the normal state (N) in Fig. 1A] enables cardiac myocytes to operate with the requisite flexibility across a wide range of oscillatory responses through small variations of RyR2 activity (between values of 1 and 1.2, i.e., a 20% alteration in RyR2 function). Progressively larger (dysfunctional) augmentation of RyR2 activity ($>1.4$, Fig. 1B) results in irregular oscillatory activity, characterized by a successive reduction in amplitude (panels I through III). Eventually, oscillations in cytosolic Ca$^{2+}$ are attenuated into stages of low complexity and amplitude (e.g., panel IV, RyR2 activity $>2.2$) that lack the inherent adaptability of healthy cardiac dynamics (cf. panel N). Note that the modeling data shown in Fig. 1B reproduce the accelerated functional decline at advanced stages of perturbation (RyR2 activity between values of 1.8 and 2.2) that were predicted to occur as a consequence of reduced dynamic range in Fig. 1A.

Figure 1A also depicts the progression from the normal state (N) to successively perturbed states (I–IV) occurring via sharp transitional points. Such sharp alteration in behavior (termed a phase-transition or crisis) is characteristic of chaotic systems (43), and this phenomenon is reproduced in our mathematical modeling (Fig. 1B, inset). Crises can be precipitated by increased noise (18), and thus the level of cellular noise may be an important determinant of the rapidity of pathologic decline i.e., transition between discrete maladapted states may be sensitive to noise in the network. Noise has been described as “unavoidable stochastic fluctuation” (36), and the functionality of a system has typically been considered dependent on its ability to attenuate (damp) and filter noise (128). Recent experimental evidence strengthens the argument that the failure to attenuate cellular noise via altered protein expression or mislocalization accelerates network dysfunction (96, 97, 175) (see also the section Dynamic Control of Signal Transmission in Cell Signaling Networks). Moreover, given the generalized abnormalities in Ca$^{2+}$ signaling protein expression and activity in chronic heart disease (65), a causal link between the progressive alterations in protein and accelerated functional decline in the later stages of disease can be envisaged. Not all noise is bad though; noise may also be canalized for constructive purposes (36, 72, 116, 118, 119), enabling cellular processes to maintain a high sensitivity to signals over a wide dynamic range (128) or enhancing weak stimuli to cross biological thresholds and entrain large-scale fluctuations (stochastic resonance) (168). Thus a basal level of cellular noise may fundamentally contribute to the wide dynamic range of the “normal” signaling network.

Figure 1 also introduces the concept of genetic accelerators—mutations in signaling components that perturb the homeostatic state and establish a persistent network-level basal dysfunction. We propose that mutations create an intrinsically different basal homeostatic state that is characterized by reduced complexity and diminished plasticity. Consistent with this idea, there are several examples of genetic mutations that provoke malignant arrhythmias in response to normal physiological stimuli. For example, mutations in calsequestrin (CSQ) (74), PLB (132), LTCC (143), and ankryin B (102) are associated with severe arrhythmias in response to moderate exercise or stress. Recent studies on the mechanisms of mutation-linked RyR2 dysfunction in stress-induced VT have shown that mutations perturb intramolecular interactions that stabilize RyR2 (48, 153). Subsequently, the resultant channel instability provokes an altered basal Ca$^{2+}$ handling state (38) that reduces the threshold for severe stress-induced arrhythmia (47, 115, 149, 173).

Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State

We have proposed a model in which the trajectory to ever greater phenotypic dysfunction is a consequence of the inability of an inherently chaotic system, restricted by diminished dynamic range, to reestablish the normal steady state. In this section we consider possible mechanisms that may initiate the transition from the normal plastic, adaptable state into a maladapted state characterized by desynchronization of cellular processes and reduced functional plasticity.

The remarkable plasticity and adaptability of the cardiac Ca$^{2+}$ signaling machinery is exemplified by the cardiac phenotypes in mice following the cardiac-specific ablation of NCX (61) and the near-total elimination of SERCA (4). In NCX-null mice there is a profound adaptation of sarcolemmal ion fluxes that maintains functional output and apparently normal cardiac function (61, 114). In the SERCA2-deficient mouse, in which SERCA levels are reduced by $\sim95\%$ four weeks after excision of the serca2 locus, effectively normal heart function is preserved because of the concomitant augmentation of LTCC and NCX activities (4).

Indeed, central to the perspectives offered in this review is that within the framework of highly interconnected cellular pathways such extraordinary levels of functional adaptation in Ca$^{2+}$ cycling can only be achieved by altering the behavior of
other intimately linked processes. Accordingly, the normalization of steady state via negative and positive feedback loops and cross-talk can lead to “undershooting” and “overshooting” readjustment (dynamical hysteresis) that eventually settles into a new oscillatory steady state (functional compensation). This new state may be associated with a perceptibly normal phenotype, but it is fundamentally distinct from the “normal” basal state. Put more simply, functional (mal)adaptation of the signaling network in an attempt to maintain normal steady state introduces a systems-level perturbation. We have termed this maladapted state the “pseudo-stable state” (44). This concept is further illustrated in Fig. 2 in which the functional readjustment (dynamical hysteresis) that eventually settles into a new oscillatory steady state (functional compensation). This new state may be associated with a perceptibly normal phenotype, but it is fundamentally distinct from the “normal” basal state (Fig. 2A). Data from the SERCA knockout mouse serve to reinforce the idea that apparently normal cardiac function can be underscored by a different mode of Ca\(^{2+}\) signaling that predisposes the system to dysfunction [i.e., the “4 week” SERCA phenotype represents a pseudo-stable state (see Fig. 2D)]. There are equivalently low levels of SERCA abundance (<5% normal levels) between weeks 4 and 7 post-SERCA knockout, yet there is a steep decline in cardiac performance from near-normal function at 4 weeks to severe heart failure at 7 weeks. Clearly, this is an extreme example of the pathogenic decline described in the section The Pathogenic Consequences of Reduced Network Complexity and in Fig. 1, AJP-Cell Physiol • doi:10.1152/ajpcell.00388.2011 • www.ajpcell.org

![Figure 2](image_url)

**Fig. 2.** System balance, homeostasis, and activation: the emergence of pseudo-stable states via functional (mal)adaptation. Using a simplified scheme of four components in the surface membrane (gray bar) and SR (blue bar) that mediate directional ion flux (arrows), we show that the proper transition from the basal state (A) to the fully activated state (B) depends on the synchronization of all components. The isolated perturbation of a single component in this system (triangle) normally leads to a transiently unstable state (C) that is either restored to the basal state (by subsequent modulation of this component) (A) or exists as an acute intermediate step en route to full activation (B). However, under some circumstances (e.g., persistent changes in abundance or activity of a component), functional adaptation renders the system functionally rebalanced such that it appears stable (D). This rebalanced state (illustrated here as a compensatory reconfiguration of ion fluxes) is fundamentally different from the basal (homeostatic) state (A). We term the state depicted in D as a pseudo-stable state. Black = basal activity, red = reduced activity, green = augmented activity. E: the emergence of pseudo-stable states through functional (mal)adaptation was tested by the mathematical model of cellular Ca\(^{2+}\) cycling described in Fig. 1B. We modeled the functional relationship between the SERCA pump and the ryanodine-sensitive Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism by plotting a family of bifurcation diagrams (as in Fig. 1B) for increasing values of SERCA pump activity. Increasing the activity of RyR2 shifts the oscillatory activity from an assumed “normal” operating point (OP2) to a suboptimal level of activity defined as OP2 (red arrow). Compensatory upregulation of the SERCA pump is potentially able to restore the amplitude of Ca\(^{2+}\) oscillations to its original level, thus reaching a new stable operating point (OP3, green arrow). The text section Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State describes OP3-like scenarios that are characterized by apparently normal phenotype but are underscored by fundamentally different network properties (44).
but it is intriguing to note that the deterioration of cardiac function between weeks 4 and 7 in this model is accompanied by an unsustainable increase in ATP consumption (80), altered cellular ultrastructure (146), and increased apoptosis (84). This phenotypic deterioration, which would suggest the profound uncoupling/de-synchronization of cellular processes from an already altered (pseudo-stable) homeostatic state (i.e., the “4-week” SERCA-knockout mouse) provides further experimental corroboration of the scheme described in Fig. 1. Although we are not aware of any reports that the cardiac NCX-knockout mouse is predisposed to overt cardiac dysfunction, the reduced L-type Ca\(^{2+}\) current and abbreviated action potential associated with the functional adaptation in this model (114) establishes a pseudo-stable state (cf. Fig. 2D) that would be anticipated to heighten arrhythmia susceptibility in some conditions (i.e., increased tendency to revert to the unstable arrhythmogenic state depicted by Fig. 2C).

There are other examples of the adverse consequences of functional (mal)adaptation. Michael and colleagues (100) described how the chronicity of homeostatic adaptation of multiple sarcoplasmic K\(^{+}\) currents mediating the repolarization phase of the action potential (repolarization reserve) is arrhythmogenic. In the context of heart failure, Belevych and colleagues (9) recently demonstrated that reestablishing “normal” Ca\(^{2+}\) cycling through multicomponent adaptation involving CaMKII, RyR2, and L-type Ca\(^{2+}\) channels provoked cellular ionic instability. At a molecular level, the structural reconfiguration of RyR2 caused by the genetic deletion of exon 3 (85) lowers the threshold for channel activation that underlies malignant arrhythmias (17). At the cellular level, the compensatory mechanisms that normalize caffeine-evoked RyR2-dependent Ca\(^{2+}\) perturbation in isolated rat cardiomyocytes (150) augment the propensity for isoproterenol-induced Ca\(^{2+}\) dysfunction (154). Elsewhere, adaptive changes in Ca\(^{2+}\) cycling following prolonged exposure to low-dose caffeine resulted in an increased susceptibility of mouse HL-1 cardiac cells to apoptosis (50).

In Fig. 2B we present modeling data that reconcile the maladapted (pseudo-stable) state with apparently normal Ca\(^{2+}\) cycling. Recapitulating the experimental demonstration of an adaptive shift in SERCA activity as a result of increased cytosolic Ca\(^{2+}\) (169), our modeling shows that the right-shifted trajectory (red arrow) from a normal operating point (OP1) to another operating point 2 (OP2) in response to RyR2 activation can be reversed by very small alterations in SERCA activity (green arrow). However, although certain aspects of the new rebalanced operating point (OP3) may resemble the original configuration, the two states remain fundamentally apart in terms of the underlying dynamics and will therefore respond differently to further pathogenic perturbations, i.e., the network state represented by OP3 is a maladapted pseudo-stable configuration as described in Fig. 2D.

We further illustrate this concept experimentally using the spontaneous Ca\(^{2+}\) oscillatory behavior of an HL-1 cardiomyocyte syncytium (Fig. 3). Focusing on a seven-cell cluster (Fig. 3A), the basal state is characterized by a near-identical pattern of Ca\(^{2+}\) oscillations that exhibit a high level of intercellular synchronization (Fig. 3, B and C). Following the application of caffeine, an RyR2 agonist that triggers massive SR Ca\(^{2+}\) release (Fig. 3B and Supplemental Movie; Supplemental Material for this article is available online at the Journal website), a new steady state is established that is characterized by a reduced intercellular synchronization of Ca\(^{2+}\) oscillation (Fig. 3, C and D), a reduced oscillatory frequency (Fig. 3F), and a diminished Ca\(^{2+}\) release amplitude (Fig. 3G). Notably, this new oscillatory steady state exists against a background of unchanged basal Ca\(^{2+}\) levels (Fig. 3E).

Following on from the arguments presented in the section The Pathogenic Consequences of Reduced Network Complexity relating to the “predictability” of chaotic systems, we hypothesize that the response of each of these cells to a pharmacological stimulus (in this instance caffeine) could be predicted from a detailed understanding of their basal network state. To this end, we are using various mathematical techniques (i.e., modeling and numerical simulations, nonlinear statistical tools) (56, 57) to explore the overall complexity of the basal cellular state in functionally coupled cardiomyocyte syncytia (135, 136) to determine predictability of cardiac cellular response to pharmacologic modulation. We have preliminary evidence that suggests that the pro- and antiarrhythmic responses of cardiac cell networks to cardio-active compounds is modulated by basal Ca\(^{2+}\) signaling patterns (134). In other biological scenarios, there is substantial momentum behind harnessing emerging knowledge of the mechanistic basis of variability to be able to predict cellular and organ behavior (16, 73, 103, 104, 107, 120, 127, 137, 142).

In terms of identifying candidate mechanisms that promote or exacerbate network unraveling (albeit with the caveat that nonlinear systems do not strictly conform to the notion of cause and effect), it should be noted that it is also possible to decipher the signaling patterns to identify molecular culprits of cellular dysfunction. For example, the decay phase of the Ca\(^{2+}\) transient is the manifestation of the activities of SERCA, NCX, and mitochondrial uptake mechanisms and the sensitivity of RyR2 to the localized cytoplasmic Ca\(^{2+}\) environment. Consequently, in the example given in Fig. 3, the observed desynchronization of Ca\(^{2+}\) transient decay (Fig. 3, C and H) could be further investigated at a molecular level by focusing on relevant candidate proteins including those named above. In future, it is anticipated that the assignation of molecular culprits in cellular network dysfunction would be further augmented by new tools that enable the visualization of “global” cellular signaling events such as multiplexing novel probes and biosensors (e.g., reporters of PKA, cAMP and Ras activities) (37, 99, 117, 177) and the application of new imaging modalities (40, 60, 82, 98, 109, 131, 167).

Towards Therapeutic Re-Tuning of Cardiac Ca\(^{2+}\) Signaling

In this section we consider the possible implications of our model that describes the progressive deterioration of cardiac function occurring via successive maladapted (pseudo-stable)
states for the development of new therapeutic approaches for cardiac disease.

The therapeutic “damping down” of multiple signaling processes via modulation of upstream hubs [e.g., β-blockers, angiotensin-converting enzyme (ACE) inhibitors, and modulators of the renin-angiotensin-aldosterone system (RAAS)] is a widely adopted regimen in the clinical management of cardiovascular disease but does not address the causal mechanisms of dysfunction (45, 49, 105). To this end, much research is focused on the search for discrete molecular “culprits” or druggable targets (45, 92, 105, 165). From the perspectives offered in this review, it is apparent that the targeting of discrete (protein) molecules has real limitations. As described in Pathogenic Consequences of Reduced Network Complexity and in Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State, the interconnectivity of signaling components within the network means that modulating a single component or process in an effort to improve contractility or rhythmicity may directly contribute to cardiac dysfunction. Thus a system-level understanding of cardiovascular diseases may galvanize efforts towards pharmacologic pleiotropy [magic “shotguns” (125)] or adjunctive therapeutic strategies [e.g., metabolic regulators (1, 6, 151)] that could potentially modify other coupled components within the network.

Running counter to our arguments in the section Towards an Understanding of Reduced Dynamic Range, Maladaptation, and the Disease-Linked Network State that the altered configuration of signaling pathways would be anticipated to perturb signal transmission, it is important to note that in the setting of preexistent disease-linked states, further changes may actually normalize system function. For example, chronic β-blocker therapy evokes adaptive changes in the diseased heart that are beneficial (8, 70). Thus it may be feasible to manipulate disease-associated system parameters back into the normal range (129), an approach that has been described as “walking the system out of chaos” (43). Such a strategy is critically dependent on the reversibility of the process, and reports suggesting that such “reverse remodeling” may be feasible up until the very late stages of cardiac disease support this type of approach (10, 93).

However, there are potential hazards associated with “system-correction” strategies. Zhang and colleagues (176) showed that the targeted ablation of PLB in a model of CaMKII-
dependent RyR2 hyperactivation actually provoked augmented SR Ca\textsuperscript{2+} leak, mitochondrial Ca\textsuperscript{2+} dysfunction, and increased cell death despite normalizing SR Ca\textsuperscript{2+} load. This study raises an important issue, namely that the anticipated rescue of one facet of cell dysfunction (Ca\textsuperscript{2+} handling) could accelerate phenotypic deterioration if other system defects persist (e.g., sustained activation of CaMKII or the physical loss of PLB from the network) (176). In another example, SERCA-mediated normalization of excitation-contraction coupling (ECC) is gathering momentum as a potentially viable chronotropic and antiarrhythmic approach in hypertrophy and heart failure (30, 86). However, SERCA-induced rescue of ECC is dependent on the myocardial energetic status (112) and indeed SERCA up-regulation in a “normal” scenario actually worsened cardiac dysfunction (112).

Conclusions

Normal cardiac function is dependent on the complex synchronization of ion fluxes, protein turnover, metabolism, cell death, and cell-to-cell connections that underpins the stability, plasticity, and robustness of normal signaling processes. In this review we have considered evidence that supports the view of plasticity, and robustness of normal signaling processes. In this facet of cell dysfunction (Ca\textsuperscript{2+} signaling, propulsion by the synergy of communication: insights from imaging. Circulation: Insights into Cardiac Cell Networks.

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Disclosures

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Author Contributions

C.H.G. and D.P. prepared the figures; C.H.G. drafted the manuscript; C.H.G., D.P., and N.C.S. edited and revised the manuscript; C.H.G., D.P., and N.C.S. approved the final version of the manuscript.

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