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Cardiovascular Research 45 (2000) 826–842

Cardiovascular Research

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Review

Signalling via stress-activated mitogen-activated protein kinases in the cardiovascular system

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Received 23 July 1999; accepted 22 October 1999

Abstract

A number of physiological, pharmacological and pathological stimuli initiate cardiac hypertrophy. The intracellular signalling events activated by these stimuli are equally complex. Our ability to treat the hypertrophic and failing myocardium effectively will require clarification of which signalling events regulate growth, remodelling and failure. Much recent attention has focused on the regulation of the mitogen-activated protein kinase cascades (MAPKs), with the importance of these cascades in the development of cardiovascular diseases being extensively explored. These signalling pathways may provide one link from the diverse stress and pharmacological extracellular stimuli to the regulation of gene expression, contractile protein regulation and protein function. This review focuses on the recent progress made in the understanding of the regulation and function of MAPKs in the cardiovascular system, with particular emphasis being placed on the events in the cardiac ventricular myocyte.

Keywords: Cytokines; Gene expression; Growth factors; Protein kinases; Signal transduction

1. Historical perspective and introduction to the MAPKs

In 1988 a protein kinase activated in 3T3-L1 cells in response to insulin was described. By 1991, two members of this family of protein kinases had been purified, cloned and their close similarity to protein kinases involved in the yeast pheromone response recognised [1]. In the following years, the combined experimental results from mammalian cell biology, biochemistry, and genetic studies in yeast, nematodes and fruit flies allowed the identification of a superfamily of related Ser/Thr protein kinases now known as the mitogen-activated protein kinases or MAPKs. Their signature motif is a conserved amino acid sequence Thr–X–Tyr (T–X–Y) within their activation loop and phosphorylation of both Thr and Tyr results in their activation [2].

Throughout this review, the MAPKs first recognised to respond to growth factor stimulation are referred to as Extracellular Signal Regulated Kinases, or ERK MAPKs. Principles of the regulation of the ERK MAPKs including the regulation by phosphorylation within the T–X–Y motif and position within the signal transduction cascades, can be readily applied to the regulation of the other MAPKs (Fig. 1). If we first focus on the ERK MAPKs, it is apparent that the two isoforms, ERK1 and ERK2, are widely-expressed [1]. In cardiac myocytes, hypertrophic agents activate both isoforms [3]. In vascular smooth

Abbreviations: ANF, Atrial Natriuretic Factor; BMK, Big Mitogen-activated protein Kinase (=ERK5); CSBP, Cytokine Suppressive Anti-Inflammatory Drug; Binding Protein (=p38 MAPK); CT, Cardiotrophin; ERK, Extracellular signal Regulated protein Kinase; FAK, Focal Adhesion Kinase; G-protein, GTP-binding-protein; GDS, Guanine nucleotide dissociation stimulator; IL, Interleukin; JNK, c-Jun-N-terminal protein Kinase; LIF, Leukemic Inhibitory Factor; MAPK, Mitogen-Activated Protein Kinase; MAPKAPK, MAPK-Activated Protein Kinase; MEK, Mitogen-activated protein kinase(or ERK) kinase; MEKK, MEK kinase; MKK, Mitogen-activated protein Kinase Kinase; MKP, MAPK phosphatase; Mnk, MAPK interacting kinase; MSK, Mitogen- and Stress-Activated Kinase 1; Mxi2, Max interacting protein2 (=p38 MAPK); p38, protein kinase 38 kDa; $p90^{RSK}$, 90kDa Ribosomal S6 Kinase; PDGF, Platelet Derived Growth Factor; PI3K, Phosphatidylinositol 3′Kinase; PKC, Protein Kinase C; Ral.GDS, Ral guanine nucleotide dissociation stimulator; RK, Reactivating Kinase (=p38 MAPK); ROS, Reactive Oxygen Species; SAPK, Stress Activated protein Kinase; SRE, Serum Response Element; TNF, Tumour Necrosis Factor

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Time for primary review 35 days.

0008-6363/00/$ – see front matter
PII: S0008-6363(99)00386-7

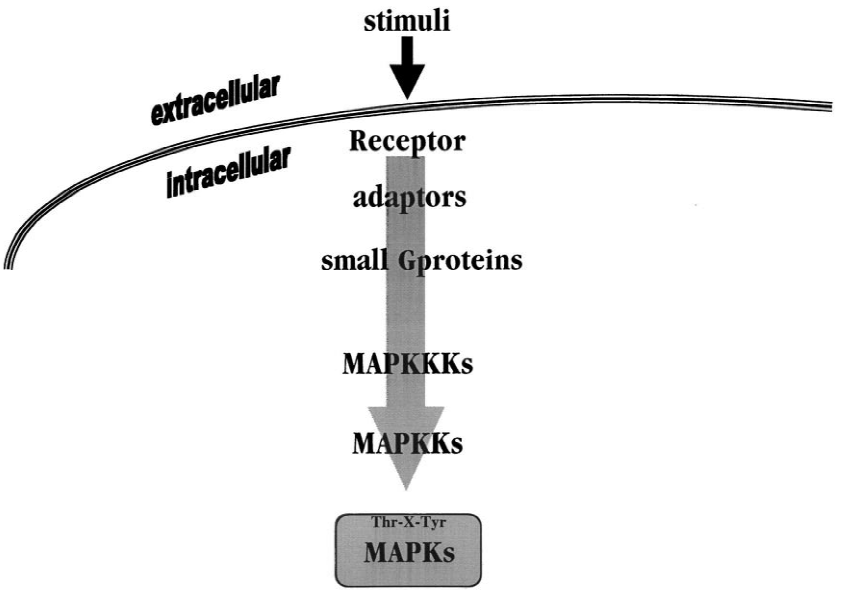

Fig. 1. Organisation of a typical Mitogen-activated Protein Kinase (MAPK) signalling pathway. Each MAPK subfamily member acts within a distinct MAPK signalling pathway and a typical pathway is shown to highlight the similarity in organisation. When a hormone or growth factor interacts with its receptor, a conserved sequence of events follows in which adaptor molecules are recruited and small G-proteins are activated. There are at least three tiers of protein kinases – the MAPKKKs that phosphorylate and activate MAPKKs which in turn activate the MAPKs by phosphorylation of both Thr and Tyr within a conserved Thr–X–Tyr sequence. Despite having this overall similarity in the tiers of organisation, each MAPK pathway does have highly specific signalling intermediates which maintain fidelity of signalling. At least four mammalian MAPK subfamilies within parallel MAPK pathways have been characterised.

muscle cells, potent proliferative and hypertrophic stimuli are also potent ERK MAPK activators [4]. Although this correlation suggests that ERK MAPKs facilitate growth-promoting effects, there are conflicting reports [5–8]. It is difficult to identify the causes for these disagreements, although subtle differences in model systems and analysis may be confounding effects mediated by complex interacting signal transduction networks. There has been a recent focus on other MAPK subfamilies and it may be predicted that the extent and duration of activation of these kinases will have consequences for the final responses in cells of the cardiovascular system. The MAPKs activated by cellular stresses are the subject of this review.

2. MAPKs activated by extracellular stresses – the SAPKs

All cells of the body are subjected to extracellular stresses such as shear stress evoked by blood flow past endothelial cells, ischemia induced by diminished blood flow, or stretch imposed upon vascular smooth muscle cells. Even before the stress-activated cytoplasmic signalling events in mammalian cells had been fully explored, the existence of multiple distinct stress-activated MAPK pathways in yeast suggested the possibility of multiple mammalian MAPK pathways. At least four groups of stress-activated MAPKs are characterized in mammalian cells (Fig. 2).

2.1. JNK MAPKs

The first identification of a mammalian stress-activated MAPK came in 1990 with the observation that intraperitoneal injection of the protein synthesis inhibitor cycloheximide activated a 54 kDa protein kinase. Subsequent characterisation revealed that both Thr and Tyr phosphorylation were essential for its activation, and that there was 40–45% sequence identity with the ERK MAPKs [9]. Ten isoforms have been since identified as the products of three genes in which alternative splicing generates the 46 kDa and 55 kDa variants commonly observed [10]. These protein kinases, the Jun N-terminal kinases or JNK MAPKs, phosphorylate the N-terminal transactivation domain of the transcription factor c-Jun [11]. They are activated by stresses such as heat shock, protein synthesis inhibition and UV irradiation, and in initial studies, most growth factors which were good ERK MAPK activators were poor JNK MAPK activators [9]. The activation of JNK MAPKs has now been observed in a variety of cell types of the cardiovascular system [12–16]. The defining motif of this group of kinases is Thr–Pro–Tyr (T–P–Y) within the activation loop.

2.2. p38 MAPKs: p38α, p38β, p38-2, SAPK-3 (p38γ) and SAPK-4 (p38δ)

A 38 kDa phosphoprotein was first reported following the exposure of pre-B cells to endotoxic lipopolysaccharides. Subsequent purification and cloning revealed a

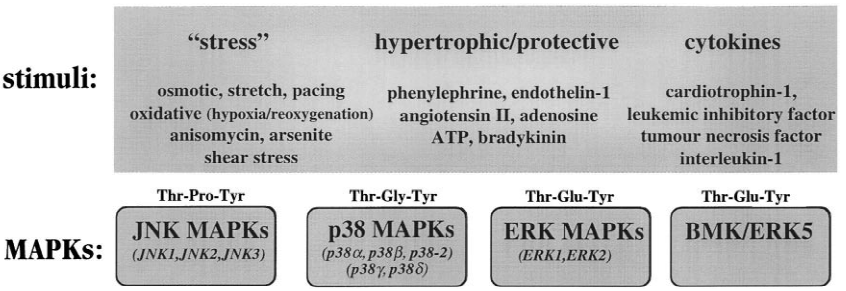

Fig. 2. Mitogen-activated Protein Kinase (MAPK) subfamilies within the cardiovascular system. Cells of the cardiovascular system may be exposed to a diversity of extracellular stimuli which lead to activation of one or more MAPK subfamilies. Most initial work focused on the regulation of ERK MAPKs by hormones and peptide growth factors (including phenylephrine, endothelin-1 or angiotensin) which are hypertrophic for ventricular myocytes. Cells of the cardiovascular system also respond to a variety of cytokines and stresses. These cytokines include those providing cardiac protection (cardiotrophin-1 and leukemic inhibitory factor) and pro-inflammatory cytokines (tumour necrosis factor and interleukin). The stresses include hyper- and hypo-osmotic shock, physical stretch or deformation, increased rates of contraction (pacing), exposure to reactive oxygen species (oxidative stress), chemical stresses (such as anisomycin or arsenite) and shear stress. To date, at least four MAPK subfamilies have been implicated in the response to these stresses. Each MAPK subfamily acts within a specific cascade (as summarised in Fig. 1). Activation of each MAPK is achieved by phosphorylation of both Thr and Tyr within its distinctive Thr–X–Tyr motif as indicated. There may be some cross-talk between these pathways but for simplicity of this representation, these multiple steps between sensing the extracellular stimuli and MAPK activation have been omitted.

protein kinase with 49% identity to ERK MAPKs [17]. Closely-related isoforms were identified by their ability to reactivate MAPK-activated protein kinase-2 (MAPKAPK2) [18], their ability to interact with the cytokine suppressive anti-inflammatory drug SB203580 [19], or their ability to phosphorylate the transcription factor Max [20]. This has led to names such as p38 MAPK, RK MAPK, CSBP, and Mxi-2. The identification of another two isoforms of p38 MAPK, p38β and p38-2, has further emphasized this subfamily complexity [21,22]. The defining motif of the p38 MAPKs is Thr–Gly–Tyr (T–G–Y) within the activation loop.

More recently, two additional p38 MAPK isoforms have been identified. These kinases, SAPK-3 (p38γ) and SAPK-4 (p38δ), are 40% identical to ERK MAPKs, 60% identical to p38 MAPKs, and 65% identical to each other [23,24]. No studies have directly investigated protein expression, but RNA analysis indicates SAPK-3 is predominantly expressed in skeletal muscle, whereas SAPK-4 is ubiquitously expressed [24]. The close similarity to p38 MAPKs is extended to the signature motif of T–G–Y within the activation loop.

2.3. BMK1/ERK5

Big MAPK or ERK5 was identified independently as a 98 kDa protein kinase [25,26]. BMK1/ERK5 is activated in vascular smooth muscle cells or aortic endothelial cells exposed to oxidative stress, osmotic shock, laminar flow or serum but not angiotensin II, platelet-derived growth factor (PDGF) or tumour necrosis factor-α (TNF-α) [27,28]. Although the expression of BMK1/ERK5 in the heart is high as judged by Northern blot analysis [26], its activation in cardiac myocytes remains uncharacterised. BMK1/ERK5 shows similarities to the ERK MAPKs, with a Thr–Glu–Tyr (T–E–Y) motif within its activation loop. The significant differences within this activation loop sequence confirm BMK1/ERK5 is activated by a distinct upstream kinase MEK5 [26].

2.4. ERK MAPKs

The essential features of the ERK MAPKs have been discussed in the preceding sections. A wide variety of growth promoting/hypertophic agents activates these kinases in cardiac myocytes, fibroblasts, smooth muscle cells or endothelial cells [29–32]. As will become clear in this review, the activation of ERK MAPKs may also follow exposure to a variety of extracellular stresses. Such activation of a potent growth factor-stimulated pathway by stress may seem contradictory, but if the ERK MAPK activation that follows cellular insults is chemically inhibited, cell death is accelerated [33]. Thus, ERK MAPKs may contribute to cardiovascular cell survival.

3. Stress stimuli and the identification of "stress-receptors"

Studies in a variety of cell types have identified a diverse range of extracellular stresses that can ultimately activate one or more MAPK pathways. The identification of ''stress-receptors'' or ''stress-sensors'' mediating these events would provide targets for therapeutic intervention.

For in vitro cultured cardiac myocytes, mechanical stretch, electrical pacing, hyperosmotic shock, or oxidative stress, rapidly activate ERK, JNK or p38 MAPKs [34–36]. The diversity of these stress stimuli and their long-term effects implies that there may also be diverse ''stress-receptors'' engaged. Of these stress stimuli, both stretch and increased electrical pacing have powerful positive growth effects on the myocyte and promote hypertrophy. It

has been tempting to propose that the MAPK pathways provide the link between the initial stimulus and the final cellular outcome. This has been supported when the co-expression of the JNK MAPK substrate c-Jun enhanced the hypertrophic effect of pacing [36]. Other physiologically-relevant stresses for the cardiovascular system accompany changes in blood flow during ischemia and reperfusion with the concomitant changes in oxygen and metabolite concentrations in the immediate environment of the cells. Accompanying the changes in oxygen and metabolite concentrations are differences in the MAPK subfamilies activated. The identification of one or more ''stress-receptors'' either within the cell cytosol or plasma membrane remains elusive. In the following sections, the ''stress-receptors'' engaged by three broad categories of stress are considered.

3.1. Diversity of chemical stress stimuli, ''stress-receptors'', and MAPK activation

Hypoxia, ischemia/reperfusion and hypertension are complex and multi-faceted stress stimuli. Therefore more defined forms of chemical stress have been used to a limited extent with cells of the cardiovascular system. For isolated adult cardiac myocytes, short-term exposure to the arsenic compound arsenite activates the p38 MAPKs [37]. The long-term effects of arsenite largely remains uncharacterised but high concentrations are toxic to cultured neonatal cardiac myocytes (M.A. Bogoyevitch and N.W. Court, unpublished). When anisomycin, a protein synthesis inhibitor and activator of both JNK and p38 MAPKs, is added at low concentrations to cultured cardiac myocytes it effectively protects against subsequent ischemic damage [38]. In these cases, the exact ''stress-receptor'' that senses arsenite or anisomycin remains undefined. Of course, for cardiac myocytes, smooth muscle cells or endothelial cells, the stresses of greatest significance are likely to include stretch, mechanical deformation or shear stress.

3.2. The identification of ''stress-receptors'' and the involvement of autocrine/paracrine growth factor signalling in mechanical stress

Cellular detection of mechanical stress most likely involves the integrins and interaction with the extracellular matrix via focal adhesion sites as shown in Fig. 3(a). Plasma membrane perturbations may then subvert the upstream activators in the growth factor-activated pathways. There is some direct support for these events. The exposure of aortic endothelial cells to shear stress results in clustering of receptor tyrosine kinases such as the vascular endothelial growth receptor Flk-1 [39]. The advantage of this model is that it simply explains how changes in the physical environment of the cell modify the physical state of the cell membrane and subsequent signalling events.

Another model which involves autocrine/paracrine release of hormones or growth factors is summarized in Fig. 3(b). Signalling by growth factor and/or cytokine receptors at the surface of the cell is therefore an integrative step, and this rationalises how stress events are transduced into signalling pathways normally activated by growth factor receptors. Thus the autocrine/paracrine release of growth factors could provide a critical signalling link even though the initial ''stress-receptor'' remains unknown. There is ample evidence to support the release of growth factors from cells subjected to various forms of stress. If we take the example of endothelial cells exposed to shear stress, genes encoding the growth factors Fibroblast Growth Factor, PDGF and endothelin-1 are upregulated [40,41]. The growth factors presumably released then act upon their specific receptors to promote endothelial cell proliferation. In a similar manner, cyclic stretch of vascular smooth muscle cells regulates an autocrine production of insulin-like growth factor-I and PDGF that stimulate proliferation [42]. It is also clear that the activation of JNK MAPKs in vascular smooth muscle cells is mediated via an autocrine release of ATP that activates P_{2Y6} purinoceptors [43]. Thus the exact identity of the released growth factors (or cytokines) dictate which MAPK cascades are activated.

The same model of autocrine/paracrine signalling can also be invoked for cardiac myocytes subjected to stress. Specifically for the heart, α_1-adrenergic agents, endothelin-1, prostaglandin $F_{2\alpha}$, angiotensin II and adenosine activate the JNK, p38 and ERK MAPKs in perfused hearts and/or cultured cardiac myocytes (Sections 4 and 5). The potential exists for any of these agents to act as autocrine and/or paracrine mediators of the stress response. Thus angiotensin II released from stretched cardiac myocytes promotes hypertrophy [44]. Whether angiotensin exclusively mediates the stretch effects has been critically re-evaluated with the development of a transgenic model that is homozygous for loss of angiotensinogen. The preservation of the response to activate ERK MAPK (but not JNK MAPK) in this transgenic model suggests that factors other than angiotensin II are important for some signalling events following cellular stretch [45]. A requirement for factors which act via G-protein coupled receptors is also supported by another transgenic model in which the overexpression of the carboxy-terminus of the Gαq subunit inhibits signalling and prevents the development of pressure overload hypertrophy [46]. The complexity of these events is further highlighted with the recent evidence that the cytokine Leukemic Inhibitory Factor (LIF, Section 4.3) plays a role in the in vitro responses of cardiac myocytes to stretch [47].

3.3. The identification of ''stress-receptors'' and the involvement of reactive oxygen species (ROS) and autocrine/paracrine signalling in oxidative stress

In the preceding examples, the initiating stress was mechanical stretch. Another well-studied example of

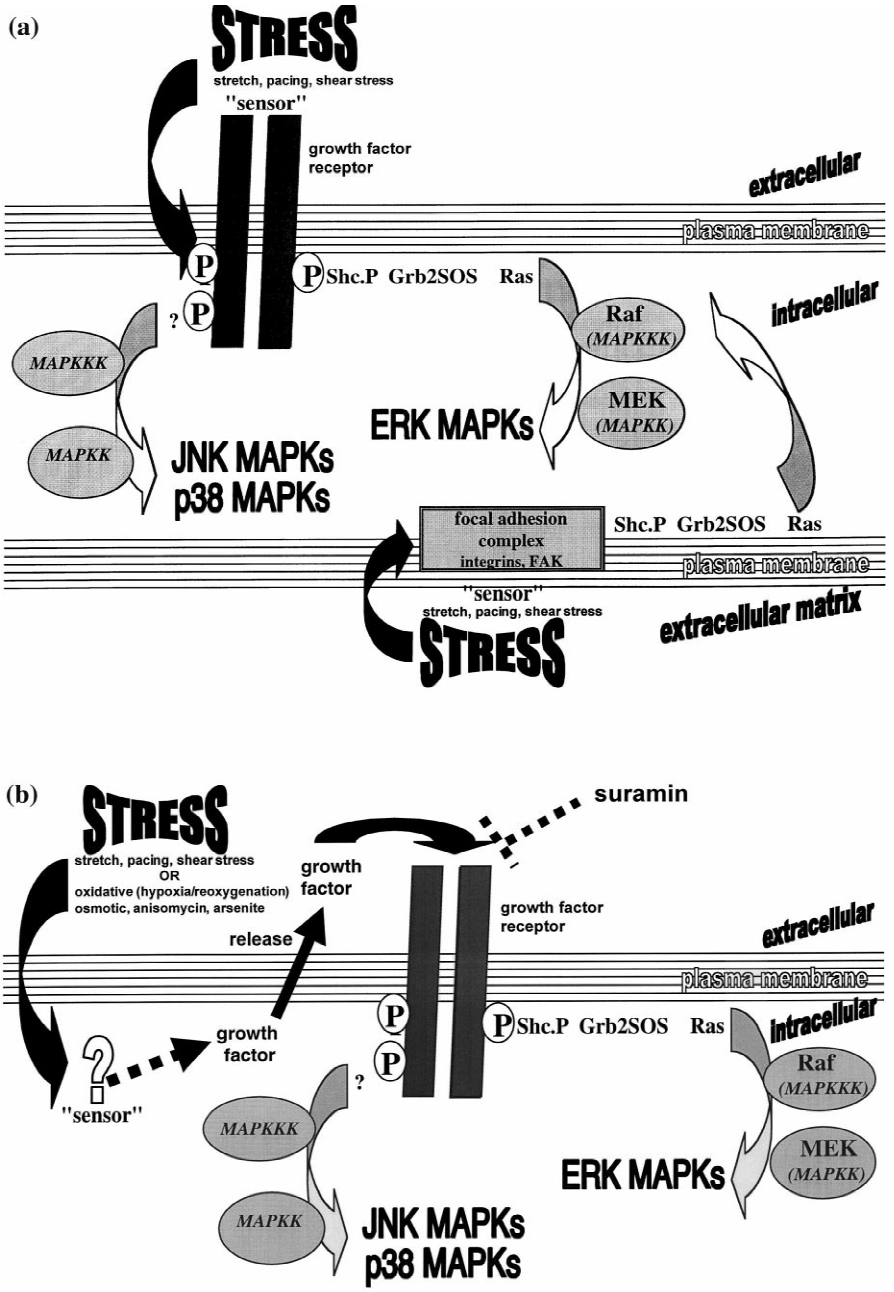

Fig. 3. Models for stress-activation of intracellular signalling pathways culminating in activation of one or more MAPK pathways. (a) The activation of MAPK pathways by mechanical stretch and other physical forces (pacing or shear stress) can be explained by events that lead to deformation of the plasma membrane. At least two inputs are likely. Following physical stress, growth factor receptors may be clustered and this promotes their activation and phosphorylation (P) in the absence of growth factors. Simultaneously, physical stress will be detected at the focal adhesion complexes that mediate interaction of the cell with the substratum, the extracellular matrix. The subsequent activation of integrins and Focal Adhesion Kinase (FAK) activates intracellular events. There are obvious links between the growth factor receptors or focal adhesion complexes and the activation of the ERK MAPK pathways. This involves phosphorylation of the adaptor Shc, recruitment of the adaptor Grb2 then the Sos-mediated activation of the small G-protein Ras. Finally the protein kinase Raf (a MAPKKK) is activated to initiate the tiers of kinases of MEK (a MAPKK) and the ERK MAPKs. The exact links between the growth factor receptors and activation of JNK and p38 MAPKs remain relatively undefined. (b) Although stresses were originally anticipated to directly activate specific signalling pathways within the cell, it has become clear that many of the events triggered by growth factor receptor engagement are also triggered following stress. A second model that rationalizes these observations incorporates the release of potent growth factors following cellular exposure to stress. Suramin, has been used extensively to implicate growth factor receptors in events following cellular exposure to stress. Although the exact sensor for the stresses is unknown (as shown by ?), the production and/or release of growth factors allows for growth factor receptor binding and initiation of complex intracellular events. Thus the activated growth factor receptor would recruit the adaptors Shc and Grb2, resulting in Sos-mediated activation of the small G protein Ras, the activation of Raf (MAPKKK), and the activation of MEK (MAPKK). A prediction made with this model is that the exact nature of the activated receptor will dictate which MAPK pathways are activated and so this explains the activation of JNK and p38 MAPK pathways following stress.

paracrine/autocrine events leading to MAPK activation is observed in cells exposed to oxidative stress in ischemia/reperfusion. The identity of the ''stress-receptors'' and the mediators of these effects of ischemia/reperfusion which activate MAPKs remain to be fully elucidated.

One mechanism that has received considerable attention incorporates the rapid production of reactive oxygen species (ROS) upon the re-exposure of ischemic cells to normal oxygen levels. Many sites of ROS generation exist in the intact heart. Neutrophils and macrophages may contribute to ROS production and myocytes can themselves generate ROS from oxidase enzymes and the mitochondrial electron transport chain. In some situations, ROS have been shown to alter pathways controlling cell proliferation, differentiation or death. ROS may also initiate the protective effects of myocardial ''preconditioning'' in which the prior exposure of the heart to brief periods of ischemia ameliorates cell death caused by longer ischemic episodes [48]. The effects of ROS may be more simply mimicked by the exposure of isolated or cultured cells to hydrogen peroxide. This activates MAPKs in the perfused heart [49] and cultured cardiac myocytes [50–52].

In numerous cell types, ROS can stimulate the release of growth factors. Therefore the model presented in Fig. 3(b) can also explain the response of cells to oxidative stress. The released factors engage receptors which activate Shc/Grb2/Sos, Ras, Raf, MEK then ERK MAPKs in the classic ERK MAPK cascade (Fig. 3(b)). This series of events follows the exposure of cultured cardiac myocytes to hydrogen peroxide [50,52]. Furthermore, the effects of hydrogen peroxide to activate ERK MAPKs, p38 MAPKs and JNK MAPKs could be inhibited by a general inhibitor of growth factor receptors, suramin [50]. This confirms that hydrogen peroxide activate can stimulate growth factor receptors to stimulate secondary signalling events. It would seem that ROS will play a major role in intracellular signal transduction mediated by both growth factors and stress, thereby providing flexibility in signalling pathways and the opportunity for cross-talk between signalling pathways.

3.4. The identification of ''stress-receptors'' and the involvement of protein phosphatases

Phosphatase regulation also plays a role in regulating intracellular signalling pathways, and agents that inhibit phosphatase activity will allow accumulation of their substrates in phosphorylated and activated forms. Evidence suggests that arsenite reacts with the active site cysteine of JNK MAPK phosphatases to slowly but directly inhibit phosphatase activity with subsequent accumulation of activated JNK MAPKs [53]. A similar inhibition of JNK MAPK phosphatase activity is also apparent after exposure of H9c2 cardiac myoblast cells to ethanol, oxidative stress and heat shock [54]. However, in adult cardiac myocytes, arsenite failed to activate JNK but activated p38 MAPK, emphasizing the potential for differences in signalling related to cell context [37].

The regulation of MAPKs by phosphatases has been more directly addressed with the use of specific inhibitors. The use of okadaic acid, a cell-permeable inhibitor of protein phosphatase 2A, has revealed activation of JNK MAPKs in angiotensin II-treated ventricular myocytes [32], and the regulation of novel kinases and ERK MAPKs in ventricular myocytes, fibroblasts and H9c2 myoblasts (Ref. [55] and M.A. Bogoyevitch and M. Thien, unpublished). It remains possible that phosphatase inhibition during ischemia will facilitate higher levels and/or prolonged phosphorylation of proteins that provide protection. Direct effects of phosphatases have also been investigated by their constitutive overexpression. In these well-defined in vitro protocols, the dual-specificity phosphatase MAPK-Phosphatase-1 (MKP-1), inhibited the expression of atrial natriuretic factor (ANF) with only small effects on cell morphology [56]. The maintenance of signalling specificity remains a problem of these overexpression studies [57–60]. Thus a role for one or more MAPKs seems critical, but it remains difficult to assign the importance of the individual MAPK subfamilies based solely on these studies.

4. Activation of stress-activated MAPKs by cytokine receptors

As mentioned in the previous section, the release of soluble factors following stress provides an attractive link to intracellular signalling events. An obvious class of mediators includes the pro-inflammatory cytokines released from cells of the cardiovascular system, the resident inflammatory cells or infiltrating inflammatory cells. If these inflammatory events accelerate progression towards cardiovascular dysfunction, then preventing the synthesis and/or release of these cytokines would be of therapeutic benefit. In support of this proposal, the cardioprotective effect of insulin-like growth factor-1 correlates with its ability to inhibit leukocyte-induced cardiac cell death [61]. So even if the ''stress-receptors'' themselves remain unidentified, the identification of which pro-inflammatory cytokine act as mediators can provide a target to prevent further damage.

Enhanced levels of expression of TNF-α, interleukin-1β (IL-1β) and interleukin-6 (IL-6) have been observed in the heart following infarction. The highest levels were recorded for IL-1β, with macrophages, endothelial cells and vascular smooth cells contributing to expression. A sequential increase suggests that IL-1β induces IL6 production [62]. Because the events triggered by TNF-α and IL-1β converge, we will concentrate on the signalling events and long-term events stimulated by these two

cytokines and then consider the effects of Leukemic Inhibitory Factor (LIF) and Cardiotrophin-1 (CT-1).

4.1. Tumour necrosis factor-α (TNF-α)

The cardiac myocytes together with resident macrophages are capable of producing TNF-α with infection or endotoxemia enhancing production. Myocardial TNF-α contributes to myocardial dysfunction via both its rapid and long-term actions on a variety of different cell types of the heart [63]. In vivo evidence from the transgenic mouse model of cardiac-specific overexpression of TNF-α, supports a role for TNF-α in cardiac failure [64]. In isolated myocytes, TNF-α inhibits L-type calcium transients, alters contractility, enhances synthesis of heat shock proteins, increases cell size and protein synthesis rates, or stimulates controlled cell death [65–70]. This range of actions of TNF-α from pro-survival to pro-death most likely reflects the differences in each experimental condition. The signalling events associated with TNF-α are initiated by two classes of TNF receptor, namely the type I (p55/60TNFR) and the type II (p75/80TNFR) receptor, and the adult heart expresses receptors of the type I class [65]. By analogy with the signalling events initiated in other tissues, it is expected that the TNF I Receptor will activate both cell death pathways in addition to cell survival pathways [71]. Multiple MAPK pathways are also activated by TNF-α, but whether these promote death or survival remains controversial [72]. It is most likely that the interplay of MAPKs and other signalling pathways determines final cellular outcome.

4.2. Interleukin-1 (IL-1)

The fibroblasts of the heart together with smooth muscle cells, endothelial cells and inflammatory cells can produce IL-1β. This can alter the growth of cultured myocardial cells, specifically decreasing cardiac fibroblast DNA synthesis whilst increasing rates of cardiac myocyte rates of protein synthesis [73–75]. The signalling events associated with IL-1 are initiated by the type I IL-1 receptor in association with an essential accessory protein [76]. By analogy with the signalling events initiated in other tissues, it is expected that the IL1 receptor will activate all three MAPK pathways. Again we expect that the interplay of these signalling pathways will determine final cellular outcome.

4.3. Leukemic inhibitory factor (LIF) and Cardiotrophin-1 (CT-1)

Together with cytokines such as IL-6, LIF and CT-1 form a family of cytokines that use a common gp130 signalling subunit to initiate intracellular signalling [77]. The identification of CT-1 as a potent hypertrophic cytokine produced by neonatal cardiac myocytes has heightened the interest in this family of cytokines [78]. Furthermore, the expression of CT-1 is enhanced in the hearts of genetically hypertensive rats in vivo [79]. The signalling events associated with LIF and CT-1 are initiated by the LIF-Receptor in association with gp130. This receptor complex activates the JAK/Stat and ERK MAPK pathways in cardiac myocytes [47,80–83]. It is currently unclear whether LIF or CT-1 activate the other stress-activated MAPK pathways in cardiac myocytes or other cells of the cardiovascular system.

5. Activation of stress-activated MAPKs by receptors for hypertrophic agents

There is now increasing evidence that receptors for growth-promoting factors may regulate the intracellular cascades leading to activation of JNK and p38 MAPKs. Of particular relevance to signalling in the cardiovascular system is the finding that receptors utilising heterotrimeric G-proteins can be potent activators of these pathways. When the effects of hypertrophic agents were examined in intact heart, p38, JNK MAPKs and ERK MAPKs were activated during perfusion with phenylephrine. These effects of phenylephrine were independent of the positive inotropic/chronotropic effects, suggesting that JNK and p38 MAPK activation was not secondary to stress associated with altered contractility or ion-handling [84]. JNK MAPKs are also activated, albeit at low levels for more than 24 h, in cultured cardiac myocytes exposed to phenylephrine [85]. A second major group of well-studied hypertrophic agents are the vasoactive peptides, endothelin-1 and angiotensin II. The exposure of neonatal cardiac myocytes to angiotensin II rapidly and transiently activated the JNK MAPKs [14]. In adult cardiac myocytes, angiotensin II in the presence of okadaic acid activated ERK MAPKs and JNK MAPKs. In the same study, the exposure of cardiac microvascular endothelial cells to angiotensin II alone activated ERK MAPKs and co-incubation with okadaic acid allowed for ERK and JNK MAPK activation [32]. The relevance of these in vitro studies to our understanding of the activation of MAPKs in vivo is confirmed in studies in which conscious rats were infused with angiotensin II. Both ERK and JNK MAPKs were activated in the heart with JNK MAPK activation occurring in both ventricles and likely to be mediated directly by angiotensin II receptor engagement. ERK MAPK activation occurred only in the left ventricle and was likely to be a result of elevation of blood pressure [86]. The challenge is to now relate these observations of protein kinase activation to long term biological effects in the cardiovascular system.

6. Possible physiological substrates for the stress-activated MAPKs in the cardiovascular system

It is clear that the MAPKs comprise a complex family of conveyers of intracellular signal information. It is likely that some of the specificity of cellular responses to any particular stimulus is imparted by the specific activation of individual MAPKs. However, some stimuli do activate multiple MAPKs. Overall there is 40 to 50% identity in the catalytic domains of these enzymes and this high degree of identity suggest the potential for overlap of functions, but distinct differences will allow for a degree of specificity of information transfer.

As experimental techniques for identifying protein kinases both in vitro and in vivo become more sophisticated, the numbers of MAPK substrates of potential biological importance have increased. Two major classes of substrates will be examined. First, transcription factors will be examined. These factors regulate gene expression and modulating long-term changes in the cell. Secondly, we will consider the protein kinases that are themselves substrates of the MAPKs. These kinase substrates allow signal diversification and amplification downstream of the MAPKs. Other important substrates such as regulators of contractility are beyond the scope of this review.

6.1. Phosphorylation of transcription factors

Transcription factors form a major group of MAPK substrates. Phosphorylation may alter DNA binding activity or interactions with other proteins to increase or decrease transcriptional activity. Consistent with this notion that MAPKs can access transcription factors, are the initial observations that, once activated, ERK MAPKs translocate from the cell cytoplasm to the nucleus [87]. Similar observations of nuclear localization have been made for JNK MAPKs in ischemic heart [88]. Studies have not examined p38 MAPK localization in the heart, but when overexpressed p38 MAPKs are localized in the nucleus [89]. This suggests that these MAPKs are all potentially able to access nuclear transcription factor substrates. The major examples of the transcription factor substrates for MAPKs are shown in Fig. 4. This review concentrates on the MEF2 family, c-Jun, ATF family and Elk which have been well-characterized and which play significant roles within cells of the cardiovascular system. The example of CREB illustrates that transcription factors may be substrates for kinases downstream of MAPKs.

The myocyte enhancer factor 2 (MEF2) family of transcription factors can bind as homodimers or heterodimers to A/T-rich DNA sequences in promoters of muscle-specific genes and additionally participate in the control of non-muscle specific gene expression. An essential role for MEF2C in cardiac development has been recently demonstrated in MEF2C homozygous null mutant [90]. Also MEF2C C-terminus phosphorylation by BMK1

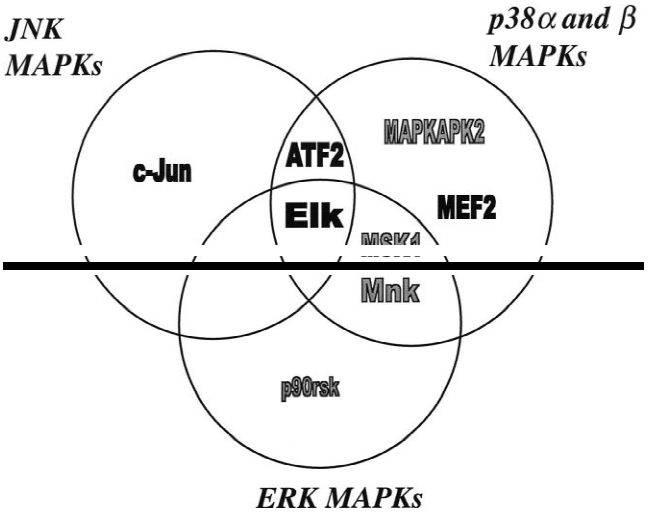

Fig. 4. Substrates for the ERK, JNK and p38α/β MAPKs. Although the MAPKs all share a high degree of sequence homology, these different subfamilies show substrate preferences. Both transcription factors (shown in black) and protein kinases (shown in gray) may act as substrates. Some substrates such as Elk can act as substrates for all three subfamilies, whilst others such as ATF2, MSK1, and Mnk are suitable substrates for only two subfamilies. Other substrates such as c-Jun, MAPKAPK2, MEF2 and $p90^{RSK}$ are suitable for only one of the three subfamilies.

or p38 MAPK increases transcriptional activity [91,92]. Potential sites for MEF2 binding also occur within the α-cardiac myosin heavy chain promoter [93]. Thus when MEF2C is phosphorylated by p38 MAPKs, it confers transcriptional inducibility in a muscle-specific context [94].

The existence of a viral form of Jun, together with the rapid induction of c-Jun expression following exposure to extracellular stimuli, emphasizes the importance of Jun transcriptional activation [95]. The c-Jun knockout mouse dies without any obvious cardiac abnormalities but recent reports do show that c-Jun is involved in heart outflow tract development [96]. For the heart, the expression of c-Jun is enhanced by a wide variety of factors including hypertrophic agents [97,98] or physical stresses [99,100]. Transcriptional activation by c-Jun is controlled by phosphorylation within its N-terminal transactivation domain catalyzed by JNK MAPKs [11]. The binding of phosphorylated Jun to Fos forms the AP-1 complex that is contained within the promoters of a number of genes of importance in the heart, namely skeletal muscle α-actin and ANF [101]. In addition, c-Jun plays an important role in regulation of cytochrome c expression during electrical pacing of myocytes. In these hypertrophied myocytes, enhanced c-Jun transcriptional activity allows for increases in mitochondria number and cytochrome oxidase activity which are vital for meeting the increased energy demand [102].

The control of transcriptional activation of ATF-2 is similar to that observed for c-Jun and phosphorylation within its transactivation domain enhances transcriptional

activity. Phosphorylated ATF-2 can form homodimers or heterodimers with ATF-3, CREB, c-Jun or NF-kB. This suggests a potential for widespread roles in promoter activation. In cardiac myocytes, ATF-6 is phosphorylated by p38 MAPK allowing for binding to the serum response factor (SRF) and participation in ANF induction in response to phenylephrine, serum or endothelin [103]. This implicates phosphorylation of ATF family members in the hypertophic gene programme.

Phosphorylation of the ternary complex factor Elk-1 within its C-terminal activation domain enhances ternary complex formation and transcriptional activity. ERK, JNK and p38 MAPKs all directly phosphorylate Elk and enhance transcription from the c-fos promoter Serum Response Element (SRE) [104]. However, skeletal α-actin, cardiac α-actin and ANF promoters possess SREs which do not have Elk-binding flanking sequences, and expression of ANF is primarily dependent on the non-consensus SRE motif [105]. In these cases, accessory proteins may play a role [103].

Lastly, we should consider transcription factors as indirect targets of the MAPK pathways. The phosphorylation of the transcription factor CREB is implicated in cardiac disease [106]. In this case, overexpression of CREB in a form that can no longer be phosphorylated at one of its critical sites results in cardiac dilation, interstitial fibrosis, and severe chronic venous congestion. The identity of the protein kinase responsible for phosphorylation of CREB remains controversial. One of the protein kinases acting downstream of the ERK MAPKs, $p90^{RSK}$, may translocate to the nucleus, phosphorylate CREB, allowing Fos expression [107]. Two protein kinases acting downstream of p38 MAPK, namely MAPKAPK2 and Mitogen- and Stress-activated protein Kinase 1 (MSK1), may phosphorylate CREB, and this most likely explains CREB activation following cellular stress [108,109]. It is clear that another degree of complexity in intracellular signalling has been introduced.

6.2. Phosphorylation of protein kinases

A second major class of MAPK substrates are themselves protein kinases. This gives the MAPK pathways additional opportunities for amplification and/or integration, and the potential to alter events in various cellular compartments.

The first MAPK substrate characterised was $p90^{RSK}$ which phosphorylates ribosomal protein S6 and has the potential to regulate protein synthesis. Within the cardiovascular system, $p90^{RSK}$ can be activated by stretch, α-adrenergic agonists, endothelin and angiotensin II [110], $p90^{RSK}$ [111]. A second protein kinase, MAPKAPK2, is a physiologically important substrate of the p38 MAPKs [18]. MAPKAPK2 is highly-expressed in heart and ischemia alone can activate this protein kinase in the heart [112]. MAPKAPK2 can directly phosphorylate the heat shock protein hsp27, which in turn prevents stress-mediated depolymerisation of actin filaments [38]. In endothelial cells, p38 MAPK and MAPKAPK2 have been implicated in actin organisation and cell migration in response to vascular endothelial growth factor [113].

Many substrates of the MAPKs may also be phosphorylated by more than one MAPK subfamily. Thus, MAPK interacting kinase (Mnk) is phosphorylated and activated by ERK and p38 MAPKs in vivo [114,115]. This integrates the signals from two pathways and results in direct phosphorylation of eukaryotic initiation factor-4E and subsequent regulation of protein synthesis [115]. The activation of the protein kinase MSK1 is also mediated by phosphorylation catalysed by either ERK MAPKs or p38 MAPKs and this again allows for signal integration [109]. This example emphasizes that there is potential for integration and reinforcement of signalling events when multiple MAPK pathways are activated.

7. Complex cellular outcomes – the balance of cell death, survival and adaptation

Although it is well established that ERK, JNK and p38 MAPKs are activated during cellular stress, one of the greatest controversies surrounds the ultimate role of these protein kinases. There are at least two possibilities. These activated protein kinases may contribute to signal transduction pathways that culminate in the death of cells following stress. However, these MAPKs may also act to prevent wide-spread cell death, limit damage or mediate adaptation.

7.1. Initiating cell death

The original studies examining a role for JNK and p38 MAPKs in regulating cell death described events following withdrawal of growth factors [116]. The correlation of apoptosis with JNK and p38 MAPK activation was strengthened when the overexpression of activators of JNK or p38 MAPKs promoted apoptosis [116]. Although withdrawing serum from cardiac myocytes also initiates apoptosis [83], subjecting cells or tissues to low oxygen concentrations is a physiologically-relevant stimulus that warrants further investigation. In response to an ischemic episode in the heart, the most severely affected myocytic cells will die, either by necrosis or controlled apoptosis. Of the stress-activated MAPKs that may mediate these events, the JNK MAPKs are activated in ischemic/reperfused areas as well as the remote areas of the myocardium [117–119]. The remote activation of JNK MAPKs correlates with enhanced myocyte apoptosis, and suggests that the JNK MAPKs contribute directly to the apoptotic cell death [119].

Specific assessment of the role of stress-activated MAPKs requires the use of specific inhibitors, overexpres-

sion of MAPKs, or transgenic approaches. This first approach using chemical inhibitors has led to the conclusion that the p38 MAPKs promote cardiac myocyte death during extended periods of ischemia or exposure to hydrogen peroxide [120,121]. More recently, studies in isolated perfused hearts have concluded that p38 MAPK plays a pivotal role in promoting myocardial apoptosis [122]. Similarly, cardiac injury in response to oxidative stress induced by hydrogen peroxide is apparently mediated by a p38 MAPK-dependent production of TNF-α [121]. Cardioprotection may therefore be correlated with the ability of a compound or intervention to inhibit p38 MAPK activation.

The second approach using overexpression of MAPK pathway members has yielded more complex results. Whilst p38 MAPKs have been implicated in the survival response of cardiac myocytes [123], it may be the p38α isoform is specifically involved in cell death [123]. To date the use of transgenic animals has not been specifically applied to evaluating the role of stress-activated MAPKs in apoptosis in the cardiovascular system, but studies on fibroblast and neuronal apoptosis have confirmed that JNK MAPKs mediate cell death [124]. One possible explanation for continuing a disagreement on the role of stress-activated MAPKs is that these kinases will play a subordinate role in the control of cell death processes.

7.2. Initiating a survival response

In the heart subjected to an ischemic episode, the myocytes surrounding the region of dying cells increase in size in an attempt to replace the lost contractile activity. Stress stimuli may provide the survival signal. This is supported by ischemic preconditioning which attenuates apoptotic cell death following ischemia and reperfusion [125]. The candidate signalling pathways promoting cell recovery should be active during either the ischemic period and/or the reperfusion period. Despite the suggestion that JNK and p38 MAPKs mediate cell death, ample evidence also supports their role in cell survival. The clearest evidence comes from targeted gene disruption of the JNK upstream activator MEKK1 which defines a role for the JNK MAPK pathway in the survival of embryonic stem cells [126]. This seemingly contradicts initial observations that MEKK overexpression leads to apoptosis, however, the effects of MEKK overexpression may be modified to promote differentiation when high levels of c-Jun are expressed [127]. In other well-defined cultured cell systems, TNF-α activation of JNK and p38 MAPKs enhances fibroblast survival [72], JNK and p38 MAPKs protect during photodynamic therapy [128], and one JNK MAPK isoform maintains survival following hypertonic shock [129].

In cells of the cardiovascular system, evidence also supports these pro-survival signalling cascades. Thus, anisomycin, an activator of JNK and p38 MAPKs, protected myocytes from ischemic injury [120] and phosphorylation of p38 MAPKs correlated with preconditioning protection [38]. Although contradictory findings of anisomycin-initiated cell death have also been reported, specific activation of p38 MAPKs by overexpression of pathway components may also mediate a survival response in cardiac myocytes [130]. This is supported by p38β MAPK mediating cell survival, sarcomeric organisation and gene expression changes [123]. In addition, there have been reports of JNK and/or p38 MAPK involvement in endothelial cell proliferation [131], myotube differentiation [132], and the expression of smooth muscle α-actin in vascular smooth muscle cells [133]. The effects of the different p38 and JNK MAPK isoforms are therefore likely to be complex and dependent on stimulus and cellular context. Both the duration and intensity of the stress may ultimately determine the regulation of MAPKs and the final cellular outcome. For example, the extent of p38 MAPK activation may dictate whether a cardiac myocyte is committed to death or survival [134].

Finally we should consider the role that ERK MAPKs play in the response to stress. The well-defined role of ERK MAPKs in modulating growth responses points to this MAPK subfamily as an obvious candidate for mediating cell survival. In support of this, cells overexpressing constitutively-activated MEK are less likely to die when exposed to hydrogen peroxide than cells expressing dominant-negative or wild-type MEK [135]. Similarly the cardioprotective properties of the cytokine CT-1 have been attributed to its ability to activate ERK MAPK [83]. Any conclusion on the role of MAPKs in cell survival is likely to be controversial. Again this may be explained by the MAPKs playing a subordinate role in cell survival.

7.3. Initiating cardiac hypertrophy

In addition to the survival response of cells in the heart post-infarction, the individual myocytes adapt to increased workload by an increase in size (hypertrophy). The MAPKs have been explored as mediators of this adaptive response under situations in which growth factors, cytokines, mechanical stress and chemical stress contribute to the ensuing cellular events. Circumstantial evidence of ERK MAPK involvement in these events might be obtained from the initial observations that the activation of this pathway correlated with hypertrophy. However, a number of agents potently activate ERK MAPKs in cardiac myocytes but fail to induce hypertrophy [7]. Most recent studies to address how MAPK pathway activation contributes to hypertrophy have relied on overexpression of MAPK pathway components.

7.3.1. The use of constitutively-active mutants

Studies utilising constitutively-active mutants of MAPK pathways have specifically addressed the consequences of ERK MAPK pathway activation. Two studies, supporting

involvement of Ras, demonstrated that overexpression of constitutively-active Ras stimulated the hypertrophy of isolated myocytes [136] and a general increase in cardiac gene expression [137]. There are a number of signalling pathways downstream of Ras, including phosphatidylinositol 3-kinase (PI3K), Ral.GDS and possibly all MAPK signalling pathways [138,139]. Therefore, the effects of Ras overexpression do not solely implicate the ERK MAPKs in the hypertrophic response. There are two approaches to confirm which Ras-effectors may contribute to the final response. The most widely-used approach has employed constitutively-active mutants of other components of the MAPK cascades. The overexpression of constitutively-activated MEK together with wild-type ERK2 MAPK enhanced the gene expression but did not alter cell size or morphology [140]. Similar effects are found upon overexpression of activated Raf [141]. A second approach has capitalized on the observation that additional mutation within the effector domain of active Ras permits selective coupling to only one of the Ras effector pathways. This approach has demonstrated that long-term activation of the ERK MAPKs via the V12RasD38E mutant, that selectively stimulates the Raf-dependent pathway, leads to alterations in calcium handling in cardiac myocytes [142]. Transfections with this and the other mutants such as V12RasE37G and V12RasY40C either alone or in combination, suggests that Raf, Ral.GDS and PI3K all contribute to Ras-induced changes in gene expression and cell size [143].

These studies have not directly addressed whether stress-activated MAPKs mediate the cellular response to constitutive Ras activation. The overexpression of activated Ras mutants activates the JNK MAPK pathway in a variety of cells including cardiac myocytes [85], potentially via the JNK MAPK activator, MEKK. Overexpression of a MEKK1 mutant that preferentially activated JNK MAPKs enhanced myocyte hypertrophy [60,85,94], but these results have not been confirmed in all subsequent studies. Low levels of expression of JNK1 or its upstream activator SEK1 (or JNKK1 or MKK4) inhibited rather than enhanced the MEKK-stimulated activation of ANF expression [144]. Whilst some studies indicate MEKK can alter the morphological features of hypertrophy [117], other studies have shown MEKK disrupts muscle fibre organisation [60]. Some of the confusion surrounding the role of MEKK, and hence JNK MAPKs, in myocyte hypertrophy may result from the loss of signalling fidelity upon overexpression of the mutant active form of MEKK (ΔN–MEKK). Increased specificity with the use of the JNK MAPK activator MKK7, resulted in increased cell size, myofilament organization and ANF expression [145]. This suggests that JNK MAPK activation is sufficient for a hypertrophic response. However, this is unlikely to be the complete story, with conflicting reports suggesting that JNK suppresses hypertrophy [144].

The results of overexpression of p38 MAPKs and constitutively-activated mutants of their specific activators, MKK3 and MKK6 indicate that this pathway also plays a complex role in the cardiac myocyte. Whilst the overexpression of MKK3 or MKK6 increased surface area, sarcomeric organisation and ANF expression, the co-expression of MKK3 with p38α prevented these changes and induced cell death. In contrast, p38β expression in the presence of MKK3 augmented hypertrophy and promoted cell survival [123]. Thus p38α apparently promotes cell death whilst p38β promotes cell hypertrophy. The effects of LIF, endothelin-1 and phenylephrine to stimulate expression of ANF expression is also enhanced by co-transfection with MKK6 and p38 MAPK [144], suggesting that the levels of expression of these protein kinases may limit the extent of signalling in the cardiac myocyte.

As mentioned previously, many stimuli are now known to co-activate the p38, JNK and ERK MAPK pathways in the heart. It would be anticipated that this could be modelled by the co-expression of a number of different pathway components. The co-activation of p38 and JNK MAPKs as it occurs in ischemia/reperfusion and pressure overload hypertrophy could therefore be mimicked by the co-expression of activators of these pathways [117]. It would be initially hypothesised that the co-expression of two positive modulators of hypertrophy would result in a more striking response. Surprisingly the co-expression of the JNK activator MKK7 with the p38 activator MKK6 inhibited the hypertrophic response whereas the coexpression of MKK7 with the MKK3 resulted in cell death [145]. However, in these studies signalling pathways stimulated by constitutive overexpression are chronically activated. The effects of transient activation under physiological conditions may not be faithfully reproduced by prolonged overexpression and further work must use systems that rapidly turn these pathways on and off.

7.3.2. The use of dominant-negative mutants or chemical inhibitors

There are two additional strategies to identify which MAPKs, if any, are most likely to contribute to the development of hypertrophy. The first method that has been widely used entails the overexpression of dominant-negative mutants of the ERK MAPK cascade. Again initial studies examined upstream events by the microinjection of a dominant-interfering Ras demonstrated to block the phenylephrine-induced increases in ANF protein and cell size [136]. Other conflicting studies have demonstrated that a dominant-interfering mutant of Raf could not block the phenylephrine-stimulated ANF luciferase activity [85]. Differences in culture conditions, expression levels of the constructs or other experimental protocols may explain the differences in results. Thus, the importance of these pathways must be evaluated at events further downstream.

When an interfering mutant of ERK1 MAPK blocked ANF reporter activation induced by phenylephrine exposure, this confirmed the significant role that ERK MAPKs

play in hypertrophy [5]. But again there have been differences of results, and dominant-negative (kinase-inactive) mutants of ERK1 or ERK2 MAPKs failed to prevent phenylephrine stimulated ANF-luciferase, but inhibited phenylephrine-stimulated Fos expression and Ras activation of ANF-luciferase [7]. Furthermore, the expression of activated MEK paradoxically inhibited ANF expression [146]. This conflict in results has led to the examination of other MAPK pathways. Specifically, a dominant-negative MEKK mutant inhibited phenylephrine-induced ANF reporter gene expression [85], suggesting a role for MEKK-dependent pathways. Confirming this, an inactive JNK MAPK mutant inhibited ANF expression [60], but potentiated ANF expression in a separate study [144]. These contradictory results suggest that overexpression of dominant-negative regulators may not have the desired level of specificity. Recent advances in development of protein kinase inhibitors showing greater degrees of specificity have accelerated progress. As with all inhibitors, care should be exercised to not exceed the doses at which specificity can be achieved.

The ERK MAPK pathway inhibitor, PD098059, at a concentration sufficient to completely inhibit ERK MAPK activation, has failed to inhibit phenylephrine-induced ANF expression [7] and only partially inhibited the ouabain-induction of ANF and fos mRNA whilst not altering skeletal muscle α-actin expression [147]. This suggests that ERK MAPK activation contributes to some, but not all, of the hypertrophic responses. In contrast, PD098059 enhanced ANF-luciferase expression stimulated in response to MEKK transfection, implying that ERK MAPKs inhibit the signalling from MEKK to hypertrophic changes [60].

Specific inhibitors of p38-α and p38-β MAPKs, SB203580 and the related compound SB202190, have also been extensively used. Incubation in the presence of SB202190 alters the responses to endothelin-1, LIF or phenylephrine [144]. Thus the p38 MAPKs have been proposed as regulators of cardiac hypertrophy. More recently studies with SB203580 have demonstrated its lack of effect on the early changes in cell size stimulated by phenylephrine or endothelin-1, implying that the role of p38 MAPKs may actually be in the maintenance of the hypertrophic response/survival over a long period of time [148].

8. Examining physiological roles in vivo

As can be seen from the preceding sections, most work addressing the activation and functional consequences of the MAPK pathways activation has utilised cell culture models. Although these results have made significant contributions to our understanding of MAPK pathway function, it is clear that the hypotheses developed in simple in vitro models must ultimately be tested in vivo.

In some of the first studies to address the cardiovascular functions of the MAPKs in vivo, transgenic animals have been used. In this way, a defined initiating signal can be examined in an intact animal model. Targeting expression of activated Ras to the ventricular myocyte has been sufficient to elicit the development of the hypertrophy in vivo [149]. The phenotype most closely resembled compensated hypertensive heart disease with diastolic dysfunction and concentric chamber hypertrophy [149]. The JNK MAPKs (not the ERK MAPKs) were activated [85]. This suggests that chronic low levels of JNK MAPK stimulation may be sufficient to mediate the long-term changes. However, the major difficulty in assessing in vivo MAPK activation is in selecting the time-points for sampling. In the model of Ras-overexpression, it may be that the activation of ERK MAPKs precedes JNK MAPK activation. Thus, ERK MAPKs may play a role in the initial stages of the development of hypertrophy but without samples at earlier time points during the development of hypertrophy this is impossible to confirm or refute.

Despite these problems, MAPK activation can accompany a hypertrophic response in other in vivo models. In the hearts of stroke-prone spontaneously hypertensive rats, left ventricular ERK and JNK activities are significantly elevated in the mild hypertension, and remained elevated during the development of left ventricular hypertrophy. Furthermore, antihypertensive treatment prevented hypertrophy and also decreased the left ventricular JNK activities, thereby implicating the JNK MAPKs in the development of hypertrophy [150]. In hypertension elicited by acute angiotensin II infusion, the ERK and JNK MAPKs were rapidly activated in the left ventricles. In the right ventricles of these rats, only JNK MAPKs were activated whereas both MAPKs were activated in the aorta [86]. This implies that activation of ERK MAPKs maybe predominantly mediated by changes in blood pressure, whereas the activation of JNK MAPKs is more direct and mediated by the actions of angiotensin II and its specific receptor.

9. General conclusions on the roles of MAPKs activated during stress

Physiologically-important stimuli activate a number of families of MAPKs in the cardiovascular system. The major focus in recent years has moved from the traditional growth factor-activated pathway, the ERK MAPK pathway, towards the JNK and p38 MAPK pathways. The investigation of the role of the BMK1/ERK5 pathway will take on greater importance with the observations of its stress-activation in vascular smooth muscle cells exposed to stress.

The overall conclusion on the contributions of these pathways to the cellular response to stress remains controversial with different reports variously demonstrating the

importance of activation of ERK, JNK or p38 MAPKs. The situation in vivo obviously has added complexity, and the interactions between these pathways must be more thoroughly addressed. Studies must also evaluate the roles of these protein kinases in dysfunction and disease in vivo in transgenic mice that conditionally express or fail to express components of these complex protein kinase cascades. Only then, can the specific effects of these protein kinases on cardiovascular function be assessed. What is clear from all of these studies is that the signalling pathways initiated in the cardiovascular system are complex, inter-connected and possibly redundant. With the American Heart Association and World Health Organization statistics clearly showing that cardiovascular diseases remain a major cause of death world-wide, the search for understanding stress-activated signalling cascades in the cardiovascular system will certainly continue well into the next millennium.

Acknowledgements

The work in the author's laboratory is supported by Project Grants from the National Heart Foundation of Australia (G98P0203), and the National Health and Medical Research Council (0035566), and a Seeding Grant from Merck Sharp and Dohme.

References

[1] Boulton TG, Nye SH, Robbins DJ et al. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 1991;65:663–675.
[2] Cobb MH, Goldsmith EJ. How MAP kinases are regulated. J Biol Chem 1995;270:14843–14846.
[3] Sugden PH, Bogoyevitch MA. Intracellular signalling through protein kinases in the heart. Cardiovasc Res 1995;30:478–492.
[4] Force T, Bonventre JV. Growth factors and mitogen-activated protein kinases. Hypertension 1998;31:152–161.
[5] Thorburn J, Frost JA, Thorburn A. Mitogen-activated protein kinases mediate changes in gene expression, but not cytoskeletal organization associated with cardiac muscle cell hypertrophy. J Cell Biol 1994;126:1565–1572.
[6] Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ, Sugden PH. Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. Circ Res 1996;78:954–961.
[7] Post GR, Goldstein D, Thuerauf DJ, Glembotski CC, Brown JH. Dissociation of p44 and p42 mitogen-activated protein kinase activation from receptor-induced hypertrophy in neonatal rat ventricular myocytes. J Biol Chem 1996;271:8452–8457.
[8] Abdellatif M, Packer SE, Michael LH, Zhang D, Charng MJ, Schneider MD. A Ras-dependent pathway regulates RNA polymerase II phosphorylation in cardiac myocytes: implications for cardiac hypertrophy. Mol Cell Biol 1998;18:6729–6736.
[9] Kyriakis JM, Banerjee P, Nikolakaki E et al. The stress-activated protein kinase subfamily of c-Jun kinases. Nature 1994;369:156–160.
[10] Gupta S, Barrett T, Whitmarsh AJ et al. Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO J 1996;15:2760–2770.
[11] Dérijard B, Hibi M, Wu I-H et al. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell 1994;76:1025–1037.
[12] Li Y-S, Shyy JY-J, Li S et al. The Ras-JNK pathway is involved in shear-induced gene expression. Mol Cell Biol 1996;16:5947–5954.
[13] Jo H, Sipos K, Go Y-M, Law R, Rong J, McDonald JM. Differential effect of shear stress on extracellular signal-regulated kinase and N-terminal Jun kinase in endothelial cells. G_{i2} and Gβ/γ-dependent signaling pathways. J Biol Chem 1997;272:1395–1401.
[14] Kudoh S, Komuro I, Mizuno T et al. Angiotensin II stimulates c-Jun NH_2-terminal kinase in cultured cardiac myocytes of neonatal rats. Circ Res 1997;80:139–146.
[15] Shapiro PS, Evans JN, Davis RJ, Posada JA. The seven-transmembrane-spanning receptors for endothelin and thrombin cause proliferation of airway smooth muscle cells and activation of the extracellular regulated kinase and c-Jun NH_2-terminal kinase groups of mitogen-activated protein kinases. J Biol Chem 1996;271:5750–5754.
[16] Rao GN, Runge MS. Cyclic AMP inhibition of thrombin-induced growth in vascular smooth muscle cells correlates with decreased JNK1 activity and c-Jun expression. J Biol Chem 1996;271:20805–20810.
[17] Han J, Lee J-D, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 1994;265:808–811.
[18] Rouse J, Cohen P, Trigon S et al. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell 1994;78:1027–1037.
[19] Lee JC, Laydon JT, McDonnell PC et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994;372:739–746.
[20] Zervos AS, Faccio L, Gatto JP, Kyriakis JM, Brent R. Mxi2, a mitogen-activated protein kinase that recognizes and phosphorylates Max protein. Proc Natl Acad Sci USA 1995;92:10531–10534.
[21] Jiang Y, Chen C, Li Z et al. Characterization of the structure and function of a new mitogen-activated protein kinase (p38β). J Biol Chem 1996;271:17920–17926.
[22] Stein B, Yang MX, Young DB et al. p38-2, a novel mitogen-activated protein kinase with distinct properties. J Biol Chem 1997;272:19509–19517.
[23] Mertens S, Craxton M, Goedert M. SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases. FEBS Lett 1996;383:273–276.
[24] Wang XS, Diener K, Manthey CL et al. Molecular clonding and characterization of a novel p38 mitogen-activated protein kinase. J Biol Chem 1997;272:23668–23674.
[25] Lee J-D, Ulevitch RJ, Han J. Primary structure of BMK1: A new mammalian MAP kinase. Biochem Biophys Res Commun 1995;213:715–724.
[26] Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. J Biol Chem 1995;270:12665–12669.
[27] Abe J-i, Kushuhara M, Ulevitch RJ, Berk BC, Lee J-D. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. J Biol Chem 1996;271:16586–16590.
[28] Yan C, Takahashi M, Okuda M, Lee J-D, Berk BC. Fluid shear stress stimulates Big mitogen-activated protein kinase (BMK1) activity in endothelial cells. Dependence on tyrosine kinases and intracellular calcium. J Biol Chem 1999;274:143–150.
[29] Seko Y, Takahashi N, Tobe K, Ueki K, Kadowaki T, Yazaki Y. Vascular endothelial growth factor (VEGF) activates Raf-1,

mitogen-activated protein (MAP) kinases, and S6 kinase ($p90^{rsk}$) in cultured rat cardiac myocytes. J Cell Physiol 1998;175:239–246.
[30] Zou Y, Komuro I, Yamazaki T et al. Cell type-specific angiotensin II-evoked signal transduction pathways. Critical roles of Gβγ subunit, Src family, and Ras in cardiac fibroblasts. Circ Res 1998;82:337–345.
[31] Wilden PA, Agazie YM, Kaufman R, Halenda SP. ATP-stimulated smooth muscle cell proliferation requires independent ERK and PI3K signaling pathways. Am J Physiol 1998;275:H1209–H1215.
[32] Fischer TA, Singh K, O'Hara DS, Kaye DM, Kelly RA. Role of AT1 and AT2 receptors in regulation of MAPKs and MKP-1 by ANGII in adult cardiac myocytes. Am J Physiol 1998;275:H906–H916.
[33] Wang X, Martindale JL, Liu Y, Holbrook NJ. The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival. Biochem J 1998;333:291–300.
[34] Bogoyevitch MA, Ketterman AJ, Sugden PH. Cellular stresses differentially activate the c-Jun N-terminal protein kinases and the extracellular signal-regulated protein kinases in cultured ventricular myocytes. J Biol Chem 1995;270:29710–29717.
[35] Komuro I, Kudo S, Yamazaki T, Zou Y, Shiojima I, Yazaki Y. Mechanical stretch activates the stress-activated protein kinases in cardiac myocytes. FASEB J 1996;10:631–636.
[36] McDonough PM, Hanford DS, Sprenkle AB, Mellon NR, Glembotski CC. Collaborative roles for c-Jun N-terminal kinase, c-Jun, serum response factor and Sp1 in calcium-regulated myocardial gene expression. J Biol Chem 1997;272:24046–24053.
[37] Wang X, Proud CG. p70 S6 kinase is activated by sodium arsenite in adult rat cardiomyocytes: roles for phosphatidylinositol 3-kinase and p38 MAP kinase. Biochem Biophys Res Commun 1997;238:207–212.
[38] Weinbrenner C, Liu G-S, Cohen MV, Downey JM. Phosphorylation of Tyr 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart. J Mol Cell Cardiol 1997;29:2383–2391.
[39] Chen K-D, Li Y-S, Kim M, Yuan S, Chien S, Shyy JY-J. Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. J Biol Chem 1999;274:18393–18400.
[40] Malek AM, Gibbons GH, Izumo S. Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B chain in vascular endothelium. J Clin Invest 1993;92:2013–2021.
[41] Malek AM, Greene AL, Izumo S. Regulation of endothelin 1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C. Proc Natl Acad Sci USA 1993;90:5999–6003.
[42] Standley PR, Obards TJ, Martina CL. Cyclic stretch regulates autocrine IGF-I in vascular smooth muscle cells: implications in vascular hyperplasia. Am J Physiol 1999;276:E697–E705.
[43] Hamada K, Takuwa N, Yokoyama K, Takuwa Y. Stretch activates Jun N-terminal kinase/stress-activated protein kinase in vascular smooth muscle cells through mechanisms involving autocrine ATP stimulation of purinoceptors. J Biol Chem 1998;273:6334–6340.
[44] Sadoshima J-I, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 1993;75:977–984.
[45] Kudoh S, Komuro I, Hiroi Y et al. Mechanical stretch induces hypertrophic responses in cardiac myocytes of angiotensin II Type1a receptor knockout mice. J Biol Chem 1998;273:24037–24043.
[46] Akhter SA, Luttrell LM, Rockman HA, Lefkowitz RJ, Koch WJ. Targeting the receptor-Gq interface to inhibit in vivo pressure overload myocardial hypertrophy. Science 1998;280:574–577.
[47] Pan J, Fukuda K, Saito M et al. Mechanical stretch activates the JAK/STAT pathway in rat cardiomyocytes. Circ Res 1999;84:1127–1136.
[48] Baines CP, Goto M, Downey JM. Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium. J Mol Cell Cardiol 1997;29:207–216.
[49] Clerk A, Fuller SJ, Michael A, Sugden PH. Stimulation of ''stress-regulated'' mitogen-activated protein kinases (Stress-activated protein kinases/c-Jun N-terminal kinases and p38-mitogen-activated protein kinases) in perfused rat hearts by oxidative and other stresses. J Biol Chem 1998;273:7228–7234.
[50] Aikawa R, Komuro I, Yamazaki T et al. Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. J Clin Invest 1997;100:1813–1821.
[51] Sabri A, Byron KL, Samarel AM, Bell J, Lucchesi PA. Hydrogen peroxide activated mitogen-activated protein kinases and Na^+-H^+ exchange in neonatal rat cardiac myocytes. Circ Res 1998;82:1053–1062.
[52] Clerk A, Michael A, Sugden PH. Stimulation of multiple mitogen-activated protein kinase sub-families by oxidative stress and phosphorylation of the small heat shock protein HSP25/27, in neonatal ventricular myocytes. Biochem J 1998;333:581–589.
[53] Cavigelli M, Li WW, Lin A, Su B, Yoshioka K, Karin M. The tumor promoter arsenite stimulates AP-1 by inhibiting a JNK phosphatase. EMBO J 1996;15:6269–6279.
[54] Meriin AB, Yaglom JA, Gabai VL, Mosser DD, Zon L, Sherman MY. Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72. Mol Cell Biol 1999;19:2547–2555.
[55] Andersson MB, Ketterman AJ, Bogoyevitch MA. Differential regulation of parallel mitogen-activated protein kinases in cardiac myocytes revealed by phosphatase inhibition. Biochem Biophys Res Commun 1998;251:328–333.
[56] Fuller SJ, Davies EL, Gillespie-Brown J, Sun H, Tonks NK. Mitogen-activated protein kinase phosphatase 1 inhibits the stimulation of gene expression by hypertrophic agonists in cardiac myocytes. Biochem J 1997;323:313–319.
[57] Hirsch DD, Stork PJS. Mitogen-activated protein kinase phosphatases inactivate stress-activated protein kinase pathways in vivo. J Biol Chem 1997;272:4568–4575.
[58] Liu Y, Gorospe M, Yang C, Holbrook NJ. Role of mitogen-activated protein kinase phosphatase during the cellular response to genotoxic stress. Inhibition of c-Jun N-terminal kinase activity and AP-1-dependent gene activation. J Biol Chem 1995;270:8377–8380.
[59] Franklin CC, Srikanth S, Kraft AS. Conditional expression of mitogen-activated protein kinase phosphatase-1, MKP-1, is cytoprotective against UV-induced apoptosis. Proc Natl Acad Sci USA 1998;95:3014–3019.
[60] Thorburn J, Xu S, Thorburn A. MAP kinase- and Rho-dependent signals interact to regulate gene expression but not actin morphology in cardiac muscle cells. EMBO J 1997;16:1899–1900.
[61] Buerke M, Murohara T, Skurk C, Nuss C, Tomaselli K, Lefer AM. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci USA 1995;92:8031–8035.
[62] Guillen I, Blanes M, Gomez-Lechon MJ, Castell JV. Cytokine signaling during myocardial infarction: sequential appearance of IL-1β and IL-6. Am J Physiol 1995;269:R229–R235.
[63] Meldrum DR. Tumor necrosis factor in the heart. Am J Physiol 1998;274:R577–R595.
[64] Bryant D, Becker L, Richardson J et al. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-α. Circulation 1998;97:1375–1381.
[65] Krown K, Yasui K, Brooker M et al. TNFa receptor expression in rat cardiac myocytes: TNFα inhibition of L-type Ca^{2+} current and Ca^{2+} transients. FEBS Lett 1995;376:24–30.
[66] Goldhaber JI, Kim KH, Natterson PD, Lawrence T, Yang P, Weiss JN. Effects of TNF-α on $[Ca^{2+}]i$ and contractility in isolated adult rabbit ventricular myocytes. Am J Physiol 1996;271:H1449–H1455.
[67] Nakano M, Knowlon AA, Yokoyama T, Lesslauer W, Mann DL. Tumor necrosis factor-α-induced expression of heat shock protein 72 in adult feline cardiac myocytes. Am J Physiol 1996;270:H1231–H1239.

[68] Nakamura K, Fushimi K, Kouchi H et al. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. Circulation 1998;98:794–799.
[69] Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-α provokes a hypertrophic growth response in adult cardiac myocytes. Circulation 1997;95:1247–1252.
[70] Krown KA, Page MT, Nguyen C et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signalling cascade in cardiac cell death. J Clin Invest 1996;98:2854–2865.
[71] Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. Oncogene 1998;17:3261–3270.
[72] Roulston A, Reinhard C, Amiri P, Williams LT. Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor-α. J Biol Chem 1998;273:10232–10239.
[73] Palmer JN, Hartogensis WE, Patten M, Fortuin FD, Long CS. Interleukin-1β induces cardiac myocyte growth but inhibits cardiac fibroblast proliferation in culture. J Clin Invest 1995;95:2555–2564.
[74] Patten M, Hartogensis WE, Long CS. Interleukin-1β is a negative transcriptional regulator of α1-adrenergic induced gene expression in cultured cardiac myocytes. J Biol Chem 1996;271:21134–21141.
[75] Koudssi F, López JE, Vilegas S, Long CS. Cardiac fibroblasts arrest at the G_1/S restriction point in response to interleukin (IL)-1β. Evidence for IL-1β-induced hypophosphorylation of the retinoblastoma protein. J Biol Chem 1998;273:25796–25803.
[76] Wesche H, Korherr C, Kracht M, Falk W, Resch K, Martin MU. The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). J Biol Chem 1997;272:7727–7731.
[77] Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 1998;334:297–314.
[78] Wollert KC, Taga T, Saito M et al. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series via gp130/leukemia inhibitory factor receptor-dependent pathways. J Biol Chem 1996;271:9535–9545.
[79] Ishikawa M, Saito Y, Miyamoto Y et al. cDNA cloning of rat cardiotrophin-1 (CT-1): augmented expression of CT-1 gene in ventricle of genetically hypertensive rats. Biochem Biophys Res Commun 1996;219:377–381.
[80] Kodama H, Fukuda K, Pan J et al. Leukemia inhibitory factor, a potent cardiac hypertrophic cytokine, activates the JAK/STAT pathway in rat cardiomyocytes. Circ Res 1997;81:656–663.
[81] Kunisada K, Tone E, Fujio Y, Matsui H, Yamauchi-Takihara K, Kishimoto T. Activation of gp130 transduces hypertrophic signals via STAT3 in cardiac myocytes. Circulation 1998;98:346–352.
[82] Kunisada K, Hirota H, Fujio Y et al. Activation of JAK-STAT and MAP kinases by Leukemic Inhibitory Factor through gp130 in cardiac myocytes. Circulation 1996;94:2626–2632.
[83] Sheng Z, Knowlton K, Chen J, Hoshijima M, Brown JH, Chien KR. Cardiotrophin 1 (CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for cardiac cell hypertrophy. J Biol Chem 1997;272:5783–5791.
[84] Lazou A, Sugden PH, Clerk A. Activation of mitogen-activated protein kinases (p38-MAPKs, SAPKs/JNKs and ERKs) by the G-protein-coupled receptor agonist phenylephrine in the perfused rat heart. Biochem J 1998;332:459–465.
[85] Ramirez MT, Sah VP, Zhao X-L, Hunter JJ, Chien KR, Heller Brown J. The MEKK-JNK pathway is stimulated by α1-adrenergic receptor and Ras activation and is associated with in vitro and in vivo cardiac hypertrophy. J Biol Chem 1997;272:14057–14061.
[86] Yano M, Kim S, Izumi Y, Yamanaka S, Iwao H. Differential activation of cardiac c-jun amino-terminal kinase and extracellular signal-regulated kinase in angiotensin II-mediated hypertension. Circ Res 1998;83:752–760.
[87] Lenormand P, Sardet C, Pagès G, L'Allemain G, Brunet A, Pouysségur J. Growth factors induce nuclear translocation of MAP kinases (p42mapk and p44mapk) but not of their activator MAP kinase kinase (p45mapkk) in fibroblasts. J Cell Biol 1993;122:1079–1088.
[88] Mizukami Y, Yoshioka K, Morimoto S, Yoshida K-i. A novel mechanism of JNK1 activation. Nuclear translocation and activation of JNK1 during ischemia and reperfusion. J Biol Chem 1997;272:16657–16662.
[89] Ben-Levy R, Hooper S, Wilson R, Paterson HF, Marshall CJ. Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2. Curr Biol 1998;8:1049–1057.
[90] Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. Science 1997;276:1404–1407.
[91] Han J, Jiang Y, Li Z, Kravchenko VV, Ulevitch RJ. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. Nature 1997;386:296–299.
[92] Zhao M, New L, Kravchenko VV et al. Regulation of the MEF2 family of transcription factors by p38. Mol Cell Biol 1999;19:21–30.
[93] Lee Y, Nadal-Ginard B, Mahdavi V, Izumo S. Myocyte-specific enhancer factor 2 and thyroid hormone receptor associate and synergistically activate the α-cardiac myosin heavy-chain gene. Mol Cell Biol 1997;17:2745–2755.
[94] Zechner D, Thuerauf DJ, Hanford DS, McDonough PM, Glembotski CC. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. J Cell Biol 1997;139:115–127.
[95] Vogt PK, Bos TJ. The oncogene jun and nuclear signalling. Trends Biochem Sci 1987;14:172–175.
[96] Eferl R, Sibilia M, Hilberg F et al. Functions of c-Jun in liver and heart development. J Cell Biol 1999;145:1049–1061.
[97] Iwaki K, Sukhatme VP, Shubeita HE, Chien KR. α- and β-adrenergic stimulation induces distinct patterns of immediate early gene expression in neonatal rat myocardial cell. fos/jun expression is associated with sarcomere assembly; egr-1 induction is primarily an α_1-mediated response. J Biol Chem 1990;265:13809–13817.
[98] Dunnmon PM, Iwaki K, Henderson SA, Sen A, Chien KR. Phorbol esters induce immediate-early genes and activate cardiac gene transcription in neonatal rat myocardial cells. J Mol Cell Cardiol 1990;22:901–910.
[99] Komuro I, Kaida T, Shibazaki Y et al. Stretching cardiac myocytes stimulates protooncogene expression. J Biol Chem 1990;265:3595–3598.
[100] Webster KA, Discher DJ, Bishopric NH. Induction and nuclear accumulation of Fos and Jun proto-oncogenes in hypoxic cardiac myocytes. J Biol Chem 1993;268:16852–16858.
[101] Bishopric NH, Jayasena V, Webster KA. Positive regulation of the skeletal α-actin gene by Fos and Jun in cardiac myocytes. J Biol Chem 1992;267:25535–25540.
[102] Xia Y, Buja M, McMillin JB. Activation of the cytochrome c gene by electrical stimulation in neonatal rat cardiac myocytes. Role of NRF-1 and c-Jun. J Biol Chem 1998;273:12593–12598.
[103] Thuerauf DJ, Arnold ND, Zechner D et al. p38 mitogen-activated protein kinase mediates the transcriptional induction of atrial natriuretic factor gene through a serum response element. A potential role for the transcription factor ATF6. J Biol Chem 1998;273:20636–20643.
[104] Wasylyk B, Hagman J, Gutierrez-Hartmann A. Ets transcription factors: nuclear effectors of the Ras-MAP kinase signaling pathway. Trends Biochem Sci 1998;23:213–216.
[105] Hines WA, Thorburn J, Thorburn A. A low-affinity serum response element allows other transcription factors to activate inducible gene expression in cardiac myocytes. Mol Cell Biol 1999;19:1841–1852.

[106] Fentzke RC, Korcarz CE, Lang RM, Lin H, Leiden JM. Dilated cardiomyopathy in transgenic mice expressing a dominant-negative CREB transcription factor in the heart. J Clin Invest 1998;101:2415–2426.
[107] Xing J, Ginty DD, Greenberg ME. Coupling of the Ras-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 1996;273:959–963.
[108] Tan Y, Rouse J, Zhang A, Cariati S, Cohen P, Comb MJ. FGF and stress regulate CREB and ATF-1 via a pathway involving RK/p38 MAP kinase and MAPKAP kinase-2. EMBO J 1996;15:4629–4642.
[109] Deak M, Clifton AD, Lucocq JM, Alessi DR. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J 1998;17:4426–4441.
[110] Sadoshima J, Qiu Z, Morgan JP, Izumo S. Angiotensin II and other hypertrophic stimuli mediated by G protein coupled receptors activated tyrosine kinase, MAP kinase and 90 K S6 kinase in cardiac myocytes; a critical role of Ca2+-dependent signaling. Circ Res 1995;76:1–15.
[111] Erikson RL. Structure, expression, and regulation of protein kinases involved in the phosphorylation of ribosomal protein S6. J Biol Chem 1991;266:6007–6010.
[112] Maulik N, Watanabe M, Zu Y-L et al. Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts. FEBS Lett 1996;396:233–237.
[113] Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. Oncogene 1997;15:2169–2177.
[114] Fukunaga R, Hunter T. MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. EMBO J 1997;16:1921–1933.
[115] Waskiewicz AJ, Flynn A, Proud CG, Cooper JA. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. EMBO J 1997;16:1909–1920.
[116] Xia ZG, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326–1331.
[117] Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ et al. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal protein kinases are activated by ischemia/reperfusion. Circ Res 1996;79:162–173.
[118] Yin T, Sandhu G, Wolfgang CD et al. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. J Biol Chem 1997;272:19943–19950.
[119] Li WG, Zaheer A, Coppey L, Oskarsson HJ. Activation of JNK in the remote myocardium after large myocardial infarction in rats. Biochem Biophys Res Commun 1998;246:816–820.
[120] Mackay K, Mochly-Rosen D. An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia. J Biol Chem 1999;274:6272–6279.
[121] Meldrum DR, Dinarello CA, Cleveland JC et al. Hydrogen peroxide induces tumour necrosis factor α-mediated cardiac injury by a p38 mitogen-activated protein kinase-dependent mechanism. Surgery 1999;124:291–296.
[122] Ma XL, Kumar S, Gao F et al. Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion. Circulation 1999;99:1685–1691.
[123] Wang Y, Huang S, Sah VP et al. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J Biol Chem 1998;273:2161–2168.
[124] Behrens A, Sibilia M, Wagner EF. Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. Nature Genet 1999;21:326–329.
[125] Maulik N, Yoshida T, Engelman RM et al. Ischemic preconditioning attenuates apoptotic cell death associated with ischemia/reperfusion. Mol Cell Biochem 1998;186:139–145.
[126] Yujiri T, Sather S, Fanger GR, Johnson GL. Role of MEKK1 in cell survival and activation of JNK and ERK pathways defined by targeted gene disruption. Science 1998;282:1911–1914.
[127] Leppa S, Saffrich R, Ansorge W, Bohmann D. Differential regulation of c-Jun by ERK and JNK during PC12 cell differentiation. EMBO J 1998;17:4404–4413.
[128] Assefa Z, Vantieghem A, Declercq W et al. The activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signaling pathways protects HeLa cells from apoptosis following photodynamic therapy with hypericin. J Biol Chem 1999;274:8766–8796.
[129] Wojtaszek PA, Heasley LE, Siriwardana G, Berl T. Dominant-negative c-Jun NH2-terminal kinase 2 sensitizes renal inner medullary collecting duct cells to hypertonicity-induced lethality independent of organic osmolyte transport. J Biol Chem 1998;273:800–804.
[130] Zechner D, Craig R, Hanford DS, McDonough PM, Sabbadini RA, Glembotski CC. MKK6 activates myocardial cell NF-κB and inhibits apoptosis in a p38 mitogen-activated protein kinase-dependent manner. J Biol Chem 1998;273:8232–8239.
[131] Pedram A, Razandi M, Levin ER. Extracellular signal-regulated protein kinase/Jun kinase cross-talk underlies endothelial cell growth factor-induced endothelial cell proliferation. J Biol Chem 1998;273:26722–26728.
[132] Cuenda A, Cohen P. Stress-activated protein kinase-2/p38 and a rapamycin-sensitive pathway are required for C2C12 myogenesis. J Biol Chem 1999;274:4341–4346.
[133] Higashita R, Li L, van Putten V et al. $G\alpha_{16}$ mimics vasoconstrictor action to induce smooth muscle α-actin in vascular smooth muscle cells through a Jun-NH_2-terminal kinase-dependent pathway. J Biol Chem 1997;272:25845–25850.
[134] Nagarkatti DS, Sha'afi RI. Role of p38 MAP kinase in myocardial stress. J Mol Cell Cardiol 1998;30:1651–1664.
[135] Guyton KZ, Liu YS, Gorospe M, Xu QB, Holbrook NJ. Activation of mitogen-activated protein kinase by H_2O_2 – Role in cell survival following oxidant injury. J Biol Chem 1996;271:4138–4142.
[136] Thorburn A, Thorburn J, Chen S-Y et al. HRas-dependent pathways can activate morphological and genetic markers of cardiac muscle cell hypertrophy. J Biol Chem 1993;268:2244–2249.
[137] Abdellatif M, MacLellan WR, Schneider MD. p21 Ras a governor of global gene expression. J Biol Chem 1994;269:15423–15426.
[138] Katz ME, McCormick F. Signal transduction from multiple Ras effectors. Curr Biol 1997;7:75–79.
[139] Votjek AB, Der CJ. Increasing complexity of the Ras signaling pathway. J Biol Chem 1998;273:19925–19928.
[140] Gillespie-Brown J, Fuller SJ, Bogoyevitch MA, Cowley S, Sugden PH. The mitogen-activated protein kinase kinase MEK1 stimulates a pattern of gene expression typical of the hypertrophic phenotype in rat ventricular cardiomyocytes. J Biol Chem 1995;270:28092–28096.
[141] Thorburn J, McMahon M, Thorburn A. Raf-1 kinase activity is necessary and sufficient for gene expression changes but not sufficient for cellular morphology changes associated with cardiac myocyte hypertrophy. J Biol Chem 1994;269:30580–30586.
[142] Ho PD, Zechner DK, He H, Dillmann WH, Glembotski CC, McDonough PM. The Raf-MEK-ERK cascade represnts a common pathway for alteration of intracellular calcium by Ras and protein kinase C in cardiac myocytes. J Biol Chem 1998;273:21730–21735.
[143] Fuller SJ, Finn SG, Downward J, Sugden PH. Stimulation of gene expression in neonatal rat ventricular myocytes by Ras is mediated by Ral guanine nucleotide dissociation stimulator (Ral.GDS) and phosphatidylinositol 3-kinase in addition to Raf. Biochem J 1998;335:241–246.

[144] Nemoto S, Sheng Z, Lin A. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. Mol Cell Biol 1998;18:3518–3526.
[145] Wang Y, Su B, Sah VP, Heller Brown J, Han J, Chien KR. Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH_2-terminal kinase in ventricular muscle cells. J Biol Chem 1998;273:5423–5426.
[146] Thorburn J, Carlson M, Mansour SJ, Chien KR, Ahn NG, Thorburn A. Inhibition of a signaling pathway in cardiac muscle cells by active mitogen-activated protein kinase kinase. Mol Biol Cell 1995;6:1479–1490.
[147] Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, Xie Z. Multiple signal transduction pathways link Na^+/K^+-ATPase to growth-related genes in cardiac myocytes. The roles of Ras and mitogen-activated protein kinases. J Biol Chem 1998;273:15229–15256.
[148] Clerk A, Michael A, Sugden PH. Stimulation of the p38 mitogen-activated protein kinase pathway in neonatal rat ventricular myocytes by the G protein-coupled receptor agonists, endothelin-1 and phenylephrine: a role in cardiac myocyte hypertrophy? J Cell Biol 1998;142:523–535.
[149] Hunter JJ, Tanaka N, Rockman HA, Ross Jr. J, Chien KR. Ventricular expression of a MLC-2*v-ras* fusion gene induces cardiac hypertrophy and selective diastolic dysfunction in transgenic mice. J Biol Chem 1995;270:23173–23178.
[150] Izumi Y, Kim S, Murakami T, Yamanaka S, Iwao H. Cardiac mitogen-activated protein kinase activities are chronically increased in stroke-prone hypertensive rats. Hypertension 1998;31:50–56.