thematic review

*Thematic review series: Systems Biology Approaches to Metabolic and Cardiovascular Disorders*

# Network perspectives of cardiovascular metabolism

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**Abstract In this review, we examine cardiovascular metabolism from three different, but highly complementary, perspectives. First, from the abstract perspective of a metabolite network, composed of nodes and links. We present fundamental concepts in network theory, including emergence, to illustrate how nature has designed metabolism with a hierarchal modular scale-free topology to provide a robust system of energy delivery. Second, from the physical perspective of a modular spatially compartmentalized network. We review evidence that cardiovascular metabolism is functionally compartmentalized, such that oxidative phosphorylation, glycolysis, and glycogenolysis preferentially channel ATP to ATPases in different cellular compartments, using creatine kinase and adenylate kinase to maximize efficient energy delivery. Third, from the dynamics perspective, as a network of dynamically interactive metabolic modules capable of self-oscillation. Whereas normally, cardiac metabolism exists in a regime in which excitation-metabolism coupling closely matches energy supply and demand, we describe how under stressful conditions, the network can be pushed into a qualitatively new dynamic regime, manifested as cell-wide oscillations in ATP levels, in which the coordination between energy supply and demand is lost. We speculate how this state of "metabolic fibrillation" leads to cell death if not corrected and discuss the implications for cardioprotection.—** Weiss, J. N., L. Yang, and Z. Qu. **Network perspectives of cardiovascular metabolism. *J. Lipid Res.* 2006.** 47: **2355–2366.**

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Muscle cells must adapt rapidly to a much wider range of energy demands than most noncontractile cells. For example, the rate of respiration by cardiac muscle increases by a factor of 15–20 between unloaded and maximal workload conditions (1). This requires a very robust metabolic network that can deliver smooth performance over a wide range of parameters. Although we do not yet fully understand how this is accomplished, new systems biology approaches, complemented by network theory and computer modeling studies, are at the threshold of providing new insights.

Cardiovascular metabolism can be viewed as a network of interlinked energy-produced pathways (e.g., oxidative phosphorylation, glycolysis, and glycogenolysis), energy-consuming pathways [e.g., myofilament, sarcoplasmic reticulum (SR), and sarcolemma (SL) ATPases], and energy-distributing pathways [e.g., creatine kinase (CK) and adenylate kinase (AK)] (**Fig. 1A**). In this review, we begin by considering metabolism from a highly abstract perspective, as a metabolite network in which substrates/products are represented as nodes and metabolic enzymes are represented as the links between nodes. We present some fundamental concepts in network theory, including the concept of emergence, and discuss how through Darwinian evolution metabolic networks are likely to have evolved a scale-free hierarchal modular topology that ensures the robustness of this most fundamental of cell functions.

Next, we consider cardiovascular metabolism from the perspective of a physical network, in which various metabolic modules are spatially distributed throughout the interior of the cell to optimize ATP delivery to specific ATPases. We review evidence that glycolysis preferentially serves energy channeling to the SL, where glucose transport into the cell occurs, by providing this readily available substrate for glycolytic enzymes bound to sarcolemmal molecular complexes (2). Glycogenolysis, on the other hand, may preferentially serve the SR, where large amounts of glycogen are stored and used by glycogenolytic enzymes in SR multiprotein complexes to energize intracellular Ca ($Ca_i$) cycling (3–5). The SL/glycolytic and SR/glycogenolytic pathways thus represent low-capacity but high-specificity modules of the integrated metabolic network of a cardiac myocyte. Conversely, mitochondria represent a high-capacity but low-specificity module, channeling ATP mostly to the myofilaments (6) but also by bulk action throughout the cytoplasm to meet generic energy needs of

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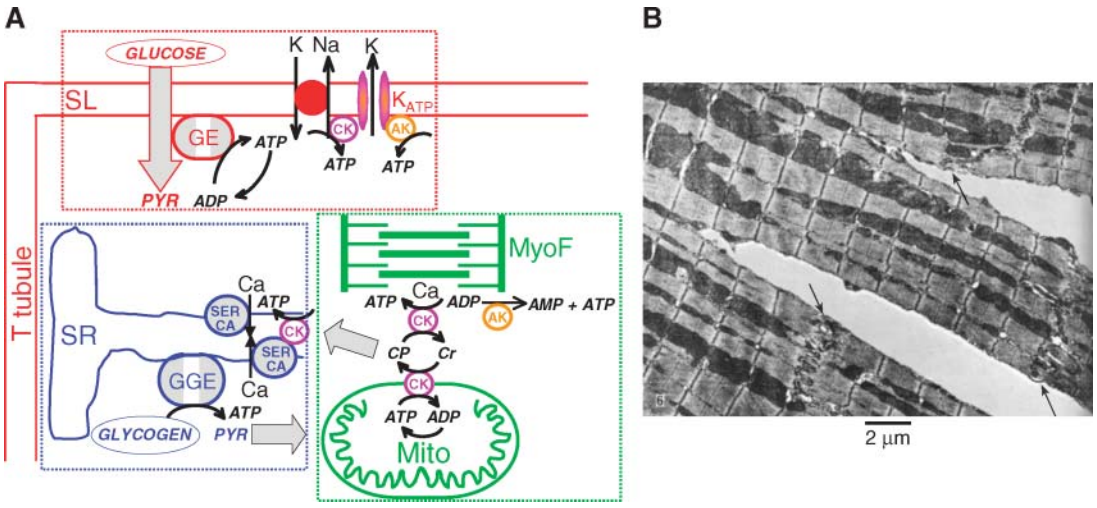


**Fig. 1.** A: Scheme of modular metabolic compartmentalization in a cardiac myocyte. Energy modules include glycolytic enzymes (GE) associated with sarcolemma (SL) ATPases in red, glycogenolytic enzymes (GGE) associated with sarcoplasmic reticulum (SR) Ca ATPases (SERCA) in blue, and oxidative phosphorylation in mitochondria (Mito) associated with myofilament (MyoF) ATPases in green. Energy-distributing modules include creatine kinase (CK) in purple and adenylate kinase (AK) in orange. CP, creatine phosphate; Cr creatine; $K_{ATP}$, ATP-sensitive K channel; PYR, pyruvate. B: Electron micrograph of a cardiac myocyte, showing rows of mitochondria (dark stripes) parallel to myofilaments. Approximately two mitochondria are spaced between each z-line.

the cell. Concomitantly, energy distribution is aided by the CK and AK systems to facilitate smooth ATP delivery throughout the cell (Fig. 1A). In this system, $Ca_i$ is the major regulator of energy consumption through its activation of various ATPases, whereas free ADP and $Ca_i$ jointly regulate energy production.

Finally, we consider cardiovascular metabolism from a dynamic perspective, focusing in particular on the ability of components of metabolic modules, such as glycolysis, oxidative phosphorylation, and SR Ca cycling, to spontaneously oscillate under appropriate conditions. For example, removing glucose or inducing localized increases in reactive oxygen species (ROS) can trigger cell-wide oscillatory mitochondrial depolarizations that deplete global ATP levels, and SR Ca ($Ca_i$) overload can cause spontaneous Ca waves attributable to Ca-induced Ca release. Based on these and other observations, we present a hypothesis describing how the oscillatory properties of the metabolic network may serve to regulate excitation-metabolism coupling. We speculate that the spatially distributed metabolic network is analogous to a system of weakly coupled oscillators, operating normally in an asynchronous mode that tightly adjusts energy production to energy needs over a wide range of physiological conditions. Under pathophysiological conditions, however, the accumulation of ROS and other factors may increase the coupling strength between the oscillators sufficiently to induce synchronization, producing cell-wide oscillations in energy production uncoupled from the energy needs of the cell. Such cell-wide oscillations in ATP levels have been observed directly in isolated cardiac myocytes subjected to metabolic stress or high ROS and recently were detected during ischemia/reperfusion in intact myocardium (7, 8). If a cell is unable to escape from this "metabolic fibrillation," it becomes increasingly susceptible to both $Ca_i$ overload and the mitochondrial permeability transition (MPT), which lead inexorably to apoptotic or necrotic cell death. This holistic view of cardiovascular metabolism as a scale-free hierarchal modular, spatially compartmentalized, dynamic network may be conceptually useful for illuminating the basis of ischemia/reperfusion injury and cardioprotection.

## PERSPECTIVE 1: CARDIOVASCULAR METABOLISM AS A METABOLITE NETWORK

When engineers design a machine, the traditional approach is based on linear logic, in which A causes B causes C, etc. Although feedback loops play essential roles in engineering design, they are incorporated mainly to regulate the flow of the linear sequence. This approach maximizes efficiency but in doing so sacrifices adaptability (because the machine is designed to perform only a specific set of tasks) and fail-safe performance (because cutting one link in a linear sequence disrupts the chain). In complex biological systems subject to Darwinian evolution, however, the latter two qualities are at least equally, if not more, important than efficiency. Complex biological systems, therefore, probably evolved using a different type of logic, incorporating a high level of redundancy in which relationships are typically reciprocal (i.e., A causes B, but B also causes A) (9). The high degree of redundancy compromises efficiency but promotes adaptability and fail-safe performance, because alternative routes to the same end point are almost always available. Moreover, this circular, or network, logic is characterized by a mysterious and fascinating phenomenon called "emergence," in which unexpected new properties can arise abruptly from the

cooperative interactions between simple individual units: that is, the whole becomes greater than the sum of the parts. Well-known examples of emergence include self–organizing pattern formation in reaction-diffusion systems [such as spontaneous patterning in the Belousov-Zhabotinsky chemical reaction (10), calcification patterns in cultured vascular smooth muscle cells produced by diffusible morphogens (11), and spiral and scroll waves causing arrhythmias in cardiac tissue (12)] and self-oscillatory behaviors (such as circadian rhythms, the cell cycle, and cardiac pacemaking). In metabolism, both glycolysis (13) and oxidative phosphorylation (14) are capable of developing emergent behavior in the form of spontaneous self-sustained oscillations. In this case, no individual metabolic enzyme provided with a constant supply of substrate has an intrinsic ability to oscillate. However, when coupled into a network with other metabolic enzymes creating positive and negative feedback loops, oscillations in metabolic fluxes (the system output) can emerge from the integrated system, even though the supply of substrates (the system input) remains constant and nonoscillatory. An important corollary is that emergent behaviors cannot be understood solely by examining the properties of individual proteins: although reductionist approaches are essential to characterize the individual components of the system, the components must be reintegrated back into the system to understand how system behavior emerges from their interactions.

The information flow in complex biological systems, such as genetic, protein-protein interaction, protein domain, and metabolic networks, can be analyzed at an abstract level by considering the system as a topological network of nodes and links (15). In the case of metabolism, the substrates/products are represented as nodes, and metabolic enzymes are represented as links mediating metabolic fluxes (i.e., conversion of substrate to product) between nodes (16) (**Fig. 2A**). Links can be unidirectional or bidirectional and assigned strengths proportional to metabolic flux rates. Network theory (also called graph theory) has been successfully applied to technology networks (e.g., the World Wide Web), social interaction networks (e.g., the six degrees of separation phenomenon), and epidemiology (e.g., the spread of epidemics) and has yielded surprising insights into universal features of complex self-organizing systems (15).

To analyze a network, the first step is to characterize its topology [i.e., the relationship between the nodes and the links (also called edges)]. If the links between nodes are distributed randomly, the probability *P(k)* that a node has *k* links follows a Poisson distribution, with most of the nodes having close to the average number of links <*k*> and very few nodes having a large number of links. This is called a random network, as illustrated in Fig. 2B. If an initially random network has been subject to growth, so that new nodes and links have been randomly added over time, older nodes will have a statistically greater chance (in proportion to their age) of acquiring new links, so that *P(k)* follows an exponential instead of a Poisson distribution (Fig. 2B). This is called an exponential network. Relative to a random network, the exponential network has a larger proportion of nodes that have many links. If an initially random network grows and while it grows is also subject to

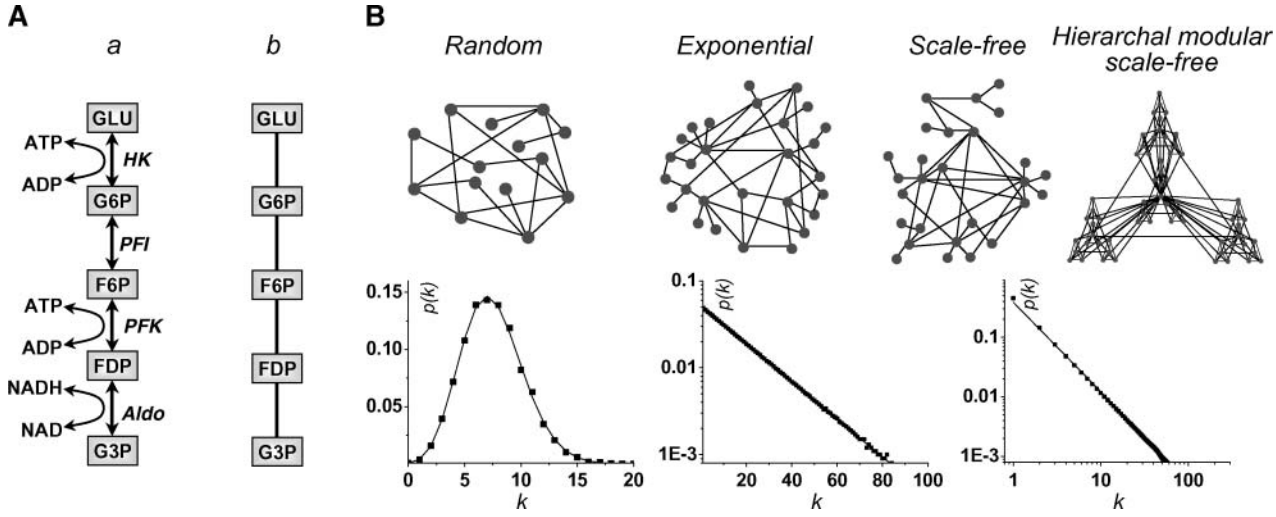


**Fig. 2.** A: Representation of a metabolic pathway as a network. *a*, the first five steps of glycolysis shown traditionally as biochemical reactions; *b*, their simplified network representation as nodes (squares) and links (lines connecting squares). GLU, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate; G3P, glyceraldehyde-3-phosphate; *HK*, hexokinase; *PFI*, phospho-fructose isomerase; *PFK*, phospho-fructose kinase; *Aldo*, aldolase. B: Upper diagrams show arrangements of nodes (circles) and links (lines) for typical examples of random, exponential, scale-free, and hierarchal modular scale-free networks. The distinctions are not necessarily intuitively obvious from the diagrams, but when analyzed according to the probability distribution *P(k)* of a given node having *k* links, the differences are obvious (graphs). For the random network, the plot of *P(k)* versus *k* on a linear scale shows a typical Poisson distribution. For the exponential network, the plot of *P(k)* versus *k* on a log-linear scale shows a straight line indicating an exponential distribution. For the scale-free networks, the plot of *P(k)* versus *k* on a log-log scale shows a straight line indicating a power law distribution. The scale-free networks enhance the prevalence of nodes with a large number of links more than either the random or monoexponential network.

selection biases, so that new links preferentially attach to already highly linked nodes (according to the principle that success begets success in competition for business or social status), then this preferential attachment produces an even greater proportion of highly connected nodes, creating a "scale-free" network (Fig. 2B). In this case, the probability $P(k)$ that a given node has $k$ links is given by a power law distribution $P(k) \approx k^{-\gamma}$. The exponent $\gamma$ is called the degree distribution of the network and typically ranges from 2 to 3 in social and biological networks. The scale-free feature arises directly from the power law relationship; for example, if one asks what is the distribution of nodes containing links on a different scale, such as links in multiples of 5, then $P(k) \approx (5k)^{-\gamma} = 5^{-\gamma} k^{-\gamma}$, which is the identical power law distribution, just multiplied by a different constant. Thus, the units in which links are measured do not affect the shape of their distribution, unlike in random or exponential networks. [For example, in an exponential network $P(k) \approx e^{-\gamma k}$, so for units of five links, $P(k) \approx e^{-\gamma 5k}$, which is a distribution with a much more rapid falloff of nodes with a large number of links.] This self-similarity feature gives scale-free networks a fractal structure, a common theme in biology.

Another way of describing the difference between a scale-free network and a random or exponential network is that no node is truly typical in a scale-free network. That is, most nodes have a small number of links, but there are always a small number of nodes with a very large number of links. The latter are called hub nodes and have critical influences on the features of a scale-free network, particularly in conferring robustness (i.e., stability in the face of random perturbations) to the network. Scale-free networks have small-world properties (15): that is, the distance between any two nodes (i.e., the minimum number of links that must be traversed to connect the nodes) is short as a result of the presence of hub nodes containing many links. For example, if every node is connected to a hub node, and all hub nodes are interconnected, then at most three links would be required to connect any two nodes in the network. In the case of metabolism, this means that if any single enzyme (link) regulating the flux of substrate X to product Y is disabled, the substrate/product flux can be maintained by an alternative, and fairly direct, pathway, allowing the organism to rapidly adapt to an environmental change. [Similarly, if a given substrate (node A) becomes limited, its product (node B) can be produced by an alternative pathway.] This high-connectivity feature makes a scale-free network robust with respect to a random disruption of nodes or links, because an efficient alternative pathway is almost always present to circumvent the disruption. Moreover, a random loss of nodes or links is unlikely to disrupt the network as a whole for the following reasons: first, the vast majority of nodes have only a few links, so the statistical likelihood that a random perturbation will disrupt a hub node or its links is small; second, hub nodes by definition have a large number of links to begin with, so that disruption of one or even several of its links may not be consequential as long as the hub node retains its connectivity to other hub nodes (preserving its ultra-small-world features). In some scale-free networks, up to 80% of nodes or links can be randomly disrupted without having a major impact on the connectivity of the network as a whole (17). Conversely, however, scale-free networks are vulnerable to a targeted attack on hub nodes. Targeted disruption of one or more hub nodes can be catastrophic for overall network function, because their influence on connectivity is so widespread. The attack vulnerability of scale-free networks, however, provides a potential advantage for therapeutic interventions in biology. That is, if the hub nodes in a complex system can be identified, their manipulation can dramatically alter system behavior.

An appealing aspect of network theory for theories of biological evolution is the feature of emergence, in which unexpected new properties abruptly emerge from the cooperative interactions between simple individual units. If the emergent behavior arising from a module of simple units confers a survival advantage, then natural selection will tend to preserve it. Moreover, if this small module later develops interactions with other small modules, new emergent behaviors conferring survival advantages can emerge from the new (larger) module of (smaller) modules. In this way, it is intuitive to imagine how an increasingly complex biological system could be built up layer by layer. Modular structure and scale-free topology are not inconsistent if modules are organized in a hierarchal structure (18), so that hub nodes remain interconnected (Fig. 2B). In network theory, hierarchical modular structure can be characterized mathematically by the network's clustering coefficient $C(k)$, reflecting the extent to which the nearest neighbors of hub nodes are interconnected to each other (15). In a study of the large-scale organization of metabolic networks from 43 different bacteria (16, 18), the clustering was significant, reflecting a hierarchal modules-within-modules (fractal) topology. Moreover, there was a generally good correlation between modules identified topologically and those defined biochemically (18). We are not aware that a formal topological analysis of mammalian cardiovascular metabolism has been performed, but the cardiovascular biochemical network has an intuitively modular structure, consisting of modules of energy production (oxidative phosphorylation, glycolysis, glycogenolysis, etc.), energy consumption (myofilament ATPases, SR Ca ATPases, SL ATPases, etc.), and energy distribution (CK and AK systems).

Although complete metabolite networks of various bacteria have been reconstructed from genomic and biochemical databases, experimental data on metabolic flux rates (i.e., rate constants) are still incomplete. Once the topological structure of a metabolite network has been defined, computational techniques based on optimality assumptions can be used to test its validity and predictive accuracy (19). Two commonly used optimality assumptions include flux balance analysis (FBA) and minimization of metabolic adjustment (MOMA). FBA assumes that the goal of metabolism is to maximize growth (i.e., maximizing the conversion of substrates into products that are essential for cell growth). With this constraint, the un-

known parameter values in the network are explored computationally and assigned optimized values that maximize growth rate. The original FBA approach applied only to the steady-state conditions of the metabolite network and did not integrate dynamic changes in metabolite concentrations, which may have important regulatory roles. Recently, the method has been extended to predict dynamic changes as well (20). The maximal growth criterion is a reasonable assumption for the evolution of metabolism in wild-type bacteria but may not be valid for engineered mutations causing acute perturbations, in which bacteria have not been exposed to long-term evolutionary pressure. Accordingly, in MOMA, the assumption is made that the metabolite network will strive to minimize metabolic flux redistribution relative to the wild-type case. Similar to FBA, the unknown parameter values are then explored computationally and assigned optimized values that minimize metabolic flux redistribution. Using these techniques, knowledge of a restricted set of parameters combined with the application of fundamental thermodynamic and evolutionary principles can generate quantitative predictions and testable hypotheses, and these have successfully predicted experimental results in microbe responses to mutations and environmental changes. Recently, a similar approach was adapted to model the metabolic responses of the mammalian myocardium to acute ischemia (21). Incorporating a dynamic MOMA approach based on the optimality assumption that during ischemia cardiac metabolism readjusts itself to minimize the fluctuation of the profile of metabolite concentrations, this study showed good agreement between predicted and experimentally measured alterations in cardiac metabolites.

## PERSPECTIVE 2: CARDIOVASCULAR METABOLISM AS A PHYSICAL, SPATIALLY COMPARTMENTALIZED NETWORK

The topological analysis of metabolite networks described above makes no assumptions about the nature of the physical and spatial interactions between nodes and links in the network. However, metabolite networks also have a physical structure. In the beating heart, the majority (60–90%) of ATP production comes from β-oxidation of fatty acids by mitochondria (22). Glycolytic and glycogenolytic enzymes directly produce only ~5% of total ATP production but provide pyruvate as a substrate for oxidative phosphorylation, from which 10–40% of total ATP is generated. It is intuitive to consider oxidative phosphorylation in terms of a physical network, because it is carried out in discrete organelles (mitochondria) that are spatially distributed in a highly organized manner throughout the cytoplasm (23) (Fig. 1B). However, despite their minor overall contribution to ATP production, abundant evidence suggests that glycolytic and glycogenolytic enzymes associate with specific ATPases in molecular complexes to serve selective functions in energy channeling.

In the past, compartmentalization of the cell's interior was believed to be largely defined by internal membranes, such as the nuclear envelope, Golgi, endoplasmic reticulum (ER), SR, vesicular trafficking systems, and mitochondria. During the past two decades, however, advances in subcellular imaging have dramatically upset this view, so that now a high degree of compartmentalization of signaling pathways within the cytoplasm is considered the norm rather than the exception. The cytoplasm is better viewed as a gel than as a well-mixed medium, in which a highly organized cytoskeleton and sophisticated molecular trafficking mechanisms direct and tether proteins into macromolecular signaling complexes at specific locations. These locations comprise microdomains with restricted diffusion, facilitating direct interactions between locally generated signaling molecules, enzymes, and their target substrates.

In metabolism, subcellular compartmentalization of energy production has been a well-accepted fact ever since mitochondria were identified as the engines driving oxidative phosphorylation. In addition, glycolytic and glycogenolytic enzyme complexes have been shown to be associated with the SL (24, 25) and specific intracellular membranes such as the SR (3, 4). On the other hand, however, energy consumption by the cell has traditionally been viewed as a democratic process, with high-energy phosphates freely diffusing throughout the cytoplasm to be consumed wherever they are needed. In tissues with high energy requirements such as muscle, the CK system is considered an important equalizer in this design, with creatine phosphate (CP) shuttling rapidly to regenerate ATP from ADP wherever CK is located, maintaining free ADP concentration at low levels to maximize the free energy of ATP hydrolysis (26–29). In heart, this role of CP as a mediator of cross-talk between ATP production and ATP use has been demonstrated for both contractile function and SL function (28, 30, 31). AK, which converts two ADP molecules to ATP and AMP, plays a similar role in facilitating local regeneration of ATP (32).

However, just as the norm for other signaling pathways is a high degree of functional compartmentalization, accumulating evidence indicates that energy consumption is less democratic than was once assumed. Evidence for preferential energy channeling has largely rested on functional studies using pharmacological and genetic manipulations. Unfortunately, imaging tools to track energy production/consumption directly at the subcellular level are still not well developed, as they are, for example, for imaging Ca microdomains or hotspots of protein kinase activity using fluorescence techniques. In addition, both traditional "grind-and-bind" biochemical methods and global measures of metabolic function such as NMR are based on averaged cytoplasmic values of various metabolites and do not easily lend themselves to investigating the subcellular compartmentalization of energy channeling. Nevertheless, a general theme emerging from functional studies in both muscle and nonmuscle cells has been that glycolytically derived ATP is used preferentially to support membrane-related ATPases (such as ion transporters and channels in the SL and SR), whereas mitochondrially generated ATP is used

preferentially in supporting functions in the cytoplasm (such as contractile protein ATPases). Several of the major observations supporting this idea are the following. Selective inhibition of anaerobic glycolysis and selective inhibition of mitochondrial oxidative phosphorylation have different functional effects, which cannot be attributed to changes in global tissue high-energy phosphate levels (2, 33, 34). Manipulations of glycolysis markedly affect functional performance and recovery during ischemia/reperfusion or hypoxia/reoxygenation, again in a manner that does not correlate with changes in global tissue high-phosphate levels (35–39). Finally, substrates for glycolysis, mitochondrial oxidative phosphorylation, or ATP regeneration via CK have differential efficacies at supporting specific membrane and/or contractile functions in heart and other tissues (33, 40–43).

Genetic models interrupting energy channeling have also proven informative. Genetically engineered mice lacking mitochondrial and cytosolic CK had nearly normal cardiac performance, up to at least moderate workloads (44–46). However, they showed increased susceptibility to ischemic injury (46) and electrical instability during stress (47) as well as a markedly reduced work capacity during voluntary exercise (48). Mice with undetectable levels of creatine phosphate attributable to knock out of a critical enzyme required for creatine synthesis also had normal resting cardiac function but a markedly impaired inotropic reserve in response to adrenergic stimulation (49). In permeabilized cardiac muscle fibers from CK knockout mice, ATP generated endogenously by mitochondria was found to be much more effective at supporting SR Ca uptake (6) than exogenous ATP delivered via the bath, indicating that competition for exogenous ATP by other ATPases limited its availability to Ca ATPases in the SR. In wild-type permeabilized fibers, on the other hand, exogenous CP plus ADP to permit local ATP regeneration by CK was equally effective as endogenous ATP generated by mitochondria. This observation is consistent with the idea that CK plays an important role in distributing ATP throughout the cytosol. These authors estimated that at normal cardiac workload, approximately one-third of ATP used by the SR Ca pump is channeled directly from the mitochondria and approximately two-thirds is provided by the CK system. In CK-deficient mice, mitochondria effectively compensated for the lack of CK up to moderate workloads, but at a cost. The hearts of these mice showed significant cytoarchitectural changes in addition to mild hypertrophy, including increased splitting of myofibrils, as if the heart were trying to decrease the average distance between mitochondria and myofilaments to compensate for the lack of CK-facilitated ATP regeneration. The roles of glycolysis and glycogenolysis in supporting SL and SR function in these mice were not specifically investigated in that study, however.

Sarcolemmal ATP-sensitive K channels ($K_{ATP}$ channels) are hypothesized to protect the heart by shortening action potential duration and reducing contractility when energy production becomes limited. In mice, knock out of SUR2a, a subunit of the cardiac sarcolemmal $K_{ATP}$ channel, compromised adaptation to exercise and promoted cardiac injury in mice by disrupting energy signaling between the cytoplasm and the SL (50). [Interestingly, a human mutation in SUR2a has been identified that causes dilated cardiomyopathy, postulated to result from similar chronically compromised energy signaling during metabolic stress (51)]. Knock out of either CK (47) or AK (32) also disrupted the efficient transmission of energy signals between mitochondria and sarcolemmal $K_{ATP}$ channels, indicating that these energy transfer systems also play a vital role in energy signaling.

In functional studies of cellular metabolism in wild-type or genetically modified animals, there are valid concerns about interpretation. Metabolic inhibitors are neither completely effective nor completely specific. Also, metabolic inhibition has global consequences, with a wide array of effects on many cellular processes, so that distinguishing the primary effects of metabolic inhibition from its secondary consequences is often difficult. Finally, in studies in which global tissue high-energy phosphate levels are used to track metabolic inhibition, these levels reflect a net effect on metabolism but do not provide direct information about how the underlying metabolic fluxes have been affected unless supplemented by other techniques. Despite these limitations, these studies have made an important contribution to our understanding of cardiovascular metabolism by providing further evidence for localized cross-talk between energy production and energy consumption in cardiovascular myocytes. Although direct visualization of this energy channeling will require the development of better metabolic imaging tools, the functional picture emerging is that metabolism shares features in common with other compartmentalized cellular signaling mechanisms: namely, the cytoplasm is far from being a well-mixed bag of water, in which high-energy phosphates diffuse readily and indiscriminately to various cellular ATPases. Like other signaling molecules, ATP has emerged as a local cellular currency, with CP as its major global intermediary. A close physical relationship is present between the mitochondrial and ER networks (52), and mitochondria participate in very localized Ca signaling with ER and SR membranes (53), so a similar relationship with respect to ATP transfer by mitochondria is not surprising. Glycolytic and glycogenolytic enzymes are also common components of molecular signaling complexes using ATP for ion transport (3, 4), metabolic signaling (24, 25), and protein kinase signaling (54) and have been demonstrated to be capable of generating ATP locally from ADP in the immediate vicinity of these ATPases (5, 24, 41, 42). Perhaps an indirect clue that the heart prefers local over global ATP generation to achieve a high efficiency in matching the output of its energy production modules to the energy consumption needs of various cellular compartments is provided by the following observation. Even when increased glucose is provided as the sole exogenous substrate to the heart, glucose oxidation accounts for only 40–50% of ATP production, with the heart diverting more than half of glucose uptake to

glycogen synthesis and consuming endogenous triglycerides to generate the majority of ATP (55, 56).

In summary, we have reviewed evidence supporting a modular organization of energy-consuming, energy-producing, and energy-distributing processes in cardiovascular myocytes. Mitochondria are regularly spaced between rows of myofilaments (Fig. 1B). SL-bound glycolytic enzymes are associated with SL ion channels and transporters, where their substrate glucose is imported into the cell. Glycogenolytic enzyme complexes are associated with the SR Ca ATPase, for which glucose import is not readily available because of diffusional barriers, but where glycogen can be stored at close range. This compartmentalization of metabolic modules is not absolute, and CK and AK act as energy-distributing systems to minimize local ATP gradients and transmit energy signals between the modules. The latter energy-distributing modules do not appear to be essential under physiologically unstressed conditions but play a critical role under stressed conditions, both in maximizing cardiac performance and protecting the heart from ischemic injury attributable to supply-demand imbalance.

In this modular network, $Ca_i$ is the major regulator of energy consumption. In the mitochondria/myofilament module, $Ca_i$ directly regulates energy consumption by myofibrillar ATPases in generating contraction. In the glycogenolysis/SR module, $Ca_i$ directly regulates energy consumption by SR/ER Ca ATPases (SERCA pumps) in recycling $Ca_i$. In the glycolysis/SL module, $Ca_i$ indirectly regulates the energy consumption by Na-K ATPase, because ~85% of total Na influx in the beating heart (which must be pumped out for Na-K ATPase to maintain ionic homeostasis) occurs via forward-mode Na-Ca exchange to remove Ca entering via L-type Ca channels in exchange for Na (57). $Ca_i$ also directly regulates energy production by oxidative phosphorylation and glycogenolysis, through Ca-sensitive metabolic enzymes such as pyruvate dehydrogenase and phosphorylase (22). However, the major local regulator of energy production is free ADP (22).

Finally, we have focused only on the three metabolic modules mentioned above, because they are responsible for the majority of energy production and consumption in working cardiovascular myocytes. However, additional metabolic energy modules also contribute to the overall cardiovascular metabolic network, including lipid, amino acid, nucleic acid, and other carbohydrate pathways. In the beating heart, these pathways account for ~20% of energy consumption (58).

## PERSPECTIVE 3: DYNAMICS OF CARDIOVASCULAR METABOLISM

In representing a complex system as a network of nodes and links, we have not yet considered how network topology affects the dynamics of the network. This is an area of active investigation (59–61) and is particularly germane to biological networks, including metabolism, because these networks contain functional modules with positive and negative feedback loops that produce rich dynamics, such as limit cycles (oscillators or clocks) and bistability (biochemical switches) (62). Such dynamics are critical to the function of biological networks, such as the cell cycle, circadian rhythms, cardiac pacemaking, protein kinase signaling, etc. The hierarchal modular topology of biological networks provides an advantage for analyzing these dynamics, because this type of network can be broken down into individual modules whose dynamics can be characterized separately before recombining them to study their interactions systematically (63, 64).

Metabolic modules have been shown to be capable of nontrivial dynamics. For example, yeast extracts containing glycolytic enzymes can spontaneously oscillate their output of metabolites when provided with a constant input of glucose as substrate (13) (**Fig. 3A**). In isolated guinea pig myocytes, removal of glucose has also been shown to induce oscillations in cellular ATP, manifested as episodes of action potential duration shortening attributable to the activation of $K_{ATP}$ channels (65) (Fig. 3B). Similarly, cell-wide oscillations in ATP levels can be triggered by localized ROS production in isolated cardiac myocytes (14, 66, 67). These oscillations have been attributed to ROS-induced ROS release triggering synchronous cell-wide mitochondrial depolarization by opening of ROS-sensitive inner membrane anion channels (IMACs) (68). Less consistently, anoxia-reoxygenation and increased extracellular Ca also have been reported to induce similar oscillations in mitochondrial membrane potential in isolated cardiac myocytes (69, 70). Apoptotic agents have also been shown to induce mitochondrial depolarization waves associated with MPT in cardiac myotubes (71). Recent confocal imaging studies suggest that similar cell-wide oscillations in mitochondrial membrane potential occur in intact tissue during ischemia/reperfusion (7, 8). Finally, single isolated mitochondria have been shown to exhibit spontaneous cyclical membrane depolarizations (Fig. 3C). Here, we discuss these data and speculate on how these dynamics may be important in cardiovascular metabolism under normal and metabolically stressed conditions such as ischemia/reperfusion.

Mitochondria are double membrane structures ~1–2 μm in diameter (**Fig. 4A**). The outer membrane is porous to solutes up to 1,000 molecular weight as a result of the abundance of voltage-dependent anion channels (also known as porin). The inner membrane, on the other hand, is impermeant, which allows electron transport to generate a matrix membrane potential ($\Delta\Psi$) of ~−200 mV by pumping protons out of the matrix. This electrochemical proton gradient is subsequently used by the $F_0$-$F_1$ ATP synthase in the inner membrane to phosphorylate ADP to ATP (Fig. 4A, detail). Less appreciated, if the matrix is depolarized, then ATP synthase will operate in the reverse direction and consume, rather than synthesize, ATP to pump protons out of the matrix to restore $\Delta\Psi$. For example, during ischemia, in which a lack of oxygen leads to mitochondrial depolarization, pretreatment with ATP synthase blockers such as oligomycin significantly reduces the rate of ATP depletion (72). A

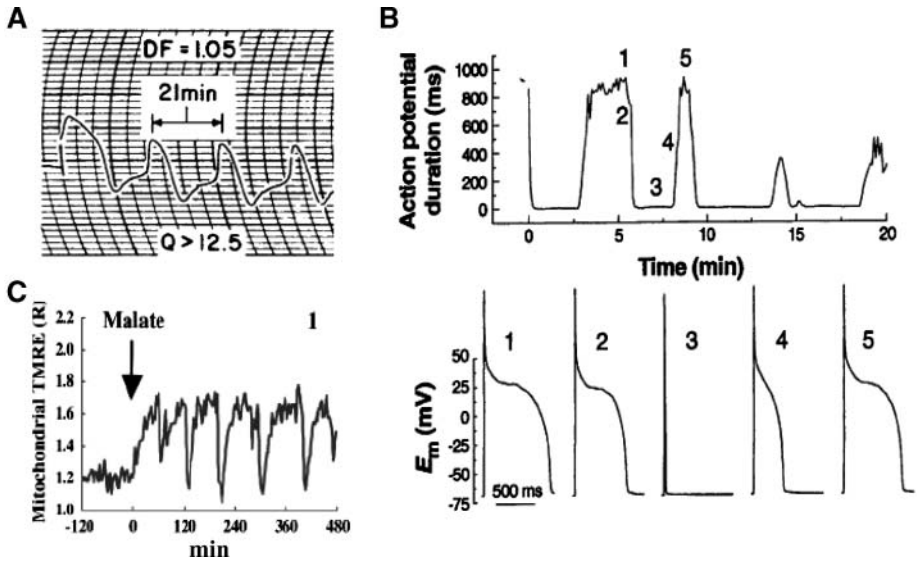


**Fig. 3.** A: Spontaneous oscillations of glycolysis in a yeast extract. The graph shows oscillations of NAD fluorescence over time. Reprinted with permission from Chance, B., B. Schoener, and S. Elsaesser. 1964. Control of the waveform of oscillations of the reduced pyridine nucleotide level in a cell-free extract. *Proc. Natl. Acad. Sci. USA.* **95:** 1618–1623. B: Spontaneous oscillations in action potential duration in a ventricular myocyte after glucose was removed from the superfusate. Representative action potentials at the points indicated are shown below. Action potential duration shortening episodes reflect global ATP depletion attributable to mitochondrial membrane potential depolarization, causing the activation of sarcolemmal $K_{ATP}$. Reprinted with permission from O'Rourke, B., B. M. Ramza, and E. Marban. 1994. Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells. *Science.* **265:** 962–966. ©1994 AAAS. C: Membrane potential oscillations imaged confocally with tetramethylrhodamine methyl ester (TMRE) in a single isolated mitochondrion elicited by the addition of malate to the superfusate. Reprinted with permission from Hattori, T., K. Watanabe, Y. Uechi, H. Yoshioka, and Y. Ohta. 2005. Repetitive transient depolarizations of the inner mitochondrial membrane induced by proton pumping. *Biophys. J.* **88:** 2340–2349.

number of ion channels are present in the inner membrane, but their conductance is normally low to avoid short-circuiting $\Delta\Psi$. However, confocal imaging of individual isolated mitochondria has revealed periodic oscillations in $\Delta\Psi$ (73–75) (Fig. 3C), and similar flickering has also been observed in intact myocytes (69), although the mechanism and relevance to mitochondrial behavior in intact cells remain controversial. Isolated mitochondria immobilized in an agar gel also cross-talk with each other sufficiently to propagate $\Delta\Psi$ depolarization waves caused by MPT in response to localized increases in Ca (76).

A model accounting for periodic $\Delta\Psi$ oscillations attributable to ROS-induced ROS release has been developed by the O'Rourke group (77) and proposed to account for cell-wide $\Delta\Psi$ oscillations induced by localized ROS production (68). In this model, a fraction of $O_2$ consumption diverted to superoxide anion (normally ~2–5% during physiological respiration) causes superoxide accumulation in the matrix and intermembrane space, beyond the capability of mitochondrial superoxide dismutase to compensate. Because of the low permeability of the inner membrane, superoxide anions are trapped in the matrix until they have accumulated sufficiently to activate ROS-sensitive IMACs. When open, IMAC channels depolarize $\Delta\Psi$ and allow the accumulated superoxide anions to be released into the cytoplasm, where they are converted to $H_2O_2$ by cytoplasmic superoxide dismutase, closing the IMAC channels and allowing $\Delta\Psi$ to be regenerated, as illustrated schematically in Fig. 4B. With the correct parameter settings in the model, $\Delta\Psi$ oscillates periodically. Normally, when cellular antioxidant levels are high, superoxide anions released by mitochondria do not activate IMAC channels in nearby mitochondria. If ROS levels are increased or antioxidant capacity is reduced, however, ROS-induced ROS release may propagate and create synchronous cell-wide $\Delta\Psi$ depolarizations. Aon, Cortassa, and O'Rourke (68) have proposed that cardiac mitochondria operate as a network of coupled nonlinear elements, in which periodic $\Delta\Psi$ depolarization events can be modeled by percolation cluster theory.

In this scenario, asynchronous ROS-induced $\Delta\Psi$ oscillations provide a mechanism for individual mitochondria to flush out toxic superoxide anions periodically. Conceptually, because mitochondria consume ATP when depolarized, the fraction of polarized versus depolarized mitochondria could also provide an effective mechanism for matching ATP production to the energy needs of the cells, as illustrated schematically in Fig. 4C. Mitochondrial ROS production is much greater in state 4 than in state 3 (78), so high workload conditions (i.e., high free ADP promoting state 3) would inhibit ROS-induced $\Delta\Psi$ oscillations, increasing the percentage of polarized mito-

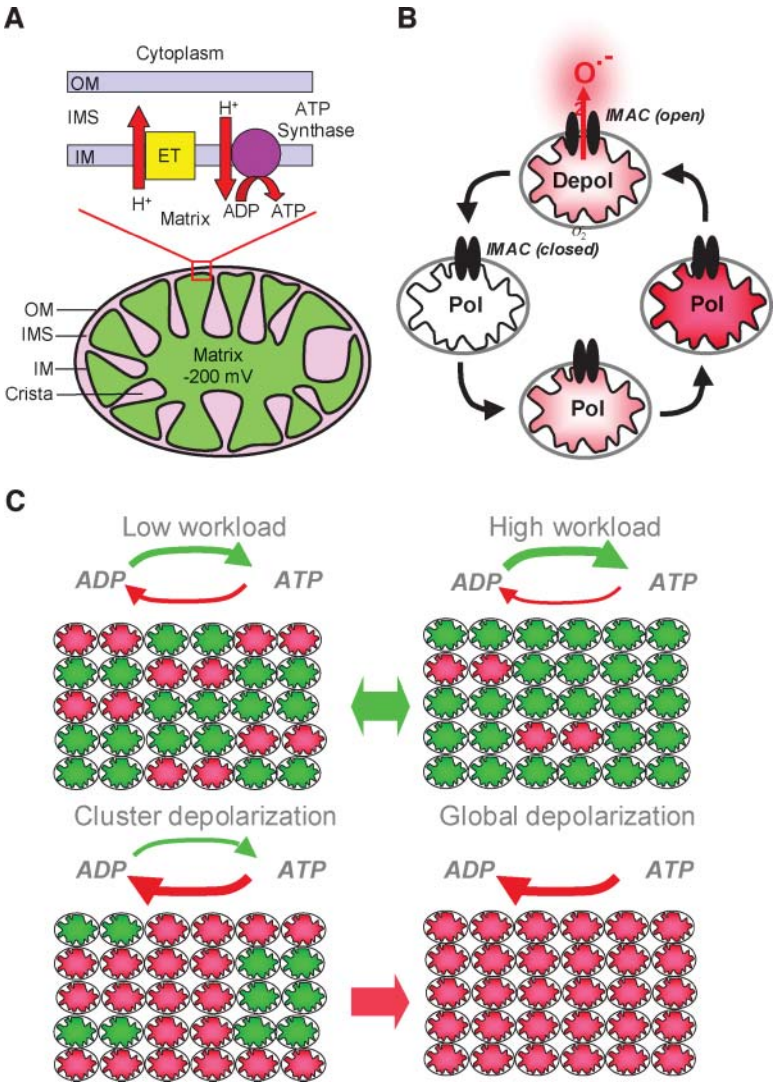


**Fig. 4.** A: Scheme of a mitochondrion (below) showing the outer membrane (OM), intermembrane space (IMS), inner membrane (IM), and matrix. The expanded cartoon (detail above) illustrates the ATP-generated system, in which electron transport (ET) pumps protons from the matrix, establishing a negative membrane potential ($\Delta\Psi$) near −200 mV. Proton influx down its electrochemical gradient is then coupled to ATP generation by ATP synthase. However, ATP synthase is reversible and consumes ATP to pump protons out of the matrix when $\Delta\Psi$ is depolarized. B: Scheme of $\Delta\Psi$ oscillations attributable to the accumulation of superoxide anion (red shading) in the matrix during respiration, eventually triggering inner membrane anion channels (IMAC) to open, releasing superoxide anions and depolarizing $\Delta\Psi$, after which the cycle repeats. Depol, depolarized; Pol, polarized. C: Hypothetical scenarios of excitation-metabolism coupling (upper) and uncoupling (lower) within the mitochondrial network in a myocyte. Red indicates depolarized mitochondria, and green indicates polarized mitochondria. In the upper panels, $\Delta\Psi$ oscillations are asynchronous, and as workload increases, more mitochondria are recruited into the polarized state. In the lower panels, reactive oxygen species (ROS)-induced ROS release causes, first, locally synchronous depolarizations of clusters of mitochondria, and then, globally synchronous $\Delta\Psi$ depolarization waves, causing cell-wide oscillations in ATP as mitochondria convert between depolarized (ATP-consuming) and polarized (ATP-producing) states. See text for further description.

chondria actively synthesizing ATP. As workload decreases (i.e., low free ADP promoting state 4), mitochondrial ROS production would increase, promoting ROS-induced $\Delta\Psi$ oscillations in a greater proportion of mitochondria and thereby decreasing net ATP synthesis to match the reduced energy requirements of the cell.

As long as ROS-induced $\Delta\Psi$ oscillations in individual mitochondria remain asynchronous, this could provide an effective mechanism for matching net ATP production to cellular energy needs over a wide range of workloads (although it would involve a degree of futile ATP cycling). However, if ROS-induced $\Delta\Psi$ oscillations in the mitochondrial network become synchronized, global cellular ATP levels would fluctuate as the bulk of mitochondria oscillate between depolarized (ATP-consuming) and polarized (ATP-producing) states, thereby uncoupling metabolism from cellular energy needs (Fig. 4C). If the myocyte were unable to escape from this "metabolic fibrillation," unregulated ROS production and the inability to handle Ca cycling by ATP-dependent processes would make mitochondria progressively more susceptible to MPT, triggering cell death by apoptosis or necrosis. We can further speculate that signaling pathways involved in cardioprotection (protein kinase C, tyrosine kinases, nitric oxide, etc.) might act through a common mechanism of modulating this synchronization threshold, whether induced by the ROS-induced ROS mechanism postulated above or a different set of pathophysiological triggers. Because the synchronization threshold in a weakly coupled oscillator network is likely to be influenced by a large number of parameters, searching for a single protein target end effector mediating cardioprotection, onto which all cardioprotective pathways ultimately converge, is likely to be fruitless. To date, this search has been very frustrating because of the bewildering plethora of pathways that all trigger cardioprotection. If viewed from the holistic perspective as an interactive dynamic network, however, we are hopeful that a deeper understanding of cardioprotection signaling might be achievable, as has been the case for other areas using systems approaches (79). Although this scenario is highly speculative at present, its feasibility can be tested quantitatively using the

appropriate computational dynamic network models that have been expanded to incorporate the interactions of the relevant signaling pathways with excitation-contraction-metabolism coupling. Based on these findings, experimental protocols can then be designed to validate model predictions. Moreover, synchronization of weakly coupled oscillators is a well-studied area in physics (80), and insights from this field may be useful for understanding the dynamics of mitochondrial and metabolic networks. [Synchronization of weakly coupled oscillators was discovered in 1665 by Christiaan Huygens, a Dutch telescope and clock maker, who, while sick in bed, noted that two clocks that he had recently fabricated and hung on the same wall were swinging with their pendulums in complete synchrony (80). When he placed the clocks on different walls, however, their motions remained asynchronous. He deduced that the weak coupling from mechanical vibrations propagating through the wall synchronized the clocks.]

## SUMMARY AND CONCLUSIONS

In this brief review, we have presented cardiovascular metabolism from three different, but highly complementary, perspectives: as a metabolite network that has been designed by nature with a hierarchal modular scale-free topology to provide a robust and reliable system of energy delivery; as a physical spatially compartmentalized network, using metabolic modules incorporating glycolysis with the SL, glycogenolysis with the SR, and oxidative phosphorylation with the myofilaments in a spatially compartmentalized manner to efficiently match energy supply to energy needs over a wide and rapidly varying range of physiological conditions; and finally, as a dynamic network of weakly coupled oscillators, whose properties are just beginning to be probed.

We are hopeful that this holistic view of cardiac metabolism as a modular interactive dynamic network may lend itself more successfully to unraveling the complex emergent pathophysiology of ischemia/reperfusion injury and its abrogation by cardioprotection. To make further progress in this area, the challenge is several-fold. We will need more detailed information cataloguing interactions between metabolic modules and cardioprotective signaling pathways. Functional proteomics approaches (81) will be essential to identify physical interactions between key signaling proteins, metabolic enzymes, and ATPases in the various metabolic modules. In addition to standard genetic approaches, genetical genomics is a novel technique for analyzing gene coexpression networks (82), which can identify cotravelers with metabolic genes by evaluating their relationships to hub genes regulating gene coexpression modules. We need to develop better bioprobes to image metabolic microdomains in wild-type and genetically engineering animals in which components of excitation-contraction-metabolism coupling have been altered. We need to understand how other cell types, such as vascular smooth muscle cells (83) and endothelial cells, interact metabolically with cardiac myocytes to decide their joint fates. Finally, we will need to develop mathematical models incorporating both the details and dynamics of excitation-contraction-metabolism coupling and their interactions with cardioprotective signaling pathways.

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