Untangling the Web
Specific Signaling From PKC Isoforms to MAPK Cascades
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The understanding of intracellular signaling pathways and their physiological effects has been confounded by the existence of numerous isoforms of the various signaling components. Thus, many families of protein kinases comprise several subfamilies, each of which may contain multiple isoforms deriving from distinct genetic loci. Furthermore, each locus may produce multiple products through alternative splicing. A rational argument maintains that each isoform has a distinct role in cellular regulation, but evidence of this is sparse. The alternative explanation, that there is redundancy, has led to the generation of web-like diagrams of interconnecting signaling pathways as investigators attempt to decipher the wiring patterns of the cell. In the heart, two superfamilies of protein kinases, the protein kinase Cs (PKCs) and the mitogen-activated protein kinases (MAPKs), are particularly implicated in the development of cardiac pathologies. Both superfamilies contain numerous isoforms, but little is known about the roles of individual isoforms. In this issue of Circulation Research, Heidkamp et al2 provide some of the first data showing that specific PKC isoforms couple to distinct MAPK pathways to regulate cardiac myocyte function.

PKC and the MAPKs
The PKC superfamily comprises the “classical” cPKCs (α, β1, β2, γ), “novel” nPKCs (δ, ε, η, θ), and PKC-related kinases (PRKs).1 In cardiac myocytes, cPKCα, nPKCδ, nPKCε, and aPKCζ are readily detectable, although cPKCβ isoforms may also be significant.4 Hypertrophic agonists such as endothelin-1 (ET-1) or the α-adrenergic agonist phenylephrine (PE) activate nPKCδ and nPKCε in cardiac myocytes, as shown by translocation from the soluble to the particulate fraction of the cell, and such studies implicate these isoforms in the hypertrophic response. Although the identities of individual PKC isoforms have been known for some years, the physiological substrates of these kinases and the downstream effects of individual PKC isoforms have remained obscure.

The MAPKs are final components of three-tiered protein kinase cascades (see Figure) and comprise at least 3 subfamilies. The extracellular signal–regulated kinases (ERK1/ERK2) are particularly implicated in growth-associated responses.1 The c-Jun N-terminal kinases (JNKs, numerous isoforms derived from 3 genes), and p38 MAPKs (at least 6 isoforms encoded by 4 genes) are generally activated by cytotoxic stresses.5 All 3 subfamilies are implicated in cardiac pathology.1 Recent studies in transgenic animals indicate that the ERK cascade promotes compensated hypertrophy.6 Stimulation of JNKs may also be hypertrophic, although this is debated.7 The role of the p38 MAPKs is unclear, and different p38 MAPK isoforms may promote hypertrophy or apoptosis.5

Considerable evidence indicates that PKCs promote activation of ERKs.1 Direct activation of cPKCs/nPKCs by phorbol esters potently stimulates ERKs in cardiac myocytes. Agonists such as ET-1 and PE stimulate ERKs, whereas 24-hour pretreatment with phorbol esters downregulates cPKCs/nPKCs rendering the ERK cascade refractory to activation by ET-1 or PE. The role of PKCs in activating JNKS or p38 MAPKs is less clear. These kinases are not significantly activated in cardiac myocytes by phorbol esters, but ET-1 and, to a lesser extent, PE, activate both JNKS and p38 MAPKs.1 Most studies rely on inhibitors to implicate PKCs in MAPK activation and in cardiac myocyte responses, but these inhibitors are not particularly selective and have effects other than inhibition of PKC.7 Furthermore, as Heidkamp et al2 demonstrate, the inhibitors themselves constitute a cellular stress sufficient to activate JNKS and p38 MAPKs.

Signaling Through Specific PKC and MAPK Isoforms
Few studies have examined which isoforms participate in specific signaling pathways or the consequences of isoform-specific signaling. These are the questions that Heidkamp et al2 have begun to address using adenoviral infection to express constitutively active (ca) nPKCδ or nPKCε in neonatal cardiac myocytes. The authors’ previous study indicated that ET-1 stimulation of ERKs is mediated by nPKCε and leads to cardiac myocyte hypertrophy.8 This conclusion is further supported in the present study.2 Here, the authors not only demonstrate selective activation of ERKs by ca-nPKCε, with minimal activation of JNKS or p38 MAPKs, but this response is isoform-specific, because ca-nPKCδ preferentially activates JNKS and p38 MAPKs with minimal activation of ERKs (see Figure). The data are consistent with studies in other cells, which also indicate that nPKCδ couples to JNKS and nPKCε couples to ERKs,10 but the study by...
Signaling from nPKCδ and nPKCε through the MAPK cascades. Overexpression of ca-nPKCε selectively activates the ERK cascade, probably through Ras activation of the MAPK kinase (MKK) Raf. Raf activates the MAPK kinases 1/2 (MKK1/2), which activate ERK1/2. This pathway is associated with cardiac hypertrophy and/or cardioprotection. Overexpression of ca-nPKCδ stimulates JNKs and p38 MAPKs, presumably by activating ill-defined MKKs, which stimulate MKK4/7 or MKK3/6. Activation of JNKs and p38 MAPKs may be direct or through an increased cellular stress generated during the apoptotic response induced by nPKCε. ET-1 activates both nPKCε and nPKCδ.

Heidkamp et al. presents clear evidence for differential wiring of intracellular signaling pathways in a single cell type.

The mechanisms involved in these selective signaling events are not understood. With respect to ERK activation, such selectivity is not apparent in Cos cells in which ERKs are activated by cPKCs, nPKCs, or PKCβ. In addition constraints within the myocyte must therefore operate to enforce specific signaling routes. Localization may be crucial, and activated PKC isoforms are anchored by a family of proteins, RACKs (receptors for activated C kinase) presumably in specific subcellular compartments. Thus, the RACK for nPKCα in myocytes may not be in an appropriate context for activation of ERKs. Alternatively, the fidelity of signal transmission may be conferred by preformed signaling complexes and, in fibroblasts, nPKCε is constitutively associated with upstream components of the ERK cascade, N-Ras, and c-Raf. The mechanisms involved in activation of JNKs or p38 MAPks by nPKCδ are more difficult to predict, partly because the upstream components of these pathways are ill-defined. In addition, nPKCδ promotes cardiac myocyte apoptosis, which itself constitutes a cellular stress, and activation of JNKs and p38 MAPKs may be a consequence of this. Indeed, maximal activation of JNKs and p38 MAPks by ca-nPKCδ occurs when apoptosis is already well-developed and some time after ca-nPKCδ is first expressed.2

Consequences of nPKCδ or nPKCε Signaling

In addition to specificity within the PKC—MAPK signaling pathways, it is also clear that the different pathways regulate specific cellular responses. Although there is much debate about the precise roles of the MAPK subfamilies, an emerging theme is that activation of the ERK cascade is associated with compensated hypertrophy. The roles of specific PKC isoforms have been less clear, but highly selective peptide activators or inhibitors of nPKCε have implicated this isomorph in the development of compensated hypertrophy and cardioprotection. The data from Strait et al. and Heidkamp et al. now provide a link from nPKCε through ERKs to hypertrophy, since ca-nPKCε stimulation of ERKs is associated with some aspects of the hypertrophic response. Increasing evidence implicates nPKCδ in apoptosis. nPKCδ may initiate apoptosis by translocating to the mitochondria to induce cytochrome c release but may also have an effector role because cleavage of nPKCδ by caspases generates an activated form that may direct cellular contents for proteolysis. Heidkamp et al. demonstrate that ca-nPKCδ induces cardiac myocyte apoptosis, results that are in accord with the recent demonstration that activation of nPKCδ increases myocardial damage induced by ischemia. Although overexpression of nPKCδ activates JNKs and p38 MAPks, as discussed above, activation of these MAPks may not be a direct consequence of nPKCδ signaling. Irrespective of the precise order of events, however, it is clear that activation of JNKs and/or p38 MAPks after nPKCδ activation is detrimental to myocyte survival.

Differential activation of ERKs by nPKCε leading to hypertrophy or cardioprotection and activation of JNKs/p38 MAPks by nPKCδ leading to apoptosis presents an orderly view of intracellular signaling and regulation of cell function. However, in the context of an individual myocyte in the heart, the situation is more complex. Apart from signals from multiple receptors, a single agonist activates multiple pathways. Indeed, ET-1 activates both nPKCδ and nPKCε in neonatal cardiac myocytes, stimulating JNKs and p38 MAPks in addition to ERKs. It is presumably the balance between the various signaling pathways that dictates the overall response of the cell, but the points of integration and commitment to a specific response have yet to be examined. Meanwhile, emerging studies such as those by Heidkamp et al. showing specific activation of selective signaling pathways to elicit particular responses, may be the first steps in unraveling the tangled web of signaling intermediates that constitute current concepts of intracellular signaling.

References


*Key Words:* cardiac myocytes | protein kinase C | mitogen-activated protein kinases | hypertrophy | apoptosis
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