Meeting Koch's postulates for calcium signaling in cardiac hypertrophy

Commentary

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In 1890, a country doctor in Germany revolutionized our thinking of human disease. The challenge of the day was to identify specific bacterial agents that were responsible for major disease outbreaks. Robert Koch, after careful study of disease epidemics from around the world, forged the precepts that unequivocally establish causality for a single infectious agent in the onset of a distinct disease (1). These "postulates" stated that: (a) the agent should be present in every case of the disease; (b) the agent must be isolated from the diseased host and grown in vitro; (c) the disease must be reproduced when the agent is delivered to a susceptible host; and (d) the agent should be recovered from the diseased animals. Koch's postulates formed the intellectual cornerstone for the next century of advances in microbiology and ultimately led to the discovery of the infectious basis for anthrax, cholera, and tuberculosis. In recognition of these seminal contributions, Robert A. Koch was awarded the 1905 Nobel Prize in Physiology and Medicine. As we enter a new century, it is becoming increasingly clear that the power of Koch's logic has extended beyond the boundaries of bacterial diseases. Although his views were based on the inoculation of agents involved in infectious diseases, the clarity of his thinking continues to guide a new generation of scientists who are using germline transmission in the mouse to establish causality for specific genes and pathways in the etiology of complex, acquired human heart diseases.

In this regard, the current article by Passier and colleagues is a valuable step toward fulfilling Koch's postulates, underscoring the potential importance of calcium signaling pathways in cardiac hypertrophy and failure (2). Alterations in calcium handling have long been known to be closely associated with the onset of cardiac hypertrophy and failure. However, only recently has there been substantial evidence to support the notion that these changes in calcium might underlie pathways that contribute to the progression of either cardiac hypertrophy or failure. The discovery of calcium-calmodulin–dependent protein kinases (3) and the subsequent identification of their critical role in neuronal cell signaling (4) laid the groundwork for a critical examination of the role of CaM kinase–dependent signaling in in vitro cultured cardiac myocyte models of hypertrophy (5). By utilizing reporter genes that are activated during the hypertrophic response, it was subsequently shown that the δ isoform of CaM kinase II, which is translocated into the nucleus and is the predominant form of CaM kinase in the heart (6), was both necessary and sufficient to activate features of hypertrophy in vitro (7). By overexpressing a constitutively active form of CaM kinase IV specifically in the heart of transgenic mice under the control of the well-characterized α-myosin heavy chain promoter (8), the study by Passier et al. now shows for the first time to our knowledge that the CaM kinase pathway is sufficient to activate many features of cardiac hypertrophy and failure in vivo (2). Using a *lacZ* indicator mouse line (9), the current study documents that one of the critical downstream targets in the CaM kinase pathway is the transcription factor, MEF-2, which had previously been shown to play a pivotal role in both skeletal and cardiac myogenesis (10). Previous studies in other cell types have documented that the nuclear transcription factor CREB is a major downstream target for CAM kinase, which directly activates the protein via phosphorylation (11). The discovery of this CaM kinase pathway, which extends from the cytoplasm to the nucleus represents the major new finding of this paper (2). This report joins a large body of work that has previously focused on the role of a calcineurin-NFAT signaling pathway in the control of the hypertrophic response, which appears to be a parallel calcium-dependent pathway (12, 13). Coupling these data with other recent reports that have identified a critical role for calcium cycling defects in the progression of heart failure (14), evidence is mounting that calcium can provoke heart failure and hypertrophy (15).

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This work is intriguing because of the efforts taken to extend previous in vitro observations to the in vivo context and the ability to make a new connection between cytosolic and nuclear signaling molecules. In particular, the use of the MEF-2 indicator line is ingenious (9), as it should ultimately allow the activation of this transcription factor to be monitored in the mouse during the biomechanical stress of pressure overload (16). As with all cutting-edge work, these studies of CaM kinase activation and cardiac hypertrophy suggest several other subsequent lines of experimentation. Given that CaM kinase IV is expressed at only trace levels in the heart (4), it will be of immediate interest to determine whether these observations can be extended to the predominant cardiac CaM kinase, the δ isoform of CAM kinase II, which is the predominant CaM kinase II activity in the heart (6). Recent-

ly, two independent studies have shown that increasing the peak intracellular calcium transient can inhibit progression of heart failure in both genetic and acquired forms of cardiomyopathy (14, 17). In fact, ablating the endogenous brake on calcium cycling, phospholamban, can completely prevent the onset of cardiac hypertrophy and block the induction of atrial natriuretic factor in a mouse model of dilated cardiomyopathy (14). Because the decreased contractile function seen in the CaM kinase transgenic lines most likely reflects a decrease in the calcium transient, it will be important to characterize the compartmentalization of the calcium signal in the hypertrophied and failing heart. Doubly transgenic animals have proved difficult to generate from crosses between the CaM kinase and calcineurin mouse lines, most likely because the α-myosin heavy chain promoter drives expression of both gene products at high levels in the fetal and the adult heart (18), leading to early lethality. For this reason, it will be critical to determine which aspects of the observed phenotypes in the CaM kinase transgenic line represent developmental effect on myocyte survival or morphogenesis and which derive from postnatal effects of the exogenous kinase. Finally, and perhaps most importantly, it will ultimately become necessary to determine whether the observed phenotypes reflect a role of the endogenous CaM kinase genes in the activation of biomechanical stress–induced hypertrophy, or if they arise in part from nonspecific effects of the 50- to 100-fold overexpression of a constitutively active protein.

With regard to this last point, there is a diverse and rapidly growing list of genes that can trigger features of hypertrophy and associated cardiomyopathy after their cardiac-specific expression (19–49) (Table 1), suggesting that multiple pathways can activate this complex adaptive response. As in the present study, in most of these cases, there are additional in vitro and in vivo data that support their role in the pathogenesis of hypertrophy. Accordingly, it is highly likely that these effects reflect a direct or indirect role for many of these genes in the hypertrophic response, a view that is supported by the fact that each of the cardiac phenotypes has distinct characteristics at the molecular, morphological, and physiological levels. However, discriminating between end points that arise strictly as a result of a signaling event from those that reflect nonspecific cardiac injury, presumably due to the disruption of the signaling stoichiometry in multiple pathways, could be a vexing problem. Death of cardiac myocytes may trigger not only postischemic heart failure, but also a transition between compensatory hypertrophy and dilated cardiomyopathy (50). As Izumo et al. (51) have shown, even the overexpression of the reporter gene GFP causes cardiotoxicity and cardiomyopathy when placed under the control of the α-myosin heavy chain promoter. For this reason, the precise role of many of these putative hypertrophy genes may have been obscured by extraordinarily high levels of transgene expression.

A case in point is the large body of work, which has focused on the role of the calcineurin-NFAT pathway in cardiac hypertrophy (12, 13, 33, 52–54). Compelling data in transgenic mice (12), coupled with inhibition of the in vivo pressure overload response by cyclosporin, formed a cornerstone in support of a primary role for calcineurin as part of a final common pathway in hypertrophy (13). However, the story has grown increasingly complicated, as a number of groups have shown that cyclosporin fails to block the onset of pressure overload hypertrophy (55–58). The equivocal nature of the data raises the possibility that a portion of the in vivo effects observed in the calcineurin transgenics might be due to the nonspecific effects of a massive increase in cellular phosphatase levels. Supporting this notion, it was recently documented that *NFAT3* knockout mice display a completely normal response to pressure overload hypertrophy, again raising the issue as to the precise role of the calcineurin pathway in the in vivo hypertrophic response (J.M. Leiden, personal communication). Recently, Tsao et al. reported a tenfold decrease in the level of calcineurin expression in the failing human heart (59). In addition, it has been noted that cyclosporin has little effect on in vivo cardiac hypertrophy in humans that receive chronic therapy during renal transplantation. However, cyclosporin is not an ideal agent to establish Koch's postulates for any specific pathway, given its pleiotropic effects. Of note, the calcineurin-NFAT pathway has also been shown to mediate skeletal muscle hypertrophy, indicating that components of this pathway may represent a conserved mechanism for hypertrophic signaling in other striated muscle cells. (60, 61). Taken together, it becomes apparent that although calcineurin may contribute to an important hypertrophic signaling pathway, its precise role in cardiac hypertrophy remains to be fully established. Definitive proof of causality awaits the engineering of a complete loss of calcineurin function specifically in heart cells. A similar caveat could apply to virtually all of the genes listed in Table 1.

The use of the mouse in the study of cardiac hypertrophy was initially envisioned as a tool to connect in vivo cardiac physiology with the role of specific genes, i.e., a direct means to examine whether single gene effects could activate all of the features of a hypertrophic response that would ordinarily be seen

Table 1
Transgenic models of cardiac hypertrophy[A]

Ligands	:	TNF-α; MCP-1; NGF (32, 39, 41, 43, 49)
Receptors	:	β-2 Adrenergic receptors; angiotensin II type I receptor (22, 47)
Channels/Transporters	:	KV4.2; GLUT-1 (ref. 48; W. Dillman, personal communication)
G proteins	:	Ras; RhoA; Gi; Gs; Gq; Gh (19, 20, 21, 23, 26, 33)
Kinases	:	PKCβ-2; P38; CaM Kinase IV (refs. 2, 44; Y. Wang, and K.R. Chien)
Phosphatases	:	Calcineurin (12)
Transcriptional factors	:	MYF5; NFAT; CREB; RXRα; RARα (12, 24, 38, 42, 46)
Sarcomeric or cardiac structural proteins	:	α-Myosin heavy chain; α-tropomyosin; tropomodulin; Troponin T; myosin binding protein C; myosin light chain-1; myosin light chain-2 (25, 28–31, 34, 35, 37, 40, 45)
Sarcoplasmic reticular proteins	:	Calsequestrin (27)
Other	:	Enteroviral genome, GFP (36, 51)

[A]Partial listing of mice which display cardiac hypertrophy after the cardiac-restricted overexpression of either wild-type or mutant forms of the proteins noted.

in response to a complex stimulus, such as hypertension (for review, see ref. 62). The concept was modeled after the logic of Koch, with the thought that single gene effects might directly activate pathways that can specifically activate all of the features of the hypertrophic response. The past 5 years have seen an exponential growth in our understanding of this important adaptive response of the heart. However, along the way, it has become increasingly clear that hypertrophy is a multigenic, integrative response (15, 63). In addition, environmental and genetic modifiers may be more important in determining the phenotypic outcome than the effects of any single gene. In short, to unravel this complex physiological response of the heart, we may now need to move beyond Koch's postulates, because heart disease is often not initiated by a single stimulus or pathway. The challenge remains to identify the nodal points in the multiple pathways that govern critical functions of the cardiac muscle cell. Ultimately, it will become imperative to examine the consequences of the specific loss of function of these nodal genes in the setting of authentic, complex environmental stimuli that lead to hypertrophy: pressure overload, hypertension, postischemic injury, and hypoxia, among others. Refined single-cell physiological end points could be extremely useful, as we need to move beyond effects on single genes. Precision engineering of hypomorphic alleles, cell-type specific mutations, inducible mutagenesis, and genetic complementation should prove valuable in the next leg of the journey. There is an old Chinese saying that "a journey of a thousand miles begins with one step." For the next generation of cardiovascular physicians and scientists, half the fun will be getting there.

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