Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular and Cellular Cardiology 35 (2003) 871–886
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Review Article

An overview of endothelin signaling in the cardiac myocyte ☆

Peter H. Sugden *

Cardiac Medicine, Faculty of Medicine NHLI Division, Imperial College of Science, Technology and Medicine, Flowers Building 4th Floor, Armstrong Road, London SW7 2AZ, UK

Received 24 February 2003; received in revised form 23 April 2003; accepted 24 April 2003

Abstract

Three endothelin (ET) isopeptides have been identified: ET-1, ET-2 and ET-3. These have two well-established gross effects on the cardiac myocyte. They affect the contractile properties and they stimulate myocyte growth and myofibrillogenesis. There may be other effects that are less fully characterized (e.g. increased resistance apoptosis). The changes in myocyte biology are brought about by modulation of intracellular signaling pathways. ET-1 binds to the ET_A receptor on the cell surface and stimulates hydrolysis of phosphatidylinositol 4', 5'-bisphosphate to diacylglycerol and inositol 1', 4', 5'-trisphosphate. Diacylglycerol remains in the plane of the membrane and this causes translocation of the δ– and ϵ-isoforms of protein kinase C (PKC) to that compartment, an event thought to be indicative of PKC activation. The next events (probably associated with PKC activation) are the activation of the small G–protein Ras and of the extracellular signal-regulated kinase 1/2 (ERK1/2) cascade. Over a longer time course, two protein kinase cascades related to the ERK1/2 cascade, the c–Jun N–terminal kinase and p38-mitogen-activated protein kinase (p38–mitogen) cascades, also become activated. As the signals originating from the ET_A receptor are transmitted through these protein kinase pathways, other signaling molecules become phosphorylated, thus changing their biological activity. Such molecules include nuclear transcription factors (e.g. GATA-4, c–Jun), protein kinases (e.g. 90–kDa ribosomal protein S6 kinase, MAPK-activated protein kinase 2), and ion exchangers/channels (e.g. the Na^+/H^+ exchanger 1). These changes are responsible for the overall biological effects of ET isopeptides on the myocyte.

Keywords: Cardiac myocyte; Phospholipid-dependent signaling; Protein kinase C; Mitogen-activated protein kinases; Transcription; Ion movements

Abbreviations: 4E–BP, eIF4E–binding protein; ANF, atrial natriuretic factor; ASK, apoptosis signal-regulating protein kinase; BNP, B–type natriuretic peptide; CaM, calmodulin; DAG, diacylglycerol; EGF, epidermal growth factor; eIF, eukaryotic initiation factor; ERK, extracellular signal-regulated kinase; ET, endothelin; FAK, focal adhesion kinase; G–protein, guanine nucleotide-binding protein; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GPCR, G-protein-coupled receptor; Hsp, heat shock protein; Ins, inositol; Ins(1, 4, 5)P_3, inositol 1', 4', 5'-trisphosphate; JNK, c–Jun N–terminal kinase; MAPK, mitogen-activated protein kinase; MAPKAPK, MAPK-activated protein kinase; MEK, MAPK (or ERK) kinase; MKK, MAPK kinase; MLCK, myosin light chain kinase; MSK, mitogen– and stress-activated protein kinase; NHE, Na^+/H^+ exchanger; p90RSK, 90–kDa ribosomal protein S6 kinase; PIE, positive inotropic effect; PI-PLCβ, Ptd(4,5)InsP_2–dependent phospholipase Cβ; PI3K, phosphoinositide 3'-OH kinase; PLD, phospholipase D; PMA, phorbol 12–myristate 13–acetate; polI, RNA polymerase I; Ptd, phosphatidyl; PtdIns, phosphatidylinositol; Ptd(4, 5)InsP_2, phosphatidylinositol 4', 5'-bisphosphate; PtdIns(3, 4, 5)P_3, phosphatidylinositol 3', 4', 5'-trisphosphate; PKC, protein kinase C; PTK, protein tyrosine kinase; PYK2, Pro-rich Tyr-kinase 2; RGS, regulator of G–protein signaling; SAPK, stress-activated protein kinase; TCF, ternary complex factor; UBF, upstream-binding factor.

☆ This review was originally delivered as a landmark lecture at the International Society for Heart Research 24th Annual North American Section Meeting.

* Correspondance author: Tel.: +44-20-7594-3410; fax: +44-20-7594-3419.
E-mail address: p.sugden@imperial.ac.uk (P.H. Sugden).

DOI: 10.1016/S0022-2828(03)00153-6

A PubMed search (April 2003) reveals that, since their discovery in 1988 as potent vasoconstrictors produced by vascular endothelial cells [1], over 14,000 publications have referred to endothelins (ETs), indicating the high levels of interest and activity in this area. Three ET isopeptides (ET-1, ET-2 and ET-3) have been identified. This review is primarily concerned with the actions of ET-1 on the cardiac myocyte, though the signaling pathways activated by ET-2 and ET-3 should not be qualitatively different. General advances in ET biology and pathophysiology have been reviewed recently [2], as have advances more specifically related to the failing heart and its pharmacotherapy [3–6]. In addition to their vasoactive effects (which is now recognized can be vasodilatory as well as vasoconstrictory depending on the vessels involved), ET isopeptides regulate a variety of biological processes in non-vascular tissues. In the cardiac myocyte, they regulate the movements and intracellular concentrations of ions, and thus affect its contractile properties [7]. They induce hypertrophy of cultured myocytes (see, e.g. Ref. [8]) and they may play a role in the development of pressure overload-induced cardiac hypertrophy in vivo [9]. Their regulation of intracellular signaling pathways is central to these actions and is the topic of this review. With respect to the ET isopeptides and cardiac pharmacotherapy, there has been a general expectation that ET-receptor antagonism may ameliorate the problems associated with heart failure. However, the results of a clinical trial (REACH-1) with the orally administered generalized ET-receptor antagonist bosentan were equivocal with worsening heart failure compared with placebo in the short term (<3 months), but with improvement in the longer term (>6 months) [10]. The trial was ended prematurely because of bosentan hepatotoxicity. These disappointing results should not be allowed to impede the development of more selective orally administered ET-receptor antagonists and further clinical trials.

1. Stimulation of diacylglycerol and inositol 1, 4, 5–trisphosphate formation by ET-1

The initial stages of ET signaling involve proteins associated with the sarcolemma. ET isopeptides signal through transmembrane *g*uanine nucleotide-binding *p*rotein–*c*oupled *r*eceptors (GPCRs), a large receptor family, which shares a number of common features [11]. Their N–terminal region is extracellular and their C–terminal region is intracellular. Seven transmembrane (7TM) α-helices, which are comparatively rich in hydrophobic residues, connect three cytoplasmic and three extracellular loops with the N– and C–termini (hence these receptors are also known as 'heptahelical' or 7TM receptors). On binding of their cognate agonists, GPCRs interact with heterotrimeric guanine nucleotide-binding (G) proteins, and the two more C–terminal cytoplasmic loops, and the C–terminal tail in the GPCRs are generally thought to be important in these interactions. ET isopeptides exert their effects through two GPCR-receptor subtypes in mammals, ET_A and ET_B, both of which have been cloned [12]. The ET_A receptor predominates in neonatal rat cardiac myocytes (the principal model for signaling studies) [13]. Although there is evidence that there may be coupling to the $G_{i/o}$ subfamily of heterotrimeric G–proteins [13–15], the best characterized signaling interaction of the ET_A receptor is with Gq subtypes [2].

As with all heterotrimeric G–proteins, Gq consists of an α-subunit (αq, or a related α-subunit, such as α11), a member of the β-subunit family, and a member of the γ-subunit family (Fig. 1). As for most heterotrimeric G–proteins, Gq is membrane associated, and this is probably mediated through hydrophobic N–terminal regions of αq and palmitoylation of this region [16], and through prenylation and C–terminal carboxymethylation of the γ-subunits [17]. In the biologically inactive Gq heterotrimer, αq is ligated to GDP. GqPCR activation stimulates exchange of GDP for GTP on αq, and the heterotrimer dissociates into αq(GTP) and βγ dimers [11]. The activated GqPCR can, thus, be considered as being a guanine nucleotide exchange factor (GEF) for αq. Both αq(GTP) and βγ dimers remain associated with the membrane, and it is generally thought that their dissociation leads to activation of phosphoinositide-specific phospholipase Cβ (PI-PLCβ) species [18,19]. There are four isoforms of PI-PLCβ with PI-PLCβ_1 and PI-PLCβ_3 being the most widely expressed. Activation of PI-PLCβ_1 and PI-PLCβ_3 can be mediated by either αq(GTP) or by βγ dimers, the rank order of efficacy for activation by αq(GTP) being PI-PLCβ_1 ≥ PI-PLCβ_3 > PI-PLCβ_2. In contrast, PI-PLCβ_2 (which is found primarily in hematopoietic tissues) exhibits the highest-binding affinity for βγ dimers. It is still not clear which PI-PLCβ-isoforms are expressed in heart. mRNA for PI-PLCβ_3 has been detected in neonatal rat cardiac myocytes [20]. In contrast, mRNA for PI-PLCβ_1 was not detectable under control conditions, but was induced by serum or suitable growth factors [20]. Confusingly, both PI-PLCβ_1 and PI-PLCβ_3 proteins have detected in adult guinea pig and rat ventricles, respectively [21,22]. Following activation, PI-PLCβ hydrolyzes the membrane phospholipid, phosphatidylinositol 4', 5'-bisphosphate [PtdIns(4, 5)P_2] to two 'second messengers': hydrophobic diacylglycerol (DAG), which remains in the plane of the membrane, and soluble inositol 1', 4', 5'-trisphosphate [Ins(1, 4, 5)P_3]. ET_A receptor-stimulated formation of Ins(1, 4, 5)P_3 (and, by implication, DAG), is detectable within seconds of exposure of myocytes to ET-1 [23], though the PI-PLCβ-isoform(s) responsible for the acute hydrolysis of PtdIns(4,5)P_2 in the heart remain unclear. Activation of PI-PLCβ is terminated by the innate GTPase activity of the αq(GTP)–subunits (an activity which can be stimulated by GTPase-activating proteins (GAPs) known as regulator of G–protein signaling (RGS) proteins [24]), and the subsequent re-association of αq(GDP) with βγ dimers.

2. Biological roles of Ins(1, 4, 5)P_3 and DAG

Following its formation at the sarcolemma, Ins(1, 4, 5)P_3 diffuses into the cytoplasm and, through receptors located

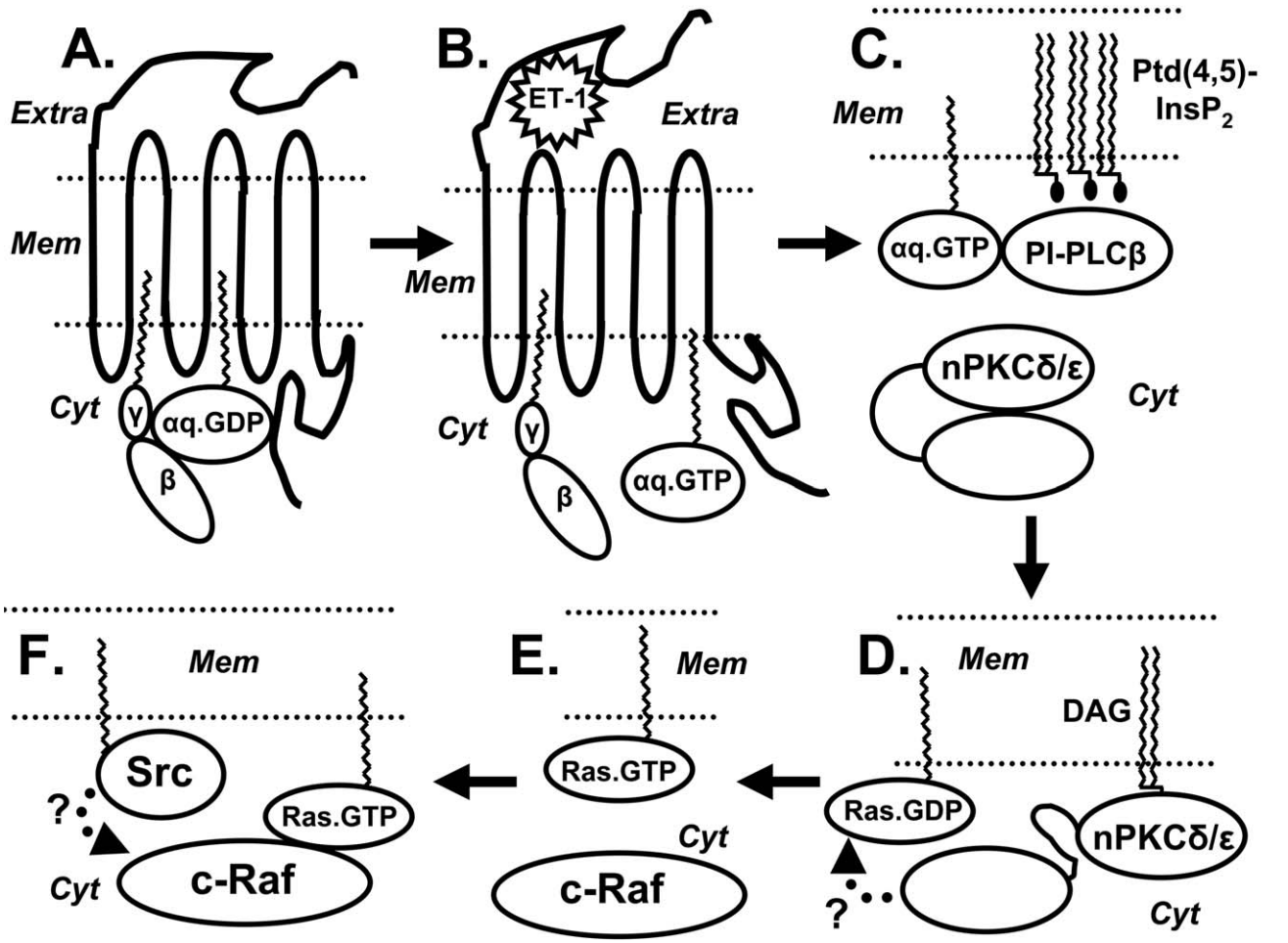

Fig. 1. Signaling from the ET_A receptor to c–Raf. Extra: extracellular, mem: membrane, cyt: cytoplasmic. (A) The basal state with Gq in its GDP-ligated heterotrimeric αq(GDP). βγ state. (B) ET-1 binds to the extracellular region of the ET_A receptor, causing a conformational change. This results in exchange of GDP for GTP on αq(GDP). βγ and dissociation of αq(GTP) and βγ. (C) αq(GTP) (and possibly βγ, not shown) activates PI-PLCβ which hydrolyzes PtdIns(4, 5)P_2 to DAG and Ins(1, 4, 5)P_3 (not shown). (D) The increase in the DAG content of the sarcolemma causes the translocation of cytoplasmic nPKCδ and nPKCϵ to that compartment, and the binding to DAG changes the conformation of nPKCδ and nPKCϵ, thereby activating them. (D, E) In some unidentified manner, nPKC activation results in the activation (Ras, GTP) of the small G–protein, Ras. (F) Cytoplasmic c–Raf migrates to the sarcolemma by binding to Ras.GTP. Other processes (e.g. phosphorylation of c–Raf by Src family PTKs) effect the full activation of c–Raf.

intracellular membrane systems, regulates intracellular Ca^{2+} movements in many cells types. However, Ins(1, 4, 5)P_3 receptors are unlikely to be of any significance in regulating the Ca^{2+} movements associated with myofibrillar contraction in cardiac myocytes. These are regulated primarily through the L–type Ca^{2+} channel, the sarcoplasmic reticulum Ca^{2+}–release channel-2 (the ryanodine receptor-2), Na^+/Ca^{2+} exchange, and the sarco/endoplasmic reticulum Ca^{2+} ATPase 2a. This does not exclude the possibility that Ins(1, 4, 5)P_3 may regulate Ca^{2+} movements that are not associated with contraction. As described in detail in the next section, DAG is a physiological regulator of several isoforms of the phosphatidylserine-dependent protein kinase, protein kinase C (PKC) [25].

3. Protein kinase C

As the sarcolemmal content of DAG increases following PI-PLCβ activation, DAG-sensitive PKCs translocate from the cytoplasm and associate with the membrane fraction and this is thought to be indicative of their activation (Fig. 1). DAG is rapidly phosphorylated by DAG kinases to phosphatidate, thus terminating activation of DAG-sensitive PKCs. PKCs are single polypeptide chains and these can be structurally subdivided into an N-terminal-regulatory domain (containing the autoinhibitory 'pseudosubstrate' site, and the cofactor/activator-binding sites), a 'hinge' region that is susceptible to proteolysis, and a C–terminal catalytic region. There are three PKC subfamilies, the '*c*lassical' or '*c*onventional' PKCs (which require DAG and Ca^{2+} in addition to phosphatidylserine for activity), the '*n*ovel' PKCs (the activities of which are DAG dependent though probably Ca^{2+} independent), and the '*a*typical' PKCs (the activities of which are independent of DAG and Ca^{2+}) [25]. Of the DAG-sensitive PKCs, cardiac myocytes express nPKCδ and nPKCϵ [26]. Whether they express bona fide cPKCα has been controversial, though it is now clear that cPKCα is actually presented at about a 10–fold higher relative molar

abundance than nPKCε [27]. Tumor-promoting phorbol esters, such as phorbol 12–myristate 13–acetate (PMA) or phorbol 12, 13–dibutyrate, act as pharmacological mimics of DAG and partition to the membrane, but they are not metabolized. They thus produce a very strong and long-lasting association of DAG-sensitive PKCs (cPKCα, nPKCδ and nPKCε in the myocyte) with the membrane fraction and this is followed by the loss of DAG-sensitive PKCs from the cell over 24 h [26]. This contrasts with the situation when myocytes are exposed to high (100 nM) concentrations of ET-1. Here, nPKCδ and nPKCε translocate almost stoichiometrically to the membrane fraction with 15–30 s [28]. nPKCδ begins to return to the soluble fraction in 1–2 min (presumably as DAG is phosphorylated), though nPKCε remains associated with the membrane for 5–15 min [28]. It follows that exposing myocytes to PMA cannot always be expected to induce the same responses even acutely as physiological agonists, although PKC may be activated in both situations. The EC_{50} of ET-1 for translocation of nPKCε is about 1 nM but that for nPKCδ is significantly greater (10 nM) [28]. In contrast to PMA, cPKCα is not detectably translocated by ET-1 in cardiac myocytes [28]. The propensity of nPKCε to translocate in myocytes (and its other properties) has led to the view that nPKCε is perhaps the most 'crucial' PKC-isoform in this cell, and this may well be justified. By way of example, moderate cardiac myocyte-directed overexpression of constitutively activated nPKCε in transgenic mice induces a 'compensated' cardiac hypertrophy [29] (though higher levels of expression cause deterioration in cardiac function [30]). A caveat needs to be added to the effect that, assuming that cPKCα and nPKCε have similar specific activities, translocation of the entire nPKCε pool would produce the same effect in terms of activity as (an experimentally undetectable) translocation of 10% of the cPKCα pool. Indeed the situation with respect to the insignificance of cPKCα may require reappraisal. It has been recently suggested that cPKCα may uniquely mediate cardiac myocyte hypertrophy [31], and it has been shown that downregulation of cPKCα by antisense deoxyoligonucleotide methodology inhibits the advent of some of the molecular criteria of hypertrophy in response to ET-1 [32].

4. Phospholipase D

Phospholipase D (PLD) hydrolyzes phosphatidylcholine to phosphatidate and choline with essentially instantaneous hydrolysis of phosphatidate to DAG, and thus could represent an alternative pathway of DAG formation [33,34]. However, the biological function of the PLD pathway may be to produce phosphatidate rather than DAG, because phosphatidate may function as a signaling intermediate in its own right [35,36]. Phosphatidate is also formed by the action of DAG kinase, which terminates the signaling function of DAG formed by PI-PLCβ. Because of the differences in degree of saturation of the acyl side chains of PtdIns(4, 5)P_2 and phosphatidylcholine, DAG and phosphatidate formed by the PI-PLCβ/DAG kinase pathway differ from PLD-derived phosphatidate and DAG [35]. For DAG, only the polyunsaturated species derived from PtdIns(4, 5)P_2 functions as a second messenger, whereas for phosphatidate, only the phosphatidylcholine-derived saturated/mono-unsaturated species function in this way [35]. The biological role(s) of the PLD pathway in cardiac myocytes, as in other cells, is obscure. Although PLD is stimulated in myocytes by ET-1 [23], it is activated significantly more slowly than PI-PLCβ, and its stimulation by PMA indicates a potential role for DAG-sensitive PKC-isoforms [23]. Somewhat unusually, activation of PLD by PKC appears to be mediated by an interaction between PLD and PKCs, rather than by any phosphorylation of PLD [33]. Other pathways of PLD activation, which may be relevant involve 'small' G–proteins (see below) and phosphorylation of Tyr-residues in PLD [33,34], but these have not been studied in cardiac myocytes.

5. Activation of small G–proteins by ET-1

In temporal terms, the next process associated with exposure of myocytes to ET-1 is the activation of a variety of small G–proteins [37] (Fig. 1) that, as with heterotrimeric G–proteins, involves exchange of GDP for GTP. Owing to the 'on–off' nature of the response, the term 'molecular switch' is frequently applied to the small G–proteins. As with Gα, GDP/GTP exchange is normally relatively slow but is enhanced dramatically by Ras GEFs [38], and activation of GEFs (rather than inhibition of GAPs) probably accounts for small G–protein activation when it occurs rapidly (i.e. within 5 min). For some small G–proteins, GDP/GTP exchange is inhibited by GDP dissociation inhibitors. Activation of small G–proteins is terminated by the innate GTPase activity of the small G–proteins. This is also normally slow, but is dramatically enhanced by GAPs.

The archetypal small G–proteins are the Ha-Ras, Ki-Ras and N–Ras members of the Ras family [37,39], all of which possess a molecular mass of about 21 kDa. They are membrane associated largely by virtue of lipid modification (irreversible C–terminal farnesylation and carboxymethylation, and, except for Ki-Ras, reversible palmitoylation). Although Ki-Ras is not palmitoylated, it contains a C–terminal polybasic region, which may promote association with polar phosphate head groups of phospholipids in the membrane lipid bilayer. ET-1 rapidly (maximal at 3 min) activates Ha-Ras and N–Ras in myocytes, though it has not been possible to establish unambiguously that Ki-Ras is activated because of the lack of a suitable antibody [40]. Study of Ha-Ras and Ki-Ras in particular is very active at the moment because of their differential partitioning to membrane sub-regions (caveolar and non-caveolar lipid rafts, and disordered plasma membrane) [41], and the specificity with which the different Ras-isoforms signal to their downstream effectors [42]. However, these topics will not be discussed here. In addition to the Ras family, several other small G–protein families have been identified: Rho, Rab, Ran and ARF/SAR1 [37]. In

myocytes, ET-1 rapidly activates RhoA and Rac1 [43], both members of the Rho family. The Rho family is involved inter alia in the regulation and re-organization of cell shape, though it is not clear whether these are their major functions in cardiac myocytes. Whether the remaining small G–proteins are activated in cardiac myocytes is not known, and even their expression characteristics have not been investigated systematically. While not directly relevant to the topic of this review, it should be noted that, in terms of myocardial responses, cardiac myocyte-specific expression of constitutively active Ha-Ras in isolated cells or transgenic mice induces cardiac myocyte hypertrophy or cardiac hypertrophy, respectively, though there are levels of complexity in the in vivo response that are not understood [44]. Rac1 and RhoA have also been implicated in the development of hypertrophy in isolated myocytes, though some of the experiments in transgenic mice are rather difficult to interpret from this viewpoint [44].

6. Mechanisms of ET-1-mediated activation of Ras

The rapid activation of Ras by ET-1 in cardiac myocytes suggests that the net activation of a GEF is likely to be more critical than inhibition of a GAP. nPKCϵ is rapidly translocated to the membrane fraction by low concentrations of ET-1 [28], suggesting that a link exists between nPKCϵ translocation and Ras activation (Fig. 1), and there is some evidence supporting an involvement of PKC in Ras activation in cardiac myocytes [40]. The nature of the connection between the two processes is obscure and other pathways may operate. Additional receptors for DAG and phorbol esters have been identified, one of which, Ras.GRP, is a GEF for Ras [45,46]. Thus, the activation of Ras may not be a consequence of translocation of nPKCϵ following activation of the ET_A receptor. However, Ras.GRP mRNA appears to be expressed primarily in neuronal tissues and its significance in the heart is difficult to assess.

It is well known that peptide growth factors, such as epidermal growth factor (EGF), bind to extracellular domains of their transmembrane receptors and induce receptor activation by increasing the innate protein Tyr-kinase (PTK) activities of those receptors, thus increasing autophosphorylation of specific Tyr-residues in the intracellular domains [47]. This promotes a series of receptor PTK–signaling protein interactions mediated by signaling protein domains (SH2, PTB) which recognize phospho-Tyr-containing sequences in receptor PTKs, ultimately resulting in the activation of Sos, a GEF for Ras, and activation of Ras [47]. Indirect GqPCR-mediated modulation of GEFs through the EGF receptor has been demonstrated in a number of cell types [48], and evidence has been presented that ET-1 and other GqPCR agonists induce EGF-receptor-dependent signaling in cardiac myocytes [49–51]. The mechanism may involve matrix metalloproteinases, cleavage and shedding of extracellular surface-bound pro-heparin-binding EGF, followed by activation of the EGF receptor by heparin-binding EGF [49,51,52]. It has been suggested that this receptor transactivation pathway may be responsible for the hypertrophic response of the myocyte to ET-1 and other interventions, and hence matrix metalloproteinase inhibition could have therapeutic value [49]. To me, it seems that there are two problems with receptor PTK transactivation as a signaling pathway to Ras in cardiac myocytes. First, from a biological point of view, it represents a particularly tortuous means of activating Ras. Secondly, it seems inconceivable that, given the complexity of the pathway, it could be responsible for the very rapid activation of Ras in cardiac myocytes [40]. In addition to shedding of heparin-binding EGF, other mechanisms of receptor PTK transactivation have been proposed. In cardiac myocytes, GqPCR agonist-stimulated Ca^{2+} movements or Ca^{2+}/calmodulin (CaM)–dependent mechanisms have been implicated [53,54], whereas production of reactive oxygen species [48] may also be involved in other cells. Reactive oxygen species are recognized mimics of receptor PTK agonists (e.g. insulin), a property that is probably attributable to their ability to increase Tyr-phosphorylation of receptor PTKs and other receptor PTK-associated signaling proteins by inhibiting protein Tyr-phosphatases [55].

7. Effectors of Ras

Three effectors of the aforementioned Ras species have been identified: protein kinases of the Raf family, lipid kinases of the phosphoinositide 3'-OH kinase (PI3K) family and Ral.GDS (a GEF for Ral, a member of the Ras family) [37,39]. More recently, a fourth signaling protein, PI-PLCϵ, has been identified as a Ras effector, though it may also serve to activate Ras or Ras-related proteins by acting as a GEF [19,38]. The Raf-isoforms (c–Raf, A–Raf and B–Raf) are 'initiator kinases' for the extracellular signal-regulated kinase 1/2 (ERK1/2) cascade. When Ras becomes activated (Ras.GDP → Ras.GTP), it is able to bind c–Raf, which translocates from the cytoplasm to the membrane (Fig. 1). Although binding of c–Raf to Ras.GTP is essential for its activation, it is probably only one of a number of steps that are necessary, others being c–Raf phosphorylation and interaction with 14–3–3 proteins [56]. Though less explored than c–Raf, it seems likely that the activation of A–Raf and B–Raf also involves interaction with Ras.GTP [57].

ERK1 and ERK2 were originally identified as '*m*icrotubule–*a*ssociated *p*rotein *k*inases', a term that was easily adapted to mitogen-activated protein kinases (MAPKs) once it was recognized that they were activated by known mitogens. Much of the confusion in this field can be attributed to the complex terminology and a more logical classification has been developed by Philip Cohen (Table 1). However, this has not yet achieved widespread usage ensuring continuing confusion and I will use the common trivial terminology (Table 1) here. MAPK cascades are composed of three sequentially acting protein kinases. In the ERK 1/2 cascade (Fig. 2), the MAPK kinase kinase Raf phosphory-

Table 1
Terminology of MAPKs and SAPKs

Cohen terminology [60]	Phosphorylation motif	Common trivial terminology and molecular mass	Other terminology
MAPK1	Thr–Glu–Tyr	ERK1 (44 kDa)	p44–MAPK
MAPK2	Thr–Glu–Tyr	ERK2 (42 kDa)	p42–MAPK
SAPK1a	Thr–Pro–Tyr	JNK (~46 or ~54 kDa)	JNK2, SAPKα
SAPK1b	Thr–Pro–Tyr	JNK (~46 or ~54 kDa)	JNK3, SAPKβ
SAPK1c	Thr–Pro–Tyr	JNK (~46 or ~54 kDa)	JNK1, SAPKγ
SAPK2a	Thr–Gly–Tyr	p38–MAPK (38 kDa)	p38–MAPKα, p38 and other now obsolete terms
SAPK2b	Thr–Gly–Tyr	p38–MAPKβ (38 kDa)	
SAPK3	Thr–Gly–Tyr	p38–MAPKγ (42 kDa)*	ERK6
SAPK4	Thr–Gly–Tyr	p38–MAPKδ (42 kDa)*	
SAPK5	Thr–Glu–Tyr	'Big' MAPK1 (~100 kDa)	ERK5, BMK1

* These proteins run anomalously at about 40–50 kDa on SDS-polyacrylamide gel electrophoresis.

lates two Ser-residues in MAPK kinases 1 and 2 (MKK1 and 2, also known as MAPK [or ERK] kinases 1 and 2, MEK1 and 2), thereby activating them. MKK1/2 subsequently phosphorylate and activate ERK1/2. One feature of ERK1/2 is that they are activated by the dual-phosphorylation of a Thr– and a Tyr-residue in a Thr–Glu–Tyr motif. In terms of biological responses, the activation of the ERK1/2 cascade is thought to be anabolic and, in relation to the heart, stimulates growth to promote an adaptive hypertrophy [58]. All three stages of the ERK1/2 cascade (c–Raf and A–Raf, MKK 1/2 and ERK1/2) are powerfully and rapidly (within3–5 min) activated by ET-1 in cardiac myocytes [59–61]. For ERK1/2, the extent of activation/phosphorylation induced by ET-1 is similar to that produced by PMA and is essentially stoichiometric. In a variety of cell types, activation of ERK1/2 leads to the phosphorylation and the modulation of the biological activities of a number of signaling proteins including protein kinases (e.g. the 90–kDa ribosomal protein S6 kinases, p90RSKs [62], which are also known as MAPK-activated protein kinase (MAPKAPK)–1s (MAPKAPK1s)), other signaling proteins (e.g. cytoplasmic phospholipase A_2 [63]), and transcription factors (e.g. Elk-1 [64]). With respect to transcription factor phosphorylation, the rapid appearance of activated ERK1/2 in the cardiac myocyte nucleus following its activation is consistent with their role in the regulation of transcription and growth in this cell [40].

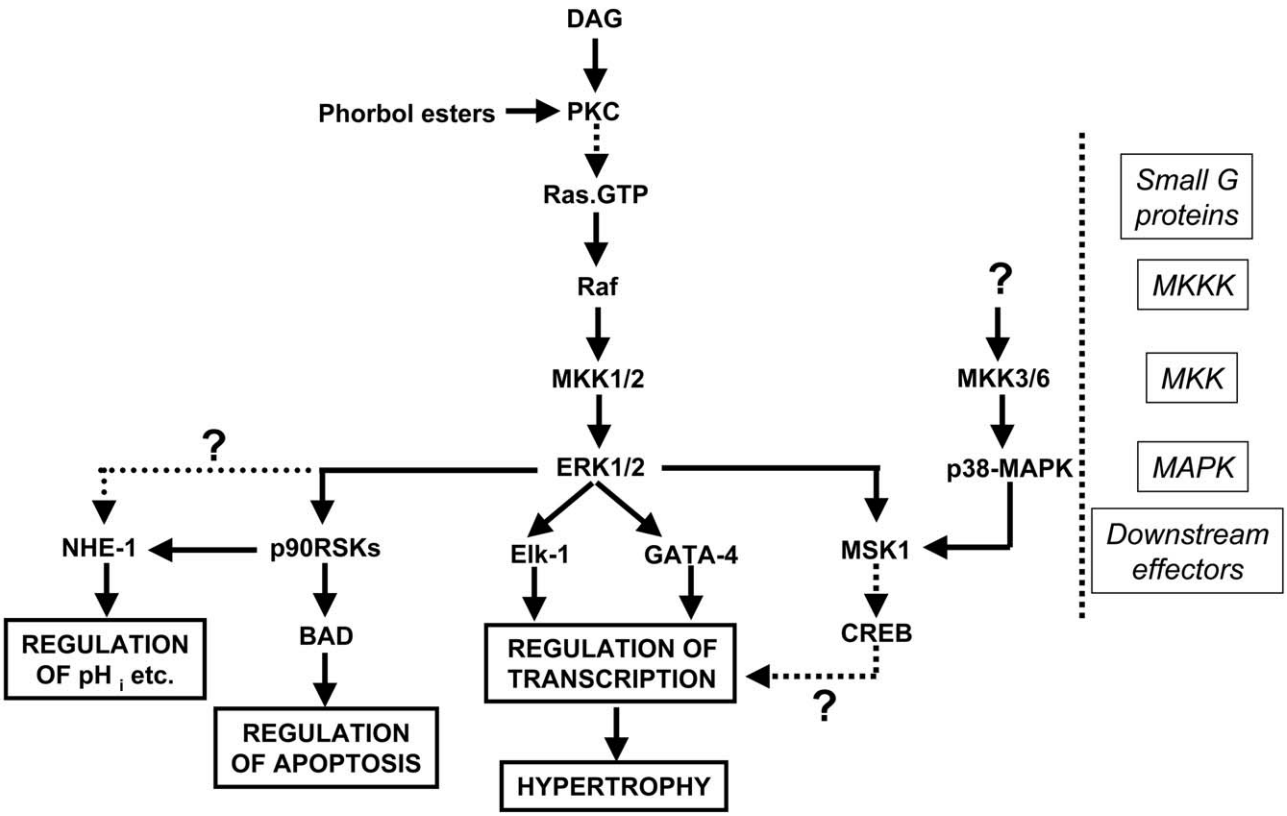

Fig. 2. The ERK1/2 cascade. Ras.GTP is instrumental in the activation of Raf-isoforms, the MAPK kinase kinases (MKKKs) of the ERK1/2 cascade. These then phosphorylate and activate MKK1/2, which in turn phosphorylate and activate ERK1/2. ERK1/2 then phosphorylate downstream effectors changing their biological activities. Downstream effectors include transcription factors and other protein kinases (p90RSKs, MSK1). The ERK1/2–related p38–MAPK may also activate MSK1.

Though activation of the ERK1/2 cascade is the best characterized response to increased Ras.GTP loading, there may be additional effectors. The PI3K group of lipid kinases catalyze the formation of PtdIns(3, 4, 5)P_3 from PtdIns(4, 5)P_2, as well as other 3'-OH phosphorylated phosphoinositides from PtdIns species [65]. Although PI3K signaling is strongly coupled to receptor PTK signaling, the γ-isoform of the PI3K catalytic subunit is activated by the βγ dimers resulting from the dissociation of heterotrimeric G–proteins [65]. PI3Ks may be effectors of Ras (though this is still controversial), and this is brought about by the binding of Ras.GTP to the catalytic subunits of PI3K (of which there are four isoforms) [65]. Formation of PtdIns(3, 4, 5)P_3 is particularly important in the activation of the Akt (also known as protein kinase B) protein kinase family [66]. Association of Akt with membrane 3–phosphoinositides and its subsequent phosphorylation by 3-phosphoinositide-dependent kinase(s) are required for its activation. In cardiac myocytes (as in other cells), insulin strongly stimulates the activity and phosphorylation of Akt [67,68]. Although ET-1 also stimulates its phosphorylation, the extent is much less than with insulin [68,69], and the significance of the PI3K/PtdIns(3, 4, 5)P_3/Akt pathway in terms of the biological actions of ET-1 in the myocyte is perhaps relatively minor. The relative ineffectiveness of ET-1 in stimulating phosphorylation of Akt in myocytes is somewhat surprising as glycogen synthase kinase 3, an established substrate of Akt [70], becomes phosphorylated (and inactivated) in a PI3K–dependent manner on exposure of myocytes to ET-1 [71]. Indeed, inactivation of glycogen synthase kinase 3 has been implicated in the development of ET-1-induced hypertrophy [71]. However, the possibility that ET-1 stimulates formation of PtdIns(3, 4, 5)P_3 or other 3'-phosphoinositides in cardiac myocytes, and this (or indeed another process) leads to phosphorylation of GSK3 in a Akt-independent manner should not be excluded. Very little is known about the function of the two other Ras effectors (Ral.GDS and PLCε) in cardiac myocytes, though there is evidence that Ral.GDS participates in the transcriptional changes that typify the hypertrophic response [72], and mRNA for PLCε is expressed in relatively high abundance in whole heart [73].

8. Activation of stress-activated protein kinases

After identification of ERK1/2, related protein kinases (c–Jun N–terminal kinases (JNKs), p38–MAPKs) were identified which were also activated by phosphorylation of Thr–Xaa–Tyr motifs (Thr–Pro–Tyr in JNKs, Thr–Gly–Tyr in p38–MAPKs) [74]. Although these kinases are most strongly activated by cytotoxic cellular stresses and are better classed as stress-activated protein kinases (SAPKs, see Table 1), they were also rather misleading grouped under the 'MAPK superfamily' epithet. ET-1 activates the JNK and p38–MAPK cascades in cardiac myocytes, though the activation of JNK or p38–MAPK by ET-1 is not as great as that by cytotoxic stresses [75,76]. How this activation is achieved is unclear, though experiments with activated nPKCδ suggest that this PKC-isoform may be involved [77]. In contrast, only ERK1/2 were strongly activated in analogous experiments with nPKCε [77]. Thus, the low concentrations of ET-1 normally encountered physiologically should lead only to activation of the ERK1/2 cascade, since nPKCε is activated preferentially over nPKCδ [28]. However, although not compared directly, we could not detect any difference in the EC_{50} values for ET-1 (~1 nM) between nPKCε activation and p38–MAPK phosphorylation [28,76]. Given that nPKCδ is minimally activated at 1 nM ET-1, the two findings are somewhat inconsistent. The current view of the biological roles of JNKs and p38–MAPKs is that they are probably pro-apoptotic [78], though their roles in the cardiac myocyte are unclear, with conflicting evidence presented for their being cytoprotective, hypertrophic or pro-apoptotic [58,79,80].

9. Biological effects of ET-1

There are two clear biological effects of ET isopeptides in the cardiac myocyte: (i) they are positively inotropic and negatively lusitropic [7] and (ii) they are hypertrophic [58]. The effects of ET isopeptides on the contractile properties of the cardiac myocytes involve modulation of ion movements [7], which themselves are regulated by activation of protein kinases (Fig. 3). It is important to realize that interrelationships and interdependencies exist between these (Fig. 3), and it is hence difficult to disentangle cause and effect. ET-1-induced cardiac myocyte hypertrophy involves alterations in the patterns and rates of gene expression, and increases in cell protein content. These growth responses are determined by a number of interdependent factors. Expression of mRNA is dependent on the rates and patterns of synthesis of the mRNA transcripts, the processing and export of mRNA from the nucleus, and the stability of the transcripts. Protein accumulation is influenced by the rates and specificity of mRNA translation, by the size and activity of the ribosomal pool available, and by protein degradation. With respect to the hypertrophic response of ET-1, all three MAPK cascades have been implicated [58] as has GSK3 [71].

10. ET-1 and the regulation of ion movements

Overall, ET isopeptides have a positive inotropic effect (PIE) but a negative lusitropic effect on (rabbit) cardiac muscle preparations [7], though there may be species differences in their ability to act as positive inotropes, which may be related to the abundance of ET receptors [81]. The effects of ET isopeptides on contractile properties differ from those of the β-adrenergic agonists, which are positively inotropic and positively lusitropic. One effect of ET isopeptides is to increase the $Ca^{2+}{}_i$ transient in cardiac myocytes [7], and this probably contributes to its PIE (Fig. 3). What is less clear is whether the changes in Ca^{2+} movements induced by ET

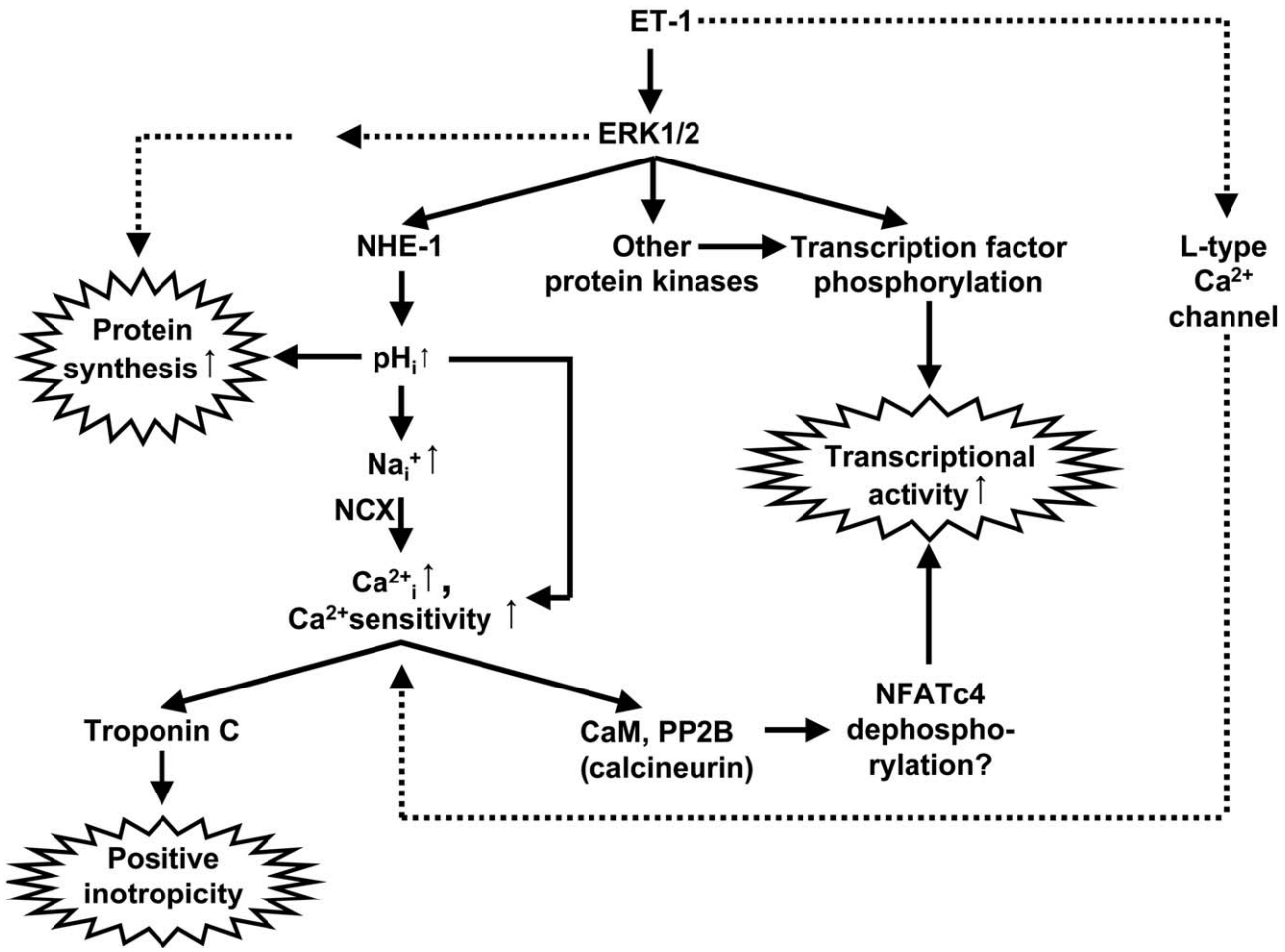

Fig. 3. Interdependence of ET-1-induced activation of ERK1/2 signaling, movement of ions and overall biological responses. NCX, Na^+/Ca^{2+} exchange; PP2B, protein phosphatase 2B, also known as calcineurin.

isopeptides affect only contraction or whether the subsequent consequences might include changes in the activities of Ca^{2+}–dependent signaling pathways, particularly those regulated by the Ca^{2+}–binding protein/Ca^{2+}–'sensor', CaM. Increased binding of Ca^{2+} to CaM should activate CaM-sensitive signaling molecules, such as calcineurin (protein phosphatase 2B), the Ca^{2+}/CaM-dependent protein kinases, and myosin light chain kinase (MLCK). From the point of view of cardiac myocyte hypertrophy, all three of these signaling proteins have been implicated [82–84], though the resulting phenotypes in transgenic mouse models may not inevitably resemble a compensated myocardial hypertrophy [83,85].

The changes in Ca^{2+} movements induced by the ET isopeptides are thought to be brought about by at least two mechanisms, stimulation of Na^+/H^+ exchanger 1 (NHE1) and of the sarcolemmal L–type Ca^{2+} channel (Fig. 3) [7]. Stimulation of Na^+/H^+ exchange through the cardiac myocyte NHE1 [86] leads to an intracellular alkalinization (pH_i is increased by as much as 0.12–0.13 units) and an increase in Na^+_i [87,88]. The increase in Na^+_i potentially reverses the normal direction of sarcolemmal Na^+/Ca^{2+} exchange (normally Ca^{2+}_i exchanged for Na^+_o), and thus increases Ca^{2+}_i [7]. An increase in pH_i may also explain the fact that, for a given degree of PIE, the ET isopeptide-induced effect requires a smaller increase in the Ca^{2+} transient than that induced by β-adrenergic agonism and why ET isopeptides are negatively lusitropic [7]. By increasing the dissociation of the carboxylic acid protons of the 'EF Hands' Glu-residues in troponin C (which is involved in the chelation of Ca^{2+} and the regulation of myofibrillar contraction), intracellular alkalinization will increase the affinity of troponin C for Ca^{2+}. This sensitizes the actomyosin ATPase to Ca^{2+} (positive inotropicity) and decreases the rate of dissociation of Ca^{2+} from the troponin C–Ca^{2+} complex (negative lusitropicity). In the same way, increased Ca^{2+}_i availability and pH_i could favor the activation of other EF Hands proteins, such as CaM.

Activation of the sarcolemmal L–type Ca^{2+} channel by ET-1 should, in a manner similar to 'normal' contractile activity, lead to enhanced Ca^{2+}–induced Ca^{2+} release through the sarcoplasmic reticulum Ca^{2+}–release channel. How L–type Ca^{2+} channel activation is achieved is unclear. The most obvious pathway would involve PKC, but, apart from some early work in *Xenopus* oocytes expressing L–type Ca^{2+} channel subunits [89], there has been relatively little published on the regulation of L–type Ca^{2+} channel current by

PKC. However, by using selective inhibitors of NHE1, 'reverse mode' Na^+/Ca^{2+} exchange and the L–type Ca^{2+} channel, there is evidence of the importance of all three of these processes in the ET isopeptide-induced PIE [90–92], and it is probably reasonable to conclude that inhibition of both NHE1 and the L–type Ca^{2+} channel is necessary to inhibit the ET isopeptide-induced PIE completely [90]. In addition, events indirectly related to or independent of ion movements may participate in the ET-1-induced changes in contractile function. For example, MLCK is regulated by CaM, and phosphorylation of the regulatory myosin light chain is potentially positively inotropic [93]. The PIE of ET-1 is sensitive to MLCK inhibition [94]. Furthermore, mutation of the PKC phosphorylation sites in cardiac troponin I diminishes the negative lusitropic effects of ET-1 and thus reduces the ET-1-induced increase in twitch duration [95].

11. ET-1-dependent activation of NHE1 by phosphorylation

As mentioned, activation of NHE1 is an important factor in the regulation of ion movements by ET-1 in the cardiac myocyte and in other cells [86,96]. NHE1 is a protein of about 813–822 amino acids in length, with (probably) 12 membrane-spanning α-helices [96]. The major intracellular domain consists of the C–terminal region of about 300 amino acids and this region is largely responsible for the regulation of NHE1 activity. Early work using PMA and other phorbol esters implicated PKC in activation of NHE in lymphocytes. Work in NIH 3T3 cells showed that NHE activity was increased in Ras-transformed cells, and further studies implicated the ERK1/2 cascade. Although NHE1 does not appear to be a direct substrate for ERK1/2, ERK1/2 effectors of the p90RSK family phosphorylate NHE1 on Ser-703 (which lies in a p90RSK Arg–Xaa–Arg–Xaa–Xaa–Ser/Thr consensus recognition sequence) in vascular smooth muscle cells and mutation of Ser-703 to Ala prevents stimulation of NHE1 by serum [97]. Almost simultaneously with the appearance of these data, p90RSKs were identified as being responsible for the activation of NHE1 by ET-1 in cardiac myocytes, though additional direct phosphorylation by ERK1/2 was not excluded [88]. Whether other MAPKs can activate NHE1 in cardiac myocytes is not clear. Withdrawal of trophic interleukins in a lymphocyte line induces phosphorylation of NHE1 by p38–MAPK and this may activate NHE1 [98]. Furthermore, NHE1 is not the only protein involved in controlling of pH_i in cardiac myocytes, which may be regulated by ERK1/2. These kinases have also very recently been implicated in the intracellular alkalinization mediated through Na^+/HCO_3^-–co-transport [99].

12. ET-1 and the regulation of gene transcription

Nuclear transcription factors regulate gene transcription and they comprise one major group of substrates of the MAPKs. It seems likely that modulation of gene transcription is a significant factor in the hypertrophic response. Transcription factors usually bind to non-coding-regulatory regions of the genome (often to short oligonucleotide consensus sequences within longer sequences in promoter regions lying 5' to the transcribed sequences). Though several transcription factors are direct substrates of MAPKs, only those demonstrably phosphorylated in cardiac myocytes in an ET-1-dependent manner will be discussed in any detail.

12.1. Regulation of c–jun expression and c–Jun activity

c–Jun binds to at least two consensus sequences frequently found in the promoter regions of genes, the AP-1 site (TGAGTCA, to which it binds preferentially, but not exclusively, as a heterodimer with the c–Fos transcription factor) and the CRE-like site (TGAGCTCA, to which it binds preferentially as a heterodimer with the ATF2 transcription factor). The transactivating activity of c–Jun is stimulated by phosphorylation of two Ser-residues (Ser-63 and Ser-73) in its N–terminal transactivation domain [100]. There may also be two further phosphorylation sites in the N–terminal part of the protein (Thr-91 and Thr-93) whose function is less clear [101], and there are also sites phosphorylated by GSK3 in the C–terminal DNA-binding domain whose dephosphorylation induced by inhibition of GSK3 increases the DNA-binding activity of c–Jun [102].

The regulation of expression of c–*jun* mRNA and c–Jun protein by ET-1 has been examined in cardiac myocytes in some detail (Fig. 4) [103]. The c–*jun* gene is normally relatively weakly expressed in myocytes, but c–*jun* mRNA and c–Jun protein are rapidly and transiently induced by ET-1, and it is possible that this is important in the development of the hypertrophic phenotype. The ERK1/2 cascade is apparently involved in both of these responses (shown by inhibitor studies), though it is probable that the ET-1-induced increase in c–Jun protein is a reflection of the ET-1-induced increase in c–*jun* mRNA. The ability of the ERK1/2 cascade to stimulate expression of c–*jun* mRNA is presumably related to the phosphorylation of transcription factors that transactivate at the c–*jun* promoter region. There are several different consensus sequences in this region (including two CRE-like sites), and thus c–Jun could potentially upregulate expression of c–*jun* mRNA. Ser-63 and Ser-73 of c–Jun can be phosphorylated by ERK1/2 (indeed, these kinases were initially thought to be responsible), but the kinases now thought to be primarily responsible are the JNKs [104]. It is thus possible that JNKs, by phosphorylating c–Jun, could upregulate c–*jun* transcription. Although ET-1 increases c–Jun phosphorylation and although inhibition of JNKs reduces this stimulation, inhibition of JNKs does not reduce stimulation of c–*jun* transcription in cardiac myocytes [103]. By implication, it would appear that stimulation of c–*jun* transcription by the ERK1/2 cascade involves transcription factors other than c–Jun, or that pathways other than modulation of transcription factor transactivating activities (e.g. mRNA stabiliza-

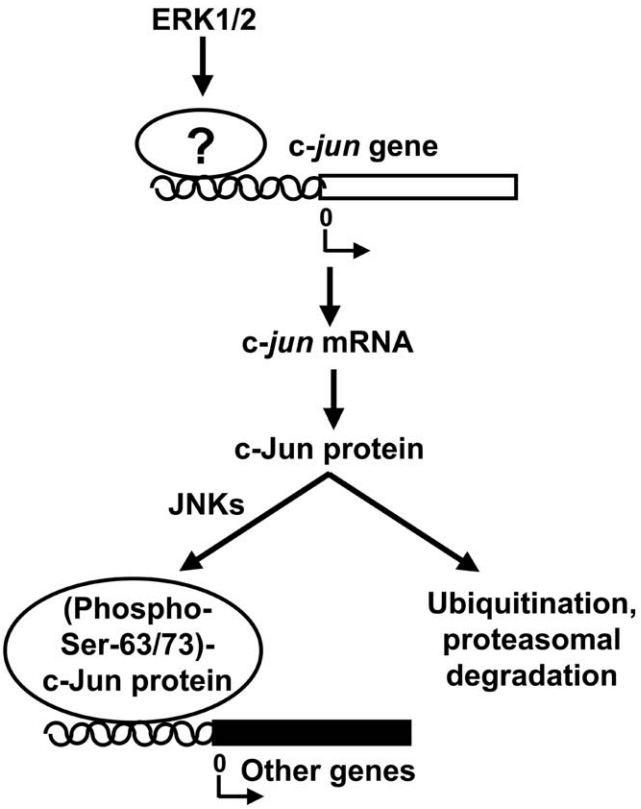

Fig. 4. A scheme for the regulation of c–*jun* mRNA and c–jun protein abundances by ET-1 in the cardiac myocyte. 0: transcriptional initiation site. ERK1/2 upregulate expression of c–*jun* mRNA presumably through phosphorylation of a transcription factor other than c–Jun. The expression of c–Jun protein is largely dependent on the increase in c–*jun* mRNA. As well as activating ERK1/2, ET-1 activates JNKs in cardiac myocytes. The JNKs phosphorylate c–Jun on two Ser-residues and this reduces the overall rate of c–Jun degradation by the proteasome, thus increasing the c–Jun protein abundance. Concurrently, phosphorylation of c–Jun increases its transactivating activity and, in combination with the increase in c–Jun abundance, increases the rate of c-Jun-dependent transcription.

tion) are involved. However, inhibition of JNKs does reduce expression of c–Jun protein and the simplest explanation of this, for which there is experimental evidence [105], is that the phosphorylation of c–Jun may increase its stability by reducing its rate of degradation [103]. The overall regulation of c–Jun abundance and transactivating activity by MAPK cascades is clearly a complex matter, but this is likely to be true for many transcription factors.

12.2. GATA-4

GATA-4 is another transcription factor that has been studied extensively in heart in relation to its regulation by ET-1 through the MAPKs (Fig. 2) [106,107]. GATA-4 is one of a six-membered family of Zn^{2+}–finger-containing transcription factors (GATA-1 to GATA-6) which recognize (A/T)GATA(A/G) consensus sequences in promoter regions. It contains two independent transactivation domains, two Zn^{2+} fingers, and a nuclear localization signal. GATA-4 binds to its consensus sequence in conjunction and cooperation with the binding of other transcription factors to their neighboring or nearby consensus sequences (e.g. NFATc4 [previously known as NFAT3], Nkx2.5, Nkx3.2, serum response factor) or in conjunction with proteins that do not bind directly to DNA (e.g. friend-of-GATA proteins) [106–108]. GATA sites are important in the regulation of the expression of genes typifying cardiac hypertrophy (e.g. atrial natriuretic factor [ANF], B–type natriuretic peptide [BNP]) [109]. Thus, the ANF promoter region contains nearby GATA-4, serum response factor and Nkx consensus sequences. These transcription factors cooperate to transactivate the ANF gene [110], with GATA-4 and serum response factor being demonstrably involved in the ET-1-stimulated transcription of this gene and in the morphological changes associated with ET-1-induced hypertrophy [111,112]. Exposure of cardiac myocytes to ET-1 also increases binding of GATA-4 to the BNP promoter [113], as does in vivo pressure overload (induced by infusion of [Arg–]vasopressin) [114]. In the case of pressure overload, increased GATA-4/BNP promoter interaction was inhibited by bosentan, implicating the ET receptor in the response [114]. However, the situation has become a little confused recently with the finding that decoy deoxyoligonucleotides for GATA-4 do not inhibit the stimulation of ANF or BNP expression by ET-1 in cardiac myocytes, even though they block the binding of GATA-4 to GATA sites [115].

Disregarding the debate about the importance of GATA-4 in ET-1 signaling, it is agreed that MAPK cascades are involved in GATA-4 activation, though a consensus view of which MAPK is involved has not yet been reached. In experiments carried out using the GqPCR-coupled α-adrenergic agonist phenylephrine, the ERK1/2 cascade was initially implicated, with phosphorylation of Ser-105 (which lies between the two transactivation domains) promoting both the transactivating activity and DNA-binding activity of GATA-4 [116]. No evidence of significant JNK or p38–MAPK involvement could be detected. However, further experimentation suggested that although p38–MAPKα and ERK1/2 promote Ser-phosphorylation of GATA-4 (the site[s] was not identified), p38–MAPKα/β activation was the more closely associated with the ET-1-stimulated binding of GATA-4 to the appropriate BNP promoter region [113]. These findings confirmed the evidence that RhoA stimulates phosphorylation of Ser-105 (and other Ser/Thr-residues) in GATA-4 in a p38-MAPK-dependent manner in cardiac myocytes [112]. Linking these findings is the finding that RhoA is rapidly activated in myocytes by ET-1 [43]. However, it is not clear whether activation of RhoA can lead to activation of p38–MAPK. Initially, it was thought that it could not [117], although, more recently, RhoA has been shown to activate p38–MAPKγ in two non-cardiomyocytic cell lines [118].

12.3. The ternary complex factors

The ternary complex factor (TCF) group of transcription factors are members of the large ETS family, and bind to ETS consensus motifs in promoter regions of genes [64]. The DNA-binding and the transactivating activity of the TCF

Elk-1 are increased by MAPK-mediated phosphorylation (Fig. 2), and by interaction with the serum response factor (another DNA-binding protein) when a serum response factor-binding site (a serum response element) is near the ETS motifs in promoter regions. The archetypal Elk-1-regulated gene is the c–*fos* immediate-early gene whose expression is rapidly but transiently stimulated when cardiac myocytes are exposed to hypertrophic agonists [119], though the BNP gene may additionally be regulated through ETS transcription factors [120]. Phenylephrine induces phosphorylation of Elk-1 in cardiac myocytes in an ERK1/2 cascade-dependent manner [121], and thus the prediction would be that ET-1 would induce a similar response. Recently, the involvement of Elk-1 in transcription of the BNP gene in response to ET-1 has been demonstrated, but in this instance its appears that p38–MAPK is the kinase responsible for Elk-1 phosphorylation and for activation of BNP transcription [120].

12.4. NF-κB

NF-κB is a transcription factor whose activation is normally associated with cell survival [122]. In unactivated cells, it is retained in the cytoplasm by binding to its protein inhibitor IκB. Phosphorylation of IκB targets it for ubiquitination and proteasomal degradation, allowing NF-κB to enter the nucleus. ET-1 stimulates IκB degradation in cardiac myocytes, activates NF-κB-dependent gene transcription, and the response may be important in myocyte hypertrophy [123,124]. Activation of NF-κB appears to involve apoptosis signal-regulating kinase 1 (ASK1) [124], a MAPK kinase kinase of SAPK cascades [78]. It has been proposed that high levels of ASK1 activation in response to cellular stresses promote apoptosis whereas more moderate levels are involved in opposition or adaptation to stresses [78]. In the cardiac myocyte, ET-1 activates ASK1 and inhibitory ASK1 constructs prevent the increases in cell surface area and NF-κB-dependent gene expression associated with exposure of myocytes to ET-1 [124].

13. ET-1 and the regulation of protein synthesis

Increased synthesis and accumulation of proteins are the essential component of the hypertrophic response. Without discussing its more intricate complexities, translation of mRNAs into proteins is a function of the activity of the rate-controlling step of protein synthesis (initiation) and of the size of the total ribosomal pool. Acute changes in protein synthesis rate are achieved by modulation of initiation, whereas changes in ribosomal pool sizes occur more slowly. ET-1 acutely stimulates cardiac myocyte protein synthesis [125], and this is probably achieved by several means (Fig. 3). As described above, ET-1 increases pH_i by as much as 0.12–0.13 units in cardiac myocytes [87,88], and the ERK1/2 cascade probably plays a substantial role in this [88]. Increases in pH_i of this magnitude stimulate protein synthesis by an unknown mechanism to the same extent as insulin [126], the most powerful stimulator of myocardial protein synthesis yet to be identified. In addition, ERK1/2 are involved in the regulation of the translational machinery. In its dephosphorylated form, eukaryotic initiation factor 4E (eIF4E)–binding protein 1 (4E–BP1) binds to eIF4E [127,128]. This prevents eIF-4E from interacting appropriately with the m7–GTP 5'-cap structures which many mRNAs contain, and from interacting with the eIF4G initiation complex scaffold protein [127,128]. There are several phosphorylation sites on 4E–BP1 and their phosphorylation reduces the ability of 4E–BP1 to bind to eIF4E and to limit initiation. Although ERK1/2 were initially considered to be responsible for phosphorylation of 4E–BP1, Akt was subsequently identified as the principal protein kinase involved. Mindful of the influences of cell specificity, these issues were recently revisited in the cardiac myocyte by Wang and Proud [69]. These workers demonstrated that phosphorylation of 4E–BP1 by ET-1 and stimulation of protein synthesis is regulated through the ERK1/2 cascade in concert with other protein kinases, such as the 70-kDa-ribosomal S6 kinase 1 and the mammalian target of rapamycin.

In addition to these acute mechanisms, the ERK1/2 cascade may be involved in the regulation of ribosomal RNA (rRNA) synthesis, rRNA species being structural components of the 60S (28S, 5.8S and 5S rRNA) and 40S (18S rRNA) ribosomal subunits. Transcription of the genes encoding the 45S rRNA precursors of 28S and 18S rRNA occurs from single promoters in the nucleolar region, is catalyzed by RNA polymerase I (polI), and involves the binding of protein factors to rDNA promoter regions which increase recruitment of polI. Overexpression of one of these protein factors, upstream-binding factor 1 (UBF1), increases rRNA expression in cardiac myocytes [129]. ET-1 also increases rDNA transcription and this is associated with hyperphosphorylation of UBF1 [130]. The ERK1/2–dependent enhancement of rDNA transcription by growth factors in non-cardiomyocytic cells may be mediated by UBF1 phosphorylation [131] and, by extrapolation, a similar pathway may operate in the case of ET-1-stimulated rDNA transcription in cardiac myocytes.

14. ET-1 and the regulation of other protein kinases

14.1. Protein tyrosine kinases

Transactivation of receptor protein tyrosine kinases (PTKs) has been mentioned above and will not be discussed further. Two families of non-receptor PTKs present in cardiac myocytes are the Src family [132], and the family consisting of focal adhesion kinase (FAK) [133] and the FAK-related Pro-rich Tyr-kinase 2 (PYK2) [134]. c–Src itself is a myristoylated membrane-bound protein and activation of members of the Src family leads activation of the ERK1/2 cascade, possibly by inducing Tyr-phosphorylation

of c–Raf (or A–Raf, but not B–Raf) complexed at the membrane to Ras.GTP [57,135]. Transfection of oncogenic (constitutively activated) v–*src* induces at least some of the characteristics of hypertrophy in cardiac myocytes [136,137], and c–Src is activated by ET-1 in these cells [137]. How this is achieved is unclear, but it must presumably involve dephosphorylation of the inhibitory phosphorylation site at c–Src(phospho-Tyr^{527}), and autophosphosphorylation of activating c–Src(Tyr^{416})–residue.

When cultured cells are plated on a suitable extracellular matrix, engagement of integrins with their extracellular ligands and/or integrin clustering stimulates the formation of 'focal adhesions' or 'focal complexes' [138]. Overall, the formation of focal adhesions leads to the activation of intracellular signaling pathways, including the ERK1/2 cascade [139]. There is still discussion about whether these entities exist in vivo or whether they are simply induced under the rather artificial conditions of cell culture. Focal adhesions consist of numerous proteins including FAK, and PYK2, as well as c–Src. PYK2 (which is also known by a variety of synonyms [RAFTK, CAKβ, CADTK]) was first identified as a Ca^{2+}–dependent Tyr-kinase present primarily in neuronal tissues which was activated by agonists, such as the GqPCR agonist, bradykinin or PMA, and potentially could be involved in the coupling of GqPCR signaling to the ERK cascade [134]. Other focal adhesion-associated proteins include docking and adapter proteins, which are also involved in the transmission of signaling events. The docking protein p130Cas binds directly to Pro-rich regions in FAK and PYK2, and becomes Tyr-phosphorylated on activation of FAK or PYK2 [140]. Adapter/scaffolding proteins (such as paxillin, itself a non-receptor PTK substrate) are also present [141]. The association of docking and adapter molecules with FAK and PYK2 and their phosphorylation provide a focus for the binding of further signaling molecules.

ET-1 promotes Tyr-phosphorylation of FAK and paxillin in cardiac myocytes [142], and disruption of focal adhesion signaling by interfering with FAK or p130Cas function attenuates the induction of hypertrophy by ET-1 [142,143]. Although relatively little work has been carried out, PYK2 has been detected in the cardiac myocyte, its expression in the neonatal rat myocyte is increased by increasing the cell density (implying that it might be induced by increased cell–cell contact), and exposing myocytes to ET-1 increases the extent of Tyr-phosphorylation of PYK2 [144]. Overall, focal adhesion-associated pathways involving FAK, PYK2, and p130Cas may be important in ET-1-dependent signaling in the cardiac myocyte.

14.2. MAPK-dependent regulation

In addition to phosphorylating transcription factors directly in an ET-1-dependent manner, MAPKs also phosphorylate and activate a number of protein kinases in cardiac myocytes. These protein kinases can also phosphorylate transcription factors and/or phosphorylate other proteins (Fig. 2). The p90RSKs [62] have already been mentioned as substrates of ERK1/2 which are activated by ET-1 in cardiac myocytes [145], and their role in the regulation of NHE1 has been described. p90RSKs are also involved in the phosphorylation of the pro-apoptotic Bcl-2 family protein, Bad, by phenylephrine in cardiac myocytes [68], thus potentially reducing its pro-apoptotic activity. The p90RSK-related protein kinase, mitogen– and stress-activated protein kinase-1 (MSK1) [62] is involved in the activation of the transcription factor CREB, which it phosphorylates on Ser-133 [146], a phosphorylation which can also be effected by the p90RSKs [62]. ET-1 stimulates phosphorylation of MSK1 in cardiac myocytes probably leading to an increase in its activity [147]. (Phenylephrine induces phosphorylation and activation of MSK1. ET-1 also causes the phosphorylation of MSK1 in a manner analogous to phenylephrine, but its effects on activity were not examined [147].) Characteristically, the phosphorylation of MSK1 was essentially completely prevented by inhibition of either the ERK1/2 or the p38–MAPKα/β cascades [146,147]. Two more protein kinases are phosphorylated and activated the related MAPKAPK2 and MAPKAPK3. These are activated by p38–MAPKs [148,149], and activation of p38–MAPKs and MAPKAPK2/3 by ET-1 is readily detectable in cardiac myocytes [76]. MAPKAPK2/3 phosphorylates the Hsp25/27 small heat shock proteins (Hsps, ~25 kDa in rat and mouse, ~27 kDa in human cells) promoting the disaggregation of multimers of the small Hsps [150,151]. Phosphorylation and disaggregation of these multimers has been detected in cardiac myocytes in response to oxidative stress [152]. ET-1 also induces phosphorylation of Hsp25 in cardiac myocytes [76], though the predicted disaggregation of the Hsp25 multimers has not been examined. In non-muscle cells, phosphorylation and disaggregation of small Hsps is thought to stabilize the actin cytoskeleton in response to stress stimuli [151], and, in the cardiac myocyte, small Hsps become phosphorylated and associated with sarcomeric structures following heat stress [153]. However, the role of Hsp25/27 phosphorylation in cardiac myocyte cytoprotection is still controversial given data which suggests that it is relatively unimportant [154].

15. Concluding remarks

ET isopeptides are clearly able to affect the contractile properties and growth of the cardiac myocyte. The signaling pathways activated by these peptides are relatively well characterized and involve modulation of protein kinase activities and modulation of ion movements. The interdependency between protein phosphorylation, ion movements, contractile activity and growth in the cardiac myocyte has been emphasized (Fig. 3). The gross consequences of the activation of the various signaling pathways are understood, though the intricacies of the mechanisms involved have yet to be fully elucidated.

References

[1] Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5.

[2] Kedzierski RM, Yanagisawa M. Endothelin system: the double-edged sword in health and disease. Annu Rev Pharmacol Toxicol 2001;41: 851–76.

[3] Giannessi D, Del Ry S, Vitale RL. The role of endothelins and their receptors in heart failure. Pharmacol Res 2001;43:111–26.

[4] Greenberg BH. Endothelin and endothelin receptor antagonists in heart failure. Congest Heart Fail 2002;8:257–61.

[5] Hurlimann D, Enseleit F, Noll G, Luscher TF, Ruschitzka F. Endothelin antagonists and heart failure. Curr Hypertens Rep 2002;4:85–92.

[6] Remuzzi G, Perico N, Begnini A. New therapeutics that antagonize endothelin: promises and frustrations. Nat Rev Drug Discov 2002;1: 986–1001.

[7] Endoh M, Fujita S, Yang HT, Talukder MA, Maruya J, Norota I. Endothelin: receptor subtypes, signal transduction, regulation of Ca^{2+} transients, and contractility in rabbit ventricular myocardium. Life Sci 1998;62:1485–9.

[8] Shubeita HE, McDonough PM, Harris AN, Knowlton KU, Glembotski CC, Brown JH, et al. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. A paracrine mechanism for myocardial cell hypertrophy. J Biol Chem 1990;265:20555–62.

[9] Ito H, Hiroe M, Hirata Y, Fujisaki H, Adachi S, Akimoto H, et al. Endothelin ET_A receptor antagonist blocks cardiac hypertrophy provoked by hemodynamic overload. Circulation 1994;89:2198–203.

[10] Mylona P, Cleland JG. Update of REACH-1 and MERIT-HF clinical trials in heart failure. Cardionet.net Editorial Team. Eur J Heart Fail 1999;1:197–200.

[11] Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol 2002;3:639–50.

[12] Davenport AP. International Union of Pharmacology. XXIX. Update on endothelin receptor nomenclature. Pharmacol Rev 2000;54:219–26.

[13] Hilal-Dandan R, Ramirez MT, Villegas S, Gonzalez A, Endo-Mochizuki Y, Brown JH, et al. Endothelin ET_A receptor regulates signaling and ANF gene expression via multiple G protein-linked pathways. Am J Physiol 1997;272:H130–7.

[14] Hilal-Dandan R, Merck DT, Lujan JP, Brunton LL. Coupling of the type A endothelin receptor to multiple responses in adult rat cardiac myocytes. Mol Pharmacol 1994;45:1183–90.

[15] Jones LG. Inhibition of cyclic AMP accumulation by endothelin is pertussis toxin sensitive and calcium independent in isolated adult feline myocytes. Life Sci 1996;58:115–23.

[16] Chen CA, Manning DR. Regulation of G proteins by covalent modification. Oncogene 2001;20:1643–52.

[17] Higgins JB, Casey PJ. The role of prenylation in G–protein assembly and function. Cell Signal 1996;8:433–7.

[18] Rhee SG. Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem 2001;70:281–312.

[19] Fukami K. Structure, regulation, and function of phospholipase C enzymes. J Biochem (Tokyo) 2002;131:293–9.

[20] Schnabel P, Mies F, Nohr T, Geisler M, Böhm M. Differential regulation of phospholipase C–β isozymes in cardiomyocyte hypertrophy. Biochem Biophys Res Commun 2000;275:1–6.

[21] Hansen CA, Schoering AG, Robishaw JD. Subunit expression of signal transducing G proteins in cardiac tissue: implications for phospholipase C–β regulation. J Mol Cell Cardiol 1995;27:471–84.

[22] Jalili T, Takeishi Y, Song G, Ball NA, Howles G, Walsh RA. PKC translocation without changes in $G\alpha_q$ and PLC-β protein abundance in cardiac hypertrophy and failure. Am J Physiol Heart Circ Physiol 1999;277:H2298–304.

[23] Clerk A, Sugden PH. Regulation of phospholipases C and D in rat ventricular myocytes. Stimulation by endothelin-1, bradykinin and phenylephrine. J Mol Cell Cardiol 1997;29:1593–604.

[24] Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 2000;69:795–827.

[25] Newton AC. Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem Rev 2001;101:2353–64.

[26] Clerk A, Bogoyevitch MA, Fuller SJ, Lazou A, Parker PJ, Sugden PH. Expression of protein kinase C isoforms during cardiac ventricular development. Am J Physiol 1995;269:H1087–97.

[27] Rohde S, Sabri A, Kamasamudran R, Steinberg SF. The α_1–adrenoceptor subtype– and protein kinase C isoform-dependence of norepinephrine's actions in cardiomyocytes. J Mol Cell Cardiol 2000;32: 1193–209.

[28] Clerk A, Bogoyevitch MA, Andersson MB, Sugden PH. Differential activation of protein kinase C isoforms by endothelin-1 and phenylephrine, and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. J Biol Chem 1994;269:32848–57.

[29] Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, Walsh RA. Transgenic overexpression of constitutively active protein kinase Cϵ causes concentric cardiac hypertrophy. Circ Res 2000;86:1218–23.

[30] Pass JM, Zheng YT, Wead WB, Zhang J, Li RCX, Bolli R, et al. PKCϵ expression induces dichotomous cardiac phenotypes and modulates PKCϵ–RACK interactions and RACK expression. Am J Physiol Heart Circ Physiol 2001;280:946–55.

[31] Braz JC, Bueno OF, De Windt LJ, Molkentin JD. PKCα regulates the hypertrophis growth of cardiomyocytes through extracellular signal-regulated kinase 1/2 (ERK1/2). J Cell Biol 2002;156:905–19.

[32] Kerkelä R, Ilves M, Pikkarainen S, Tokola H, Ronkainen J, Vuolteenaho O, et al. Identification of PKCα isoform-specific effects in cardiac myocytes using antisense phosphorothioate oligonucleotides. Mol Pharmacol 2002;62:1482–91.

[33] Exton JH. Regulation of phospholipase D. FEBS Lett 2002;531:58–61.

[34] Powner DJ, Wakelam MJ. The regulation of phospholipase D by phospholipids and small GTPases. FEBS Lett 2002;531:62–4.

[35] Hodgkin MN, Pettitt TR, Martin A, Michell RH, Pemberton AJ, Wakelam MJO. Diacylglycerols and phosphatidates: which molecular species are second messengers? Trend Biochem Sci 1998;23:200–4.

[36] Andresen BT, Rizzo MA, Shome K, Romero G. The role of phosphatidic acid in the regulation of the Ras/MEK/Erk signaling cascade. FEBS Lett 2002;531:65–8.

[37] Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. Physiol Rev 2001;81:153–208.

[38] Quilliam LA, Rebhun JF, Castro AF. A growing family of guanine nucleotide exchange factors is responsible for activation of Ras-family GTPases. Prog Nucleic Acid Res Mol Biol 2002;71:391–444.

[39] Vojtek AB, Der CJ. Increasing complexity of the Ras signaling pathway. J Biol Chem 1998;273:19925–8.

[40] Chiloeches A, Paterson HF, Marais RM, Clerk A, Marshall CJ, Sugden PH. Regulation of Ras.GTP loading and Ras-Raf association in neonatal rat ventricular myocytes by G protein-coupled receptor agonists and phorbol esters. Activation of the ERK cascade by phorbol esters is mediated by Ras. J Biol Chem 1999;274:19762–70.

[41] Prior IA, Hancock JF. Compartmentalization of Ras proteins. J Cell Sci 2001;114:1603–8.

[42] Yan J, Roy S, Apolloni A, Lane A, Hancock JF. Ras isoforms vary in their ability to activate Raf-1 and phosphoinositide 3–kinase. J Biol Chem 1998;273:24052–6.

[43] Clerk A, Pham FH, Fuller SJ, Sahai E, Aktories K, Marais RM, et al. Regulation of mitogen-activated protein kinases in cardiac myocytes through the small G protein, Rac1. Mol Cell Biol 2001;21:1173–84.

[44] Clerk A, Sugden PH. Small guanine nucleotide-binding proteins and myocardial hypertrophy. Circ Res 2000;86:1019–23.

[45] Brose N, Rosenmund C. Move over protein kinase C, you've got company: alternative effectors of diacylglycerol and phorbol esters. J Cell Sci 2002;115:4399–411.
[46] Kazanietz MG. Novel 'nonkinase' phorbol ester receptors: the C1 domain connection. Mol Pharmacol 2002;61:759–67.
[47] Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103:211–25.
[48] Saito Y, Berk BC. Transactivation: a novel signaling pathway from angiotensin II to tyrosine kinase receptors. J Mol Cell Cardiol 2001; 33:3–7.
[49] Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, et al. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. Nat Med 2002;8:35–40.
[50] Kodama H, Fukuda K, Takahashi T, Sano M, Kato T, Tahara S, et al. Role of EGF receptor and Pyk2 in endothelin-1-induced ERK activation in rat cardiomyocytes. J Mol Cell Cardiol 2002;34:139–50.
[51] Thomas WG, Brandenburger Y, Autelitano DJ, Pham T, Qian H, Hannan RD. Adenoviral-directed expression of the type 1A angiotensin receptor promotes cardiac hypertrophy via transactivation of the epidermal growth factor receptor. Circ Res 2002;90:135–42.
[52] Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, et al. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature 1999;402:884–8.
[53] Murasawa S, Mori Y, Nozawa Y, Gotoh N, Shibuya M, Masaki H, et al. Angiotensin II type 1 receptor-induced extracellular signal-regulated protein kinase activation is mediated by Ca^{2+}/calmodulin-dependent transactivation of epidermal growth factor receptor. Circ Res 1998;82: 1338–48.
[54] Kawanabe Y, Hashimoto N, Masaki T. Characterization of Ca^{2+} channels involved in ET-1 induced transactivation of EGF receptors. Am J Physiol Heart Circ Physiol 2002;283:H2671–5.
[55] Heffetz D, Bushkin I, Dror R, Zick Y. The insulinomimetic agents H_2O_2 and vanadate stimulate protein tyrosine phosphorylation in intact cells. J Biol Chem 1990;265:2896–902.
[56] Dhillon AS, Kolch W. Untying the regulation of the Raf-1 kinase. Arch Biochem Biophys 2002;404:3–9.
[57] Marais R, Light Y, Paterson HF, Mason CS, Marshall CJ. Differential regulation of Raf-1, A–Raf, and B–Raf by oncogenic Ras and tyrosine kinases. J Biol Chem 1997;272:4378–83.
[58] Sugden PH. Signalling pathways in cardiac myocyte hypertrophy. Ann Med 2001;33:611–22.
[59] Bogoyevitch MA, Glennon PE, Sugden PH. Endothelin-1, phorbol esters and phenylephrine stimulate MAP kinase activities in ventricular cardiomyocytes. FEBS Lett 1993;317:271–5.
[60] Bogoyevitch MA, Glennon PE, Andersson MB, Clerk A, Lazou A, Marshall CJ, et al. Endothelin-1 and fibroblast growth factors stimulate the mitogen-activated protein kinase signaling cascade in cardiac myocytes. The potential role of the cascade in the integration of two signaling pathways leading to myocyte hypertrophy. J Biol Chem 1994;269:1110–9.
[61] Bogoyevitch MA, Marshall CJ, Sugden PH. Hypertrophic agonists stimulate the activities of the protein kinases c–Raf and A–Raf in cultured ventricular myocytes. J Biol Chem 1995;270:26303–10.
[62] Frödin M, Gammeltoft S. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol Cell Endocrin 1999;151:65–77.
[63] Gijon MA, Leslie CC. Regulation of arachidonic acid release and cytosolic phospholipase A_2 activation. J Leukoc Biol 1999;65:330–6.
[64] Sharrocks AD. The ETS-domain transcription factor family. Nat Rev Mol Cell Biol 2001;2:827–37.
[65] Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3–kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 2001;17:615–75.
[66] Leslie NR, Biondi RM, Alessi DR. Phosphoinositide-regulated kinases and phosphoinositide phosphatases. Chem Rev 2001;101: 2365–80.
[67] Pham FH, Sugden PH, Clerk A. Regulation of protein kinase B and 4E–BP1 by oxidative stress in cardiac myocytes. Circ Res 2000;86: 1252–8.
[68] Valks DM, Cook SA, Pham FH, Morrison PR, Clerk A, Sugden PH. Phenylephrine promotes phosphorylation of Bad in cardiac myocytes through the extracellular signal-regulated kinases 1/2 and protein kinase A. J Mol Cell Cardiol 2002;34:749–63.
[69] Wang L, Proud CG. Ras/Erk signaling is essential for activation of protein synthesis by Gq protein-coupled receptor agonists in adult cardiomyocytes. Circ Res 2002;91:821–9.
[70] Cohen P, Frame S. The renaissance of GSK3. Nat Rev Mol Cell Biol 2001;2:769–76.
[71] Haq S, Choukroun G, Kang ZB, Ranu H, Matsui T, Rosenzweig A, et al. Glycogen synthase kinase-3β is a negative regulator of cardiomyocyte hypertrophy. J Cell Biol 2000;151:117–29.
[72] Fuller SJ, Finn SG, Downward J, Sugden PH. Stimulation of gene expression in neonatal rat ventricular myocytes by Ras is mediated by Ral.GDS and phosphatidylinositol 3–kinase in addition to Raf. Biochem J 1998;335:241–6.
[73] Lopez I, Mak EC, Ding J, Hamm HE, Lomasney JW. A novel bifunctional phospholipase C that is regulated by $G\alpha_{12}$ and stimulates the Ras/mitogen-activated protein kinase pathway. J Biol Chem 2001; 276:2758–65.
[74] Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 2001;81:807–69.
[75] Bogoyevitch MA, Ketterman AJ, Sugden PH. Cellular stresses activate c–Jun N–terminal protein kinases (JNKs) in ventricular myocytes cultured from neonatal rat hearts. J Biol Chem 1995;270:29710–7.
[76] 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;142:523–35.
[77] Heidkamp MC, Bayer AL, Martin JL, Samarel AM. Differential activation of mitogen-activated protein kinase cascades and apoptosis by protein kinase C–epsilon and delta in neonatal rat ventricular myocytes. Circ Res 2001;89:882–90.
[78] Takeda K, Matsuzawa A, Nishitoh H, Icijo H. Roles of MAPKKK ASK1 in stress-induced cell death. Cell Struct Funct 2003;28:23–9.
[79] Sugden PH, Clerk A. 'Stress-responsive' mitogen-activated protein kinases in the myocardium. Circ Res 1998;83:345–52.
[80] Bishopric NH, Andreka P, Slepak T, Webster KA. Molecular mechanisms of apoptosis in the cardiac myocyte. Curr Opin Pharmacol 2001;1:141–50.
[81] Takanashi M, Endoh M. Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. Am J Physiol 1991;261:H611–9.
[82] Ramirez MT, Zhao XL, Schulman H, Brown JH. The nuclear δB isoform of Ca^{2+}/calmodulin-dependent protein kinase II regulates atrial natriuretic factor gene expression in ventricular myocytes. J Biol Chem 1997;272:31203–8.
[83] Molkentin JD, Lu J-R, Antos C, Markham B, Richardson J, Robbins J, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 1998;93:215–28.
[84] Aoki H, Sadoshima J, Izumo S. Myosin light chain kinase mediates sarcomere organization during cardiac hypertrophy in vitro. Nat Med 2000;6:183–8.
[85] Zhang T, Johnson EN, Gu Y, Morissette MR, Sah VP, Gigena NS, et al. The cardiac-specific nuclear δ_B isoform of Ca^{2+}/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. J Biol Chem 2002;277:1261–7.

[86] Avkiran M, Haworth RS. Regulatory effects of G protein-coupled receptors on cardiac sarcolemmal Na^+/H^+ exchanger activity: signalling and significance. Cardiovasc Res 2003;57:942–52.

[87] Krämer BK, Smith TW, Kelly RA. Endothelin and increased contractility in adult rat ventricular myocytes: role of intracellular alkalosis induced by activation of the protein kinase C–dependent Na^+–H^+ exchanger. Circ Res 1991;68:269–79.

[88] Moor AN, Fliegel L. Protein kinase-mediated regulation of the Na^+/H^+ exchanger in the rat myocardium by mitogen-activated protein kinase-dependent pathways. J Biol Chem 1999;274:22985–92.

[89] Singer-Lahat D, Gershon E, Lotan I, Hullin R, Biel M, Flockerzi V, et al. Modulation of cardiac Ca^{2+} channels in *Xenopus* oocytes by protein kinase C. FEBS Lett 1992;306:113–8.

[90] Talukder MA, Endoh M. Pharmacological differentiation of synergistic contribution of L–type Ca^{2+} channels and Na^+/H^+ exchange to the positive inotropic effects of phenylephrine, endothelin-3 and angiotensin II in rabbit ventricular myocardium. Naunyn Schmiedeberg's Arch Pharmacol 1997;355:87–96.

[91] Yang HT, Sakurai K, Sugawara H, Watanabe T, Norota I, Endoh M. Role of Na^+/Ca^{2+} exchange in endothelin-1 induced increases in Ca^{2+} transient and contractility in rabbit ventricular myocytes: pharmacological analysis with KB-R7943. Br J Pharmacol 1999;126:1785–95.

[92] Wang H, Sakurai K, Endoh M. Pharmacological analysis by HOE642 and KB-R9032 of the role of Na^+/H^+ exchange in the endothelin-1-induced Ca^{2+} signalling in rabbit ventricular myocytes. Br J Pharmacol 2000;131:638–44.

[93] Sweeney HL, Bowman BF, Stull JT. Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. Am J Physiol 1993;264:C1085–95.

[94] Andersen GØ, Qvigstad E, Schiander I, Aass H, Osnes JB, Skomedal T. α_1–AR-induced positive inotropic response in heart is dependent on myosin light chain phosphorylation. Am J Physiol Heart Circ Physiol 2002;283:H1471–80.

[95] Pi Y, Kemnitz KR, Zhang D, Kranias EG, Walker JW. Phosphorylation of troponin I controls cardiac twitch dynamics: evidence from phosphorylation site mutants expressed on a troponin I–null background in mice. Circ Res 2002;90:649–56.

[96] Putney LK, Denker SP, Barber DL. The changing face of the Na^+/H^+ exchanger, NHE1: structure, regulation and cellular actions. Annu Rev Pharmacol Toxicol 2002;42:527–52.

[97] Takahashi E, Abe J, Gallis B, Aebersold R, Spring DJ, Krebs EG, et al. $p90^{RSK}$ is a serum-stimulated Na^+/H^+ exchanger isoform kinase. Regulatory phosphorylation of serine 703 of Na^+/H^+ exchanger isoform-1. J Biol Chem 1999;274:20206–14.

[98] Khaled AR, Moor AN, Li A, Kim K, Ferris DK, Muegge K, et al. Trophic factor withdrawal: p38 mitogen-activated protein kinase activates NHE1, which induce intracellular alkalinization. Mol Cell Biol 2001;21:7545–57.

[99] Baetz D, Haworth RS, Avkiran M, Feuvray D. The ERK pathway regulates Na^+– HCO_{3-} cotransport activity in adult rat cardiomyocytes. Am J Physiol Heart Circ Physiol 2002;283:H2102–9.

[100] Smeal T, Binetruy B, Mercola D, Grover-Bardwick A, Heidecker G, Rapp UR, et al. Oncoprotein-mediated signalling cascade stimulates c–Jun activity by phosphorylation of serines 63 and 73. Mol Cell Biol 1992;12:3507–13.

[101] Papavassiliou AG, Treier M, Bohmann D. Intramolecular signal transduction in c–Jun. EMBO J 1995;14:2014–9.

[102] Boyle WJ, Smeal T, Defize LHK, Angel P, Woodgett JR, Karin M, et al. Activation of protein kinase C decreases phosphorylation of c–jun at sites that negatively regulate its DNA binding activity. Cell 1991;64:573–84.

[103] Clerk A, Kemp TJ, Harrison JG, Mullen AJ, Barton PJ, Sugden PH. Upregulation of c–jun mRNA in cardiac myocytes requires the extracellular signal-regulated kinase cascade, but c–Jun N–terminal kinases are required for efficient upregulation of c–Jun protein. Biochem J 2002;368:101–10.

[104] Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Genet Dev 2002;12:14–21.

[105] Musti AM, Treier M, Bohmann D. Reduced ubiquitin-dependent degradation of c–Jun after phosphorylation by MAP kinases. Science 1997;275:400–2.

[106] Molkentin JD. The zinc finger-containing transcription factors GATA-4, –5, and –6. Ubiquitously expressed regulators of tissue-specific gene expression. J Biol Chem 2000;275:38949–52.

[107] Patient RK, McGhee JD. The GATA family (vertebrates and invertebrates). Curr Opin Genet Dev 2002;12:416–22.

[108] Charron F, Nemer M. GATA transcription factors and cardiac development. Semin Cell Dev Biol 1999;10:85–91.

[109] McBride K, Nemer M. Regulation of the ANF and BNP promoters by GATA factors: lessons learned for cardiac transcription. Can J Physiol Pharmacol 2001;79:673–81.

[110] Nishida W, Nakamura M, Mori S, Takahashi M, Ohkawa Y, Tadokoro S, et al. A triad of serum response factor and the GATA and NK families governs the transcription of smooth and cardiac muscle genes. J Biol Chem 2002;277:7308–17.

[111] Morin S, Paradis P, Aries A, Nemer M. Serum response factor–GATA ternary complex formation are required for nuclear signaling by a G-protein-coupled receptor. Mol Cell Biol 2001;21:1036–44.

[112] Charron F, Tsimiklis G, Arcand M, Robitaille L, Liang Q, Molkentin JD, et al. Tissue-specific GATA factors are transcriptional effectors of the small GTPase RhoA. Gene Dev 2001;15:2702–19.

[113] Kerkelä R, Pikkarainen S, Majalahti-Palviainen T, Tokola H, Ruskoaho H. Distinct roles of mitogen-activated protein kinases pathways in GATA-4 transcription factor-mediated regulation of B–type natriuretic peptide gene. J Biol Chem 2002;277:13752–60.

[114] Hautala N, Tokola H, Luodonpää M, Puhakka J, Romppanen H, Vuolteenaho O, et al. Pressure overload increases GATA4 binding activity by endothelin-1. Circulation 2001;103:703–35.

[115] Pikkarainen S, Kerkelä R, Pöntinen J, Majalahti-Palvianen T, Tokola H, Eskelinen S, et al. Decoy oligonucleotide characterization of GATA-4 transcription factor in hypertrophic agonist induced responses of cardiac myocytes. J Mol Med 2002;80:51–60.

[116] Liang Q, Wiese RJ, Bueno OF, Dai YS, Markham BE, Molkentin JD. The transcription factor GATA4 is activated by extracellular signal-regulated kinase 1– and 2–mediated phosphorylation of serine 105 in cardiomyocytes. Mol Cell Biol 2001;21:7460–9.

[117] Minden A, Lin A, Claret F-X, Abo A, Karin M. Selective activation of the JNK signaling cascade and c–Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell 1995;81:1147–57.

[118] Marinissen MJ, Chiariello M, Gutkind JS. Regulation of gene expression by the small GTPase Rho through the ERK6 (p38γ) MAP kinase pathway. Gene Dev 2001;15:535–53.

[119] Chien KR, Knowlton KU, Zhu H, Chien S. Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. FASEB J 1991;5:3037–46.

[120] Pikkarainen S, Tokola H, Kerkelä R, Majalahti-Palviainen T, Vuolteenaho O, Ruskoaho H. Endothelin-1-specific activation of B–type natriuretic factor gene via p38 mitogen-activated protein kinase and nuclear ETS factors. J Biol Chem 2003;278:3969–75.

[121] Babu GJ, Lalli MJ, Sussman MA, Sadoshima J, Periasamy M. Phosphorylation of elk-1 by MEK/ERK pathway is necessary for c–fos gene activation during cardiac myocyte hypertrophy. J Mol Cell Cardiol 2000;32:1447–57.

[122] Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol 2000;18:621–63.

[123] Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, Lin A. Activation of NF-κB is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. Proc Natl Acad Sci USA 2001;98:6668–73.

[124] Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, et al. Involvement of nuclear factor-κB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced hypertrophy. Circulation 2002;105:509–15.
[125] Sugden PH, Fuller SJ, Mynett JR, Hatchett RJ, Bogoyevitch MA, Sugden MC. Stimulation of adult rat ventricular myocyte protein synthesis and phosphoinositide hydrolysis by the endothelins. Biochim Biophys Acta 1993;1175:327–32.
[126] Fuller SJ, Gaitanaki CJ, Sugden PH. Effects of increasing extracellular pH on protein synthesis and protein degradation in the perfused working rat heart. Biochem J 1989;259:173–9.
[127] Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu Rev Biochem 1999;68:913–63.
[128] Shah OJ, Anthony JC, Kimball SR, Jefferson LS. 4E–BP1 and S6K1: translational integration sites for nutritional and hormonal information. Am J Physiol Endocrinol Metab 2000;279:E715–29.
[129] Hannan RD, Stefanovsky V, Taylor L, Moss T, Rothblum LI. Overexpression of the transcription factor UBF1 is sufficient to increase ribosomal DNA transcription in noenatal cardiomyocytes: implications for cardiac hypertrophy. Proc Natl Acad Sci USA 1996;93: 8750–5.
[130] Luyken J, Hannan RD, Cheung JY, Rothblum LI. Regulation of rDNA transcription during endothelin-1-induced hypertrophy of neonatal cardiomyocytes. Hyperphosphorylationof upstream binding factor, an rDNA transcription factor. Circ Res 1996;78:354–61.
[131] Stefanovsky VY, Pelletier G, Hannan R, Gagnon-Kugler T, Rothblum LI, Moss T. An immediate response of ribosomal transcription to growth factor stimulation in mammals is mediated by ERK phosphorylation of UBF. Mol Cell 2001;8:1063–73.
[132] Martin GS. The hunting of the Src. Nat Rev Mol Cell Biol 2001;2: 467–75.
[133] Schaller MD. Biochemical signals and biological responses elicited by the focal adhesion kinase. Biochim Biophys Acta 2001;1540:1–21.
[134] Avraham H, Park S-Y, Schinkmann K, Avraham S. RAFTK/Pyk2–mediated cellular signalling. Cell Signal 2000;12:123–33.
[135] Mason CS, Springer CJ, Cooper RG, Superti-Furga G, Marshall CJ, Marais R. Serine and tyrosine phosphorylations cooperate in raf-1, but not B–Raf activation. EMBO J 1999;18:2137–48.
[136] Fuller SJ, Gillespie-Brown J, Sugden PH. Oncogenic *raf*, *src*, and *ras* stimulate a hypertrophic pattern of gene expression and increase cell size in neonatal rat ventricular myocytes. J Biol Chem 1998;273: 18146–52.
[137] Kovacic B, Ilic D, Damsky CH, Gardner DG. c–Src plays a role in endothelin-dependent hypertrophy of the cardiac myocyte. J Biol Chem 1998;273:35185–93.
[138] Juliano RL. Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol 2002;42:283–323.
[139] Howe AK, Aplin AE, Juliano RL. Anchorage-dependent ERK signaling—mechanisms and consequences. Curr Opin Genet Dev 2002;12:30–5.
[140] O'Neill GM, Fashena MJ, Golemis EA. Integrin signalling: a new cas(t) enters the stage. Trend Cell Biol 2000;10:111–9.
[141] Schaller MD. Paxillin: a focal adhesion-associated adaptor protein. Oncogene 2001;20:6459–72.
[142] Eble DM, Strait JB, Govidarajan G, Lou J, Byron KL, Samarel AM. Endothelin-induced cardiac myocyte hypertrophy: role for focal adhesion kinase. Am J Physiol Heart Circ Physiol 2000;278:H1695–707.
[143] Kovacic-Milivojevic B, Roediger F, Almeida EA, Damsky CH, Gardner DG, Ilic D. Focal adhesion kinase and p130Cas mediate both sarcomeric organization and activation of genes associated with cardiac myocyte hypertrophy. Mol Biol Cell 2001;12:2290–307.
[144] Bayer AL, Ferguson AG, Lucchesi PA, Samarel AM. PYK2 expression and phosphorylation in neonatal and adult cardiomyocytes. J Mol Cell Cardiol 2001;33:1017–30.
[145] Sadoshima J, Qiu Z, Morgan JP, Izumo S. Angiotensin II and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosine kinase, mitogen-activated protein kinase, and 90–kD S6 kinase in cardiac myocytes. The critical role of Ca^{2+}–dependent signaling. Circ Res 1995;76:1–15.
[146] 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: 4426–41.
[147] Markou T, Lazou A. Phosphorylation and activation of mitogen– and stress-activated protein kinase-1 in adult rat cardiac myocytes by G-protein-coupled receptor agonists requires both extracellular-signal-regulated kinase and p38 mitogen-activated protein kinase. Biochem J 2002;365:757–63.
[148] Rouse J, Cohen P, Trigon S, Morange M, Alonso-Llamazares A, Zamanillo D, 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;78:1027–37.
[149] McLaughlin MM, Kumar S, McDonnell PC, Van Horn S, Lee JC, Livi GP, et al. Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. J Biol Chem 1996;271:8488–92.
[150] Clifton AD, Young PR, Cohen P. A comparison of the substrate specificity of MAPKAP kiase-2 and MAPKAP kinase-3 and their activation by cytokines and cellular stress. FEBS Lett 1996;392:209–14.
[151] Dalle-Donne I, Rossi R, Milzani A, Di Simplicio P, Colombo R. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. Free Radic Biol Med 2001;31:1624–32.
[152] 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;333:581–9.
[153] van der Klundert FA, Gijsen ML, ven den Ijssel PR, Snoekx LH, de Jong WW. α_B–Crystallin and hsp25 in neonatal cardiac cells—differences in cellular localization under stress conditions. Eur J Cell Biol 1998;75:38–45.
[154] Martin JL, Hickey E, Weber LA, Dillmann WH, Mestril R. Influence of phosphorylation and oligomerization on the protective role of the small heat shock protein 27 in rat adult cardiomyocytes. Gene Expr 1999;7:349–55.