Gene Expression, Vol. 10, pp. 153–164
Printed in the USA.

1052-2166/02 $20.00 + .00

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REVIEW

Role of Mitogen-Activated Protein Kinases and Protein Kinase C in Regulating Low-Density Lipoprotein Receptor Expression

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The cell signaling pathways that culminate in induction of low-density lipoprotein (LDL) receptor transcription in response to a variety of extracellular and intracellular signals are beginning to be defined. Evidence is accumulating that LDL receptor transcription is under complex regulation and that a major pathway of induction by cytokines, growth factors, anisomycin, and phorbol esters involves the extracellular/mitogen-activated protein kinase ($p42/44^{MAPK}$) cascade. In fact, degree of $p42/44^{MAPK}$ activation determines the extent of LDL receptor induction. The suppression of LDL receptor expression by stress-activated $p38^{MAPK}$ via $p42/44^{MAPK}$ provides a potential mechanism for stress-induced hypercholesterolemia observed in humans and animals. Moreover, endogenous signals such as cholesterol regulate LDL receptor transcription through a different signaling cascade involving protein kinase Cε isoform (PKCε). The ability of cholesterol to directly bind PKCε in an isoform-specific manner strongly supports its role in sensing the cellular cholesterol levels. The emerging picture from the above studies is that regulation of LDL receptor transcription results from the activity of a number of interlinked regulatory molecules and pathways, rather than from a single linear series of events. These studies will provide the necessary framework for understanding differential responses within human populations to atherosclerosis following high-fat/cholesterol diet. This information may also provide new strategies to modulate specific gene expression with the hope to develop novel therapies for the treatment of hypercholesterolemia.

$p42/44^{MAPK}$ $p38^{MAPK}$ Protein kinase C LDL receptor Transcriptional regulation Hepatic cells

CARDIOVASCULAR disease (CVD) is the most frequent cause of death in the United States and in all developed Western nations. The most common form of CVD is atherosclerosis, a gradual deposition of fats and lipids in muscular arteries. Broad population studies have shown that of all known risk factors that promote atherosclerosis, a high serum low-density lipoprotein (LDL) cholesterol level is the major one. Its role in the pathogenesis of atherosclerosis is well established, supported by metabolic and pathologic studies in humans and selected animal models, as well as by primary and secondary prevention trials (9,26,34). The regulation of hepatic receptor that binds to LDL cholesterol, known as LDL receptor, plays a major role in maintaining the plasma LDL cholesterol levels (10,29). Recent studies have suggested that multiple endogenous pathways are engaged in restoring cholesterol homeostasis through regulation of LDL receptor expression. This particular review discusses recent advances in our under-

Accepted April 16, 2002.
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standing of the signaling pathways that collectively serve to regulate LDL receptor transcription. In particular, the involvement of mitogen-activated protein kinases (MAPKs), as well as the role of protein kinase C (PKC), specifically its ε isoform, in regulating LDL receptor transcription are discussed. A brief description on the role of MAPKs and PKC in cellular signaling is followed by a section describing their role in regulating LDL receptor transcription in response to extracellular and intracellular signals. The description of the nuclear factors that regulate LDL receptor expression and are the possible targets of signaling pathways described here have recently been reviewed elsewhere and will not be covered in this short review (11,12,29).

MAMMALIAN MITOGEN-ACTIVATED PROTEIN KINASES

MAPKs are important mediators involved in the intracellular network of interacting proteins that transduce extracellular cues to intracellular responses (15,45, 69,72,73,88). The MAPK signaling cascade is evolutionarily well conserved in cells from yeast to humans and is typically composed of three hierarchical protein kinases including MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). MAPKKK phosphorylates and thereby activates MAPKK, which in turn phosphorylates and activates MAPK. Generally, MAPKs are activated in the cytoplasm and translocate into the nucleus and either directly or indirectly (through downstream kinases) induce phosphorylation of transcription factors, coactivators, thereby controlling gene expression and cellular functions (30,31,84,85).

In mammalian systems, at least six independent MAPK signaling units appear to function (69,88); among them, the biochemical properties of p42/44^{MAPK} cascade have been characterized in greater detail and it is considered as the archetypal MAP kinase cascade. p42/44^{MAPK} belongs to the group of serine/threonine kinases that phosphorylates -Ser/Thr-Pro-motifs. Protein-tyrosine kinase receptors (27), G-protein-coupled receptors (33,63,87), and cytokine receptors (7) have been shown to activate this kinase cascade. Activation of the p42/44^{MAPK} in response to extracellular stimulation can be schematically divided into membranous and cytoplasmic phases (72). The membranous phase consists of the receptor sensing the extracellular signal, whereas the cytoplasmic phase shows activation of protein kinase cascade. In case of growth factors, ligand-induced dimerization of receptor with intrinsic protein-tyrosine kinase activity leads to the activation and autophosphorylation of tyrosine residues in the intracellular domain of growth factor receptor (24). Adaptor proteins mediate protein–protein interactions to link activated receptor to Ras signaling pathways (16,61). The SH3 domain of growth factor receptor-bound protein 2 has been shown to recruit guanine-nucleotide exchange factor Sos (son of sevenless) and to enforce its translocation to the plasma membrane. This translocation is thought to bring Sos in close proximity with Ras, a small GTP binding protein located at the cytoplasmic surface of the plasma membrane (24,44,77). Sos induces the dissociation of GDP from Ras, allowing the formation of an activated GTP–Ras complex (8).

Activated Ras binds to the NH_2-terminal portion of the serine-threonine protein kinase Raf-1, thereby recruiting Raf-1 to the plasma membrane. Once at the membrane, Raf-1 is activated by an unknown mechanism (4,80). Raf-1 exhibits high substrate specificity towards MAPKK isoforms, MEK-1 and MEK-2, and activates them by phosphorylation of specific regulatory serine and threonine residues (2,90,94). MEKs belong to the small group of dual specificity kinases that catalyze both serine/threonine as well as tyrosine phosphorylation. MEKs are highly selective activators of the MAPK $p44^{MAPK}$ and $p42^{MAPK}$ (also referred to as ERK1 and ERK2, respectively) by phosphorylation of both threonine and tyrosine regulatory sites. Both $p42^{MAPK}$ and $p44^{MAPK}$ are proline-directed protein kinases that phosphorylate Ser/Thr-Pro motifs and are usually considered to be functionally redundant. The three-dimensional structure of $p42^{MAPK}$ suggests that conformational changes are responsible for its activation in response to phosphorylation of the regulatory motif (15,92). After mitogenic stimulation, p42/44^{MAPK} is capable of translocation to the nucleus. Therefore, not only cytolplasmic but also nuclear proteins can be phosphorylated by p42/44^{MAPK}. Putative nuclear targets are several transcription factors indicating the importance of this kinase in the regulation of transcriptional activity (31,73). Phosphorylation of Elk-1 by p42/44^{MAPK} has been shown to increase its transcriptional activity (31). Other transcription factors implicated as substrates of p42/44^{MAPK} are c-Myc (78), ATF-2 (1), NF-IL6 (55), STATs (18), and sterol-regulatory element binding protein (SREBP)-2 (68). Protein kinases are another major group of substrates for this kinase. 90-kDa ribosomal kinase ($pp90^{RSK}$) was first identified as a substrate of p42/44^{MAPK} and is activated by phosphorylation on threonines (81,82,87). Another downstream kinase serving as a substrate for p42/44^{MAPK} is MAPK-activated protein kinase-2 (79).

Two other members of the MAPK family are minimally activated by classic growth factors but potently activated by cellular stresses (19,38,39). One family has been designated c-Jun N-terminal kinase (JNK),

also referred to as stress-activated protein kinase. JNK is activated by cellular stress signals such as proinflammatory cytokines, UV light, heat shock, endotoxins, ischemia, reversible ATP depletion, and genotoxic stress. Two isoforms of 46 and 54 kDa were named $p46/54^{JNK}$, respectively, and seem to be functionally redundant. Ten different isoforms of this kinase have been identified that originate from alternative splicing of three mammalian genes. $p46/54^{JNK}$ regulates c-*jun* transcriptional activity by phosphorylation of the N-terminal activating domain, Ser-63 and -73. N-terminal phosphorylation of c-*jun* induces the formation of c-*jun*/c-Fos heterodimers and c-*jun* homodimers that increase the transcriptional activity of many genes by binding to the AP-1 sites in their promoter region (63). Other nuclear targets of $p46/54^{JNK}$ are the transcription factors ATF2 and Elk-1 (1,19).

The second family includes isoforms of $p38^{MAPK}$, the mammalian homolog of HOG-1, a yeast kinase involved in response to osmolar stress (38,39). Like $p42/44^{MAPK}$, $p46/54^{JNK}$ and $p38^{MAPK}$ are proline directed and require phosphorylation on both tyrosine and threonine residues for activation. Unlike Thr-Asp-Tyr motif of $p42/44^{MAPK}$, $p46/54^{JNK}$ contain a Thr-Pro-Tyr motif and $p38^{MAPK}$ a Thr-Gly-Tyr motif within kinase subdomain VIII that, when phosphorylated, activates the kinases. Several isoforms of $p38^{MAPK}$ have also been described: $p38^{MAPK}\alpha$, also named CSBP2 or stress-activated protein kinase 2a; $p38^{MAPK}\beta$, also named stress-activated protein kinase 2β and its splice isoform βII; $p38^{MAPK}\gamma$, also termed stress-activated protein kinase 3 or Erk6; and $p38^{MAPK}\delta$, otherwise known as stress-activated protein kinase 4.

Experiments have demonstrated that $p38^{MAPK}$ lies downstream of the Ras-related GTP binding proteins Rac and Cdc42 and is directly activated by the MAPK kinases, MKK3, MKK4, and MKK6, with some preference for individual isoforms (25). p38MAPKα and β are the most homologous isoforms and are inhibited by pyridine-imidazoles SB202190 and SB203580, whereas γ and δ isoforms are insensitive to these compounds (17,40). Both of these compounds are highly specific inhibitors of $p38^{MAPK}$ and are widely utilized as tools to probe $p38^{MAPK}$ function in vitro and in vivo. They are extremely specific as SB202190 has no inhibitory activity on any of the kinases tested in vitro, including other MAPKs, at concentrations greater than 10-fold the usual treatment concentrations.

The signaling cascades resulting in $p46/54^{JNK}$ and $p38^{MAPK}$ activation have direct parallels with the $p42/44^{MAPK}$ cascade, but are relatively insulated from one another as the MEKs that activate one MAPK are much less effective in activating others. Like $p42/44^{MAPK}$, after phosphorylation and activation, the $p46/54^{JNK}$ and $p38^{MAPK}$ translocate to the nucleus where they phosphorylate and activate several targets.

MAPKs have been found to play an integral role in the regulation of gene expression of cytokines, the adhesion molecules, E-selectin, c-*jun*, c-*fos*, inducible NO synthase, and proliferating cell nuclear antigen (83). As a result, MAPKs have been implicated not only in the modulation of inflammatory and adaptive responses but also in the hypertrophic response, initiation and progression of atherosclerotic plaques, and postischemic injury in the heart and kidney cells.

PROTEIN KINASE C

PKC comprises a large family of serine/threonine protein kinases that play a critical role in many signal-transducing pathways in the cell (50,56). PKCs are activated by many extracellular and intracellular signals and are considered as "classical" mediators of many extracellular agonists that elicit the production of multiple lipid second messengers (57). They have been implicated in a multitude of physiological functions in the cell. Considerable evidence exists suggesting that PKC plays a fundamental role in signaling mechanisms leading to mitogenesis and proliferation of cells, apoptosis, platelet activation, remodeling of the cytoskeleton, modulation of ion channels, and secretion. cDNA cloning efforts over the past decade have revealed that, like many other signaling molecules, PKC also comprises a large family of isoforms each with distinct properties (21). Twelve distinct members have been discovered to date in mammalian cells, and have been subdivided into three distinct subfamilies as follows: conventional PKCs (cPKC) including α, βI and the splice variant βII, and γ; novel PKCs (nPKC), δ, ε, η, and θ; and atypical PKCs (aPKC) comprising ζ and ι/λ (also known as iota). An additional family may be considered by the more recently discovered PKCμ (also known as protein kinase D). These PKC isoforms are unique, not only with respect to primary structure, but also on the basis of expression patterns, subcellular localization, activation in vitro, and responsiveness to extracellular signals. Most importantly, these isoforms show differences in cofactor dependence and responsiveness to calcium and phospholipid metabolites due to the presence of specific homology domains. Both cPKC and nPKC contain one C1 domain that is defined by the presence of two repeated zinc-finger motifs, C1a and C1b ($H\text{-}X_{12}\text{-}C\text{-}X_2\text{-}C\text{-}X_{13/14}\text{-}C\text{-}X_2\text{-}C\text{-}X_4\text{-}H\text{-}X_2\text{-}C\text{-}X_7\text{-}C$). In addition, cPKC has a C2 domain that apparently confers calcium responsiveness to these isoforms. cPKCs α, βI/II, and γ were the first described and are activated by phosphatidylserine (PS) in a calcium-dependent manner. In addition, they bind to and

are activated by sn-1,2-diacylglycerol (DAG), which increases the specificity of the enzyme for PS and also increases the affinity of the enzyme for calcium. nPKCs are also activated by DAG and require PS as a cofactor, but have lost the requirement of calcium. Both aPKCs and PKCμ do not respond to either DAG or calcium, though apparently still require PS as a cofactor.

There has been considerable interest in determining roles of these specific isoforms in signaling pathways in the cell (21,28,52,70). The current review describes only the recent exciting observations concerning the role of PKCε, as this isoform has also been found to participate in the sterol regulation of LDL receptor expression in hepatic cells (49).

PKCε is a novel PKC family member that responds to both DAG and PS in vivo. Ohno et al. were the first to demonstrate that PKCε could be activated by mitogenic stimuli in vivo (58). At about the same time, two independent studies have demonstrated that PKCε can function as an oncogene when overexpressed in fibroblasts (13,52,62). Increased PKCε activity in these cells correlated with formation of dense foci in monolayer cultures, decreased doubling times, increased cell saturation densities, decreased serum requirements, growth in soft agar, and tumor formation in nude mice, all characteristics of neoplastic transformation. More recent studies have indicated that PKCε may function as an oncogene by enhancing the activity of the Raf-1 kinase, and thus modulating the MAPK pathway (14). Furthermore, PKCε is required for initiating DNA synthesis in mouse erythroleukemia cells stimulated with erythropoetin, leading to an upregulation of c-Myc (41).

PKCε has also been linked to the expression of certain transcription factors and the induction of immediate-early genes. It has been shown to induce transcription of c-*fos* and c-*jun* genes (65). This may account, at least in part, for its effect on cell growth. Moreover, PKCε has been shown to regulate the transcription factors NF-AT-1, NF-κB, and AP-1 in activated T cells, similar to the stimulatory effects of activated Ras in the same system (28). Several reports have suggested that stimulation of a variety of cell types with insulin leads to activation and membrane translocation of PKCε (32,78,91). Insulin has been shown to activate PKCε in fetal chick neurons, and this activation is concomitant with translocation of PKCε from the cytosol to the plasma membrane.

CRITICAL ROLE OF $p42/44^{MAPK}$ IN CONTROLLING LDL RECEPTOR INDUCTION BY A VARIETY OF TRASCRIPTIONAL MODULATORS IN HEPATIC CELLS

LDL receptor plays a central role in the regulation of body cholesterol homeostasis by determining the cellular uptake and catabolic rate of LDL and its precursors, intermediate and very low-density lipoproteins (10). Hepatic regulation of the receptor gene expression provides a mechanism by which dietary and hormonal agents alter plasma LDL levels. LDL receptor gene is induced primarily at the transcriptional level in hepatic cells by a variety of physiologic and pathophysiologic stimuli such as cytokines, growth factors, PKC modulators, and low intracellular cholesterol levels. When cells are depleted of cholesterol, they synthesize large amounts of transcript for the LDL receptor, which facilitates the uptake of exogenous cholesterol by receptor-mediated endocytosis. When cholesterol builds up within the cell, transcription of the gene is suppressed. Other mediators, including several growth factors, have been found to upregulate LDL receptor activity, presumably by their mitogenic activity. By stimulating cell division, mitogens increase cellular demand for cholesterol (46), a major component in membrane biosynthesis. On the other hand, oncostatin M (42), cytokines (37), and hepatocyte growth factor (60,75) promote LDL receptor transcription without affecting cell growth.

Research conducted during the past decade has led to an understanding of the nuclear factors and the active role they play in its regulation. Numerous transcription factors, including SREBPs (11,12), Sp1 (20), FP1 binding protein (22,47), sterol-independent regulatory element, consisting of cAMP-response element binding protein and CCAAT/enhancer binding protein (42), and YY1 (5) are known to bind to several canonical sequences within the 177-bp fragment of the LDL receptor promoter. Many of these sequences affect stimulated rather than basal transcription under different growth conditions. Only SREBPs have the essential capability of a regulator of LDL receptor expression in response to endogenous levels of cholesterol. They are membrane-bound transcription factors whose NH_2-terminal domain must be released proteolytically to enter the nucleus in response to depletion of cholesterol. For proteolysis to take place, the SREBPs must be transported from endoplasmic reticulum to their proteolysis site in the Golgi complex. This transport is accomplished by SREBP-cleavage-activating protein (SCAP), a polytopic membrane protein that escorts SREBPs to the Golgi complex. Here the SREBPs are cleaved sequentially by two proteases that release the NH_2-terminal domain into cytosol. The released fragment travels to the nucleus, where it binds to sterol regulatory element and interacts synergistically with the transcription factors bound to the neighboring sites and stimulates transcription. This proteolytic processing is under feedback control by cholesterol. Thus, when cholesterol accumulates in cells,

the SREBP–SCAP complex fails to reach to the Golgi, and SREBPs are not processed. The nuclear SREBPs are rapidly degraded by a proteosomal process, and the synthesis of SRE-containing genes in their promoter declines. It is thus evident that the different components involved in regulating LDL receptor transcription have been studied extensively. However, the signaling pathways that impinge on the above transcription factors are beginning to emerge from the recent studies described below.

As a first step towards identifying signaling pathways that impinge on nuclear factors regulating LDL receptor expression, we utilized specific inhibitors of MAPKs cascade and PKC in our studies. We showed for the first time that phorbol-esters (TPA) could not activate LDL receptor expression in the presence of either MEK-1/2 inhibitor PD98059, or specific PKC inhibitor calphostin C in human hepatoma HepG2 cells, suggesting roles for these kinases in the induction process (36). Our observation that inhibition of PKC also abolished TPA-induced p42/44^{MAPK} activation supported the involvement of a PKC-dependent p42/44^{MAPK} activation pathway in the induction of LDL receptor transcription. Subsequently, we observed that interleukin (IL)-1β-induced LDL receptor expression also requires p42/44^{MAPK}, as inhibition of MEK-1/2 by PD98059 completely blocked the induction process (37). Similarly, requirement of the p42/44^{MAPK} cascade was found for LDL receptor induction in response to a variety of extracellular signals, including hepatocyte growth factor, anisomycin, oncostatin, and insulin (23,42,68). In summary, the above studies led to the conclusion that induction of LDL receptor expression by a variety of extracellular signals requires at least p42/44^{MAPK} signaling cascade (Fig. 1).

Activation of multiple pathways by the above factors, however, complicates determining the sole contribution of the p42/44^{MAPK} pathway in the induction process. Due to the complexities of cytokine/growth factor signaling, specific contribution of this kinase cascade alone was studied by generating HepG2-derived cell lines that stably express inducible form of human ΔRaf-1:ER, a fusion protein consisting of an oncogenic form of human Raf-1 kinase (amino acids 305 to 648 that encodes all of the kinase domain contained in the conserved region 3 but none of conserved regions 1 or 2) and the hormone binding domain of the human estrogen receptor (35,89). This cell line provided a valuable tool to specifically activate Raf-1/MEK/p42/44^{MAPK} cascade on treatment of cells with antiestradiol ICI182,780 {7α-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-estra-1,3,5(10)-triene-3,17β-diol} (35). It was observed that exclusive activation of Raf-1/MEK-1/2/p42/44^{MAPK} cascade in these cells induced LDL receptor expression to the same magnitude as that induced by either cytokines or hepatocyte growth factor in HepG2 cells. Furthermore, by using different doses of ICI182,780, it was demonstrated that the degree of p42/44^{MAPK} activation dictates the level of LDL receptor induction in these cells. Interestingly, expression of the squalene synthase gene, another sterol-sensitive gene of the cholesterol biosynthetic pathway, was unaffected upon ICI182,780 treatment of HepG2-ΔRaf-1:ER cells, thus revealing basic differences in the regulatory mechanisms controlling their expression.

It has been shown earlier that the low levels of Raf-1 kinase activity elicit a mitogenic response, whereas high levels of this kinase activity cause cell cycle arrest (74,89). A relationship between cell growth and LDL receptor expression has also been suggested (46), because an increase in LDL receptor expression stimulated by cell growth is meant to provide proliferating cells with an additional cholesterol for the synthesis of new membranes. In order to understand the molecular mechanisms governing this phenomenon, the relationship between Raf-1 kinase activation, cell growth, and LDL receptor expression was investigated using HepG2-ΔRaf-1:ER cells. Effects of activated p42/44^{MAPK} cascade on cell growth were measured by several parameters. It was observed that ICI182,780 treatment arrested the growth of these cells, whereas no growth arrest was seen in the untreated cells. Moreover, inhibition of DNA synthesis tightly correlated with increases in p42/44^{MAPK} activity. Comparison of the cell cycle phase distribution of exponentially growing HepG2-ΔRaf-1:ER cells in the presence of ICI182,780 revealed a modest increase in the fraction of cells in the G_2/M phase with a concomitant decrease in S phase of the cell cycle. Side-scatter analyses of treated cells also revealed a shift of the cell population to higher scatter values, indicating much larger cell size of the growth-arrested cells. Finally, consistent with the antiproliferative character of the high-intensity Raf-1 kinase signal, a strong decrease in cyclin A expression was observed. It is thus safe to conclude that activation of the Raf-1/MEK-1/2/p42/44^{MAPK} cascade alone is sufficient to uncouple LDL receptor expression from cell growth. Thus, despite the apparent relationship between cell growth and LDL receptor expression, the processes of DNA synthesis and modulation of LDL receptor expression can occur independently of each other through p42/44^{MAPK} cascade. The growth-independent regulation of LDL receptor expression may also explain cytokine- and hepatocyte growth factor-induced LDL receptor induction via p42/44^{MAPK} without affecting cell growth (23,37,60).

ONE-WAY CROSS-TALK BETWEEN p42/44^{MAPK} AND p38^{MAPK}: IMPLICATION FOR STRESS-INDUCED HYPERCHOLESTEROLEMIA

The possible involvement of p38^{MAPK} cascade in regulating LDL receptor expression was observed while investigating the role of MAPKs in IL-1β- and tumor necrosis factor-α-induced LDL receptor expression in hepatic cells (37). It was observed that specific inhibition of p38^{MAPK} with SB202190 or SB203580 increased IL-1β-induced LDL receptor expression in HepG2 and HeLa cell types, which raised an interesting possibility of the regulation of LDL receptor expression by p38^{MAPK}. This relationship was explored further by examining the effects of p38^{MAPK} inhibition on LDL receptor expression (76). SB202190 treatments of HepG2 cells induced LDL receptor expression in a time- and dose-dependent manner, strongly supporting the above observations. The involvement of p38^{MAPK} was verified by determining the effect of a constitutively active mutant of p38^{MAPK} kinase (MKK6b) on human LDL receptor promoter activity in transfection studies. We showed that cotransfection of MKK6b together with p38^{MAPK} α isoform effectively suppressed LDL receptor activity. However, cotransfection of p38^{MAPK} β isoform with MKK6b had no effect, suggesting an isoform-specific interaction between p38^{MAPK} cascade and LDL receptor expression.

A clue regarding the mechanism of SB202190-induced LDL receptor expression came upon observing that p42/44^{MAPK} was also activated under these conditions. Consistent with its role, pretreatment with PD98059 blocked increase in p42/44^{MAPK} activation and concomitantly LDL receptor induction, suggesting that p38^{MAPK} is indeed negatively regulating LDL receptor expression via suppression of p42/44^{MAPK}. To explore the nature of this communication in detail, HepG2-ΔRaf-1:ER cells were again utilized. It was observed that ICI182,780 treatment resulted in rapid p42/44^{MAPK} activation and the activity was increased for up to 1 h that remained elevated in the presence of ICI182,780. Effects of p42/44^{MAPK} activation on the induction of p38^{MAPK} activity by a variety of stress inducers were measured. No significant effect of p42/44^{MAPK} activation on p38^{MAPK} activation by IL-1β or anisomycin was observed. Likewise, inhibition of p42/44^{MAPK} with PD98059 had no effect on p38^{MAPK} activation by cytokines. These observations suggested that p42/44^{MAPK} cascade does not regulate activity of the p38^{MAPK} cascade. Based on the above findings, it was proposed that there exists a one-way cross-talk between p38^{MAPK} and p42/44^{MAPK} in hepatic cells (48), and suppression of LDL receptor expression by p38^{MAPK} may explain stress-induced hypercholesterolemia observed in humans and animals (Fig. 1) (6,59,67). Subsequently, other groups have also found the existence of a one-way cross-talk between these kinases in Kaposi's sarcoma cells and in rat pinealocytes (43,54).

Furthermore, consistent with the above suggested one-way cross-talk between p38^{MAPK} and p42/44^{MAPK}, Zhang et al. (93) recently reported direct physical interactions between p38^{MAPK} α isoform and p42/44^{MAPK} by using affinity chromatography and coimmunoprecipitation studies. Importantly, they showed that increased activation (phosphorylation) of this isoform resulted in enhanced interaction with p42/44^{MAPK}, and the degree of this interaction correlated with the extent of inhibition of p42/44^{MAPK} activity. On the basis of these results, it was suggested that possible mechanism for one-way cross-talk between these MAPKs may involve sequestration of p42/44^{MAPK} by p38^{MAPK} and thus sterically blocking their phosphorylation by upstream kinases, MEK-1/2.

ROLE OF DIACYLGLYCEROL- AND PHOSPHOLIPID-REGULATED PKCε IN LDL RECEPTOR INDUCTION IN RESPONSE TO DEPLETION OF CELLULAR CHOLESTEROL

There is virtually no information available on the underlying signaling kinase(s) initiated by alterations in cellular cholesterol levels leading to the regulation of LDL receptor gene promoter in any cell type. A clue regarding the role of PKC in the LDL receptor induction process came from earlier studies showing that LDL itself induces a rapid and transient cytosol-to-membrane translocation of TPA-sensitive PKC isoforms in human smooth muscle cells or skin fibroblasts (53,71). In addition, suppression of TPA-sensitive PKC activity in fibroblasts from Nieman-Pick type C patients has been linked to accumulation of naturally occurring PKC modulators, such as sterols and sphingosine (51,66). These observations prompted us to investigate the role of PKC during LDL receptor induction in response to depletion of sterol (49). To assess the role of this kinase in the induction process, effects of inhibition of this kinase by using a specific inhibitor, calphostin C, were examined in HepG2 cells. Interestingly, suppression of LDL receptor expression was observed in a dose-dependent manner. Calphostin C also blocked induction of LDL receptor promoter activity following depletion of sterols, suggesting that this inhibitor exerts its effect at the transcription level.

To further resolve which of the PKC isoforms may be involved in mediating induction of LDL receptor transcription, effects of transient overexpression of

PKC isoforms on LDL receptor induction were examined. Surprisingly, overexpression of either wild-type or a constitutively active mutant of PKCε dramatically increased (approximately 18-fold) LDL receptor promoter activity in a dose-dependent manner, whereas isoforms PKCα, PKCγ, PKCδ, or PKCζ did not appreciably alter the induction levels under parallel conditions. Consistent with the role of PKCε, antisense inhibition of the endogenous isoform abolished the induction of LDL receptor promoter in response to depletion of sterols. Furthermore, sterols were unable to suppress LDL receptor promoter activity in cells overexpressing constitutively active PKCε mutant, suggesting that sterols may reduce LDL receptor promoter activity by modulating PKCε function. An additional evidence for the involvement of PKCε was obtained by using HepG2 cells in which PKCε was depleted by chronic treatment with a high concentration (1 μM) of TPA for 24 h, which caused loss of stimulation of LDL receptor expression in response to low level of sterol.

As stated earlier, PKCε is a known activator of Raf-1 kinase (14) and thus has the potential to induce LDL receptor transcription via $p42/44^{MAPK}$ activation. In view of earlier findings that activation of $p42/44^{MAPK}$ cascade alone is sufficient to induce LDL receptor transcription (35), a possible role of this pathway was examined by determining the effect of blocking MEK-1/2 on the induction process. It was observed that PKCε can induce LDL receptor promoter activity even in the presence of this inhibitor, even though $p42/44^{MAPK}$ phosphorylation is blocked, suggesting that PKCε induces LDL receptor expression in a $p42/44^{MAPK}$-independent manner.

In order to get insight of the mechanism of interaction between sterols and PKCε, the possibility of a direct interaction between PKCε and 25-hydroxycholesterol was explored. The photoaffinity labeling assays using [11,12-^{3}H]all-*trans*-retinoic acid (atRA) as a photoprobe was employed (64). atRA has been shown to bind directly to the PS binding site in the C2 domains of several PKCs and as a result can modulate their catalytic activity. For PKCs lacking the C2 domain, atRA binds to the C1 domain in close proximity to the fatty acid binding site. The ability of 25-hydroxycholesterol, the most active cholesterol derivative in suppressing LDL receptor transcription, to compete with [^{3}H]atRA for binding to PKCε (C1 domain) and PKCα (C2 domain) was examined. Both PKCs were found to be photolabeled by atRA to an equal efficiency, and the labeling was not only light sensitive, but also showed protection by unlabeled atRA, demonstrating that atRA binding to both isoform is specific. However, in contrast to PKCα, strong competition was observed between 25-hydroxycholesterol and atRA for binding to the PKCε isoform, thus supporting the possibility of a direct interaction with PKCε in the C1 domain, most likely at the PS/fatty acid binding site.

The role of interaction of 25-hydroxycholesterol with PKCε was further evaluated by measuring the effects of sterols on enzymatic activity of the above isoforms. It was observed that 25-hydroxycholesterol did not affect the activity of PKCα, whereas PKCε activity was significantly reduced in a dose-dependent manner. These results support the notion that sterols can directly modulate PKCε function.

On the basis of above results, a hypothetical model has been proposed that accounts for direct interaction between 25-hydroxycholesterol and PKCε in order to explain the role of this isoform in sterol-mediated regulation of the LDL receptor transcription (Fig. 1) (49). The central hypothesis of this model is that PKCε may act to coordinate the interaction of sterols with a variety of downstream signal-transducing molecules and senses steady-state membrane cholesterol levels through direct binding at the PS/fatty acid binding site. High endogenous cholesterol level results in an increased binding of sterol to the C1 domain of PKCε, thus hampering binding of PKC activators and causing inhibition of its activity, whereas depletion of endogenous sterol levels results in an increased binding of activators, thus modulating its function with a significant effect on catalytic activity. Considering that the formation of an active transcriptional complex at the LDL receptor promoter is a highly regulated process, the modified PKCε may target the transcriptional machinery in an SREBP-dependent manner to modulate its expression in a sterol-dependent manner.

SUMMARY AND FUTURE GOALS

In the past decade, considerable efforts have gone into understanding the precise mechanisms of activation of LDL receptor transcription by both sterol and nonsterol mediators. The nature of the nuclear factors and the possible protein–DNA and protein–protein interactions involved are now largely understood at the molecular level, so the impetus to understand how these nuclear factors are regulated by upstream signaling cascades to give rise to extraordinary complexity to LDL receptor regulation has never been stronger.

The importance of MAPKs and PKCs in physiology and signaling mechanisms is not a novel concept. The fact that these kinases regulate LDL receptor expression, which controls plasma LDL levels, underscores the essential role played by these kinases in

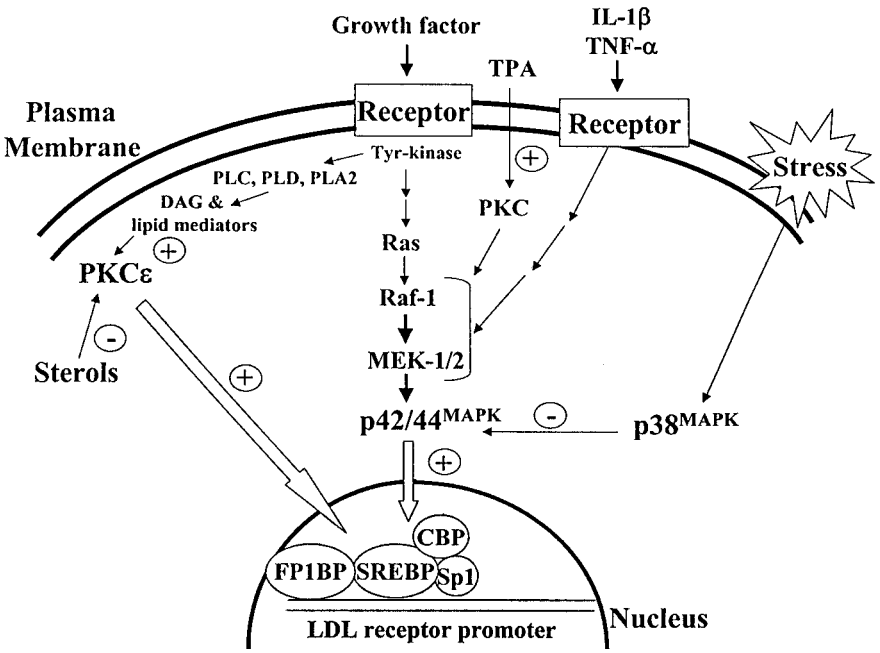

Figure 1. Schematic representation of the signaling pathways involved in regulating LDL receptor transcription in hepatic cells. Signals from a variety of extracellular agents converge on p42/44^{MAPK} cascade to induce LDL receptor transcription. Importantly, degree of p42/44^{MAPK} activation determines the extent of LDL receptor induction. Suppression of LDL receptor receptor transcription by the stress-activated p38^{MAPK} through modulating p42/44^{MAPK} cascade may explain stress-induced hypercholesterolemia observed in humans and laboratory animals. Finally, a direct interaction between PKCε and sterols may explain involvement of this kinase in sterol-regulated LDL receptor expression. One exciting possibility is that PKCε induces LDL receptor transcription via SREBPs. Identification of targeted nuclear factors should resolve a number of questions of how and why LDL receptor responds to a wide variety of cellular signals in hepatic cells. Activation is denoted by plus sign, whereas minus sign indicates suppression. CBP, cAMP-response element binding protein.

cholesterol homeostasis. The emerging picture from the above studies is that regulation of the LDL receptor transcription results from the activity of a number of interlinked regulatory molecules and pathways. The molecular links between these signaling pathways and LDL receptor promoter is not fully understood. Several questions regarding the roles of MAPKs remain elusive, including, how p42/44^{MAPK} is linked to the LDL receptor promoter, what roles nuclear cofactor(s) play in this regulatory process, and whether SREBP modification reported earlier (68) can also account for maximal LDL receptor induction by TPA, cytokines, and growth factors.

It appears that PKCε may act as a sensor localized in specific regions of the cell to respond to extracellular and intracellular cholesterol levels to control LDL receptor expression. Compatible with this conclusion is the finding that PKCε is abundant in membranes (3), possibly in lipid rafts, which allows its close proximity to the cellular structural and kinetic cholesterol pool. Clearly, the specific function of PKCε in sterol regulation is a subject of major interest, and critical issues to be addressed in future are: 1) Determine what proximal components transduce the signal from PKCε to activation of LDL receptor transcription; 2) Examine the mechanism by which induction takes place; and 3) What is the molecular basis of interaction between PKCε and intracellular cholesterol? It will be interesting to determine whether sterols can influence the subcellular location, in addition to the activity of this isoform. Examining the role of PKCε in sterol regulation of other sterol-sensitive genes is also important. Furthermore, PKC pathway often converges with other signaling pathways at the point of nuclear factors that are indispensable for gene transcription (55,86). It remains to be seen whether activation of another signaling pathway, along with PKCε, is required for maximal induction of the LDL receptor promoter activity. Although these and other related questions can be examined in vitro, ultimately the observations made in cultured cells will need to be confirmed in vivo.

In brief, the outcome of some of the recent studies reviewed here begs one important question: What are the immediate downstream targets of p42/44^{MAPK} and PKCε that are critical for the induction of human LDL receptor transcription? Identification of these proteins is likely to represent the next major area of

research in this field. Finally, considering that the cell pathways are regulated by their organization in macromolecular assemblies (69,88), elucidation of the complete picture will give us the unique opportunity to begin unraveling the intricacies of how these signals are integrated in space and time to elicit cholesterol homeostasis. This knowledge may also help us to understand how subtle perturbation of the signaling network can result in pathological situations, thus providing golden opportunities to identify novel molecular targets for pharmacological intervention in different human lipid disorders.

ACKNOWLEDGMENTS

Research in the author's laboratory was supported by grants from the National Institutes of Health (HL67760) and the Established Investigator grant (9940163) of the American Heart Association.

REFERENCES

1. Abdel-Hafiz, H.; Heasley, L. E.; Kyriakis, J. M.; Avruch, J.; Kroll, D. J.; Johnson, G. L.; Hoeffler, J. P. Activating transcription factor-2 DNA-binding activity is stimulated by phosphorylation catalyzed by p42 and p44 microtubule-activated protein kinase. Mol. Endocrinol. 6:2079–2089; 1992.
2. Alessi, D. R.; Saito, Y.; Campbell, D. G.; Cohen, P.; Sithanandam, G.; Rapp, U.; Ashworth, A.; Marshall, C. J.; Cowley, S. Identification of the sites in MAP kinase kinase-1 phosphorylated by p74raf-1. EMBO J. 13:1610–1619; 1994.
3. Anderson, R. G. The caveolae membrane system. Annu. Rev. Biochem. 67:199–225; 1998.
4. Avruch, J.; Zhang, X.; Kyriakis, J. M. Raf meets Ras: Completing the framework of a signal transduction pathway. Trends Biochem. Sci. 19:279–282; 1994.
5. Bennett, M. K.; Ngo, T. T.; Athanikar, J. N.; Rosenfeld, J. M.; Osborne, T. F. Co-stimulation of promoter for low density lipoprotein receptor gene by sterol regulatory element-binding protein and Sp1 is specifically disrupted by the yin yang 1 protein. J. Biol. Chem. 274:13025–13032; 1999.
6. Berger, D. F.; Starzec, J. J.; Mason, E. B.; DeVito, W. The effects of differential psychological stress on plasma cholesterol levels in rats. Psychosom. Med. 42: 481–492; 1980.
7. Bird, T. A.; Sleath, P. R.; De Roos, P. C.; Dower, S. K.; Virca, G. D. Interleukin-1 represents a new modality for the activation of extracellular signal-regulated kinases/microtube-associated protein-2 kinases. J. Biol. Chem. 266:22661–22670; 1991.
8. Bonfini, L.; Karlovich, C. A.; Dasgupta, C.; Banerjee, U. The Son of Sevenless gene product: A putative activator of Ras. Science 255:603–606; 1992.
9. Brown, G.; Albers, J. J.; Fisher, L. D.; Schaefer, S. M.; Lin, J. T.; Kaplan, C.; Zhao, X. Q.; Bisson, B. D.; Fitzpatrick, V. F.; Dodge, H. T. Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B. N. Engl. J. Med. 323:1289; 1990.
10. Brown, M. S.; Goldstein, J. L. A receptor mediated pathway for cholesterol homeostasis. Science 232:34–47; 1986.
11. Brown, M. S.; Goldstein, J. L. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340; 1997.
12. Brown, M. S.; Goldstein, J. L. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA 96: 11041–11048; 1999.
13. Cacace, A. M.; Guadangno, S. N.; Krauss, R. S.; Fabbro, D.; Weinstein, I. B. The epsilon isoform of protein kinase C is an oncogene when overexpressed in rat fibroblasts. Oncogene 8:2095–2104; 1993.
14. Cacace, A. M.; Ueffing, M.; Philipp, A.; Han, E. K.; Kolch, W.; Weinstein, I. B. PKC epsilon functions as an oncogene by enhancing activation of the Raf kinase. Oncogene 13:2517–2126; 1996.
15. Cobb, M. H.; Goldsmith, E. J. How are MAP kinases regulated. J. Biol. Chem. 270:14843–14846; 1995.
16. Cohen, G. B.; Ren, R.; Baltimore, D. Modular binding domains in signal transduction proteins. Cell 80:237–248; 1995.
17. Cuenda, A.; Roise, J.; Doza, Y. N.; et al. SB203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett. 364:229–233; 1995.
18. David, M.; Petericoin, E.; Benjamin, C.; Pine, R.; Weber, M. J.; Larner, A. C. Requirement for MAP kinase (ERK2) activity in interferon α- and interferon β-stimulated gene expression through STAT proteins. Science 269:1721–1723; 1995.
19. Davis, R. J. MAPKs: New JNK expands the group. Trends Biochem. Sci. 19:470–473; 1994.
20. Dawson, P. A.; Hofmann, S. L.; van der Westhuyzen, D. R.; Sudhof, T. C.; Brown, M. S.; Goldstein, J. L. Sterol-dependent repression of low density lipoprotein receptor promoter mediated by 16-base pair sequence adjacent to binding site for transcription factor Sp1. J. Biol. Chem. 263:3372–3379; 1998.
21. Dekker, L. V.; Parker, P. J. Protein kinase C-a question of specificity. Trends Biochem. Sci. 19:73–77, 1994.
22. Dhawan, P.; Chang, R.; Mehta, K. D. Identification of essential nucleotides of the FP1 element responsible for enhancement of low density lipoprotein receptor gene transcription. Nucleic Acids Res. 25:4132–4138; 1997.
23. Dhawan, P.; Bell, A.; Kumar, A.; Golden, C.; Mehta, K. D. Critical role of p42/44^{MAPK} activation in anisomycin and hepatocyte growth factor-induced LDL re-

ceptor expression: Activation of Raf-1/MEK-1/2/p42/44^{MAPK} cascade alone is sufficient to induce LDL receptor expression. J. Lipid Res. 40:1911–1919; 1999.
24. Egan, S. E.; Giddings, B. W.; Brooks, M. W.; Buday, L.; Sizeland, A. M.; Weinberg, R. A. Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. Nature 363:45–51; 1993.
25. Enslen, H.; Raingeaud, J.; Davis, R. J. Selective activation of p38 mitogen-activated protein kinase isoforms by the MAP kinase kinases MKK3 and MKK6. J. Biol. Chem. 273:1741–1748; 1998.
26. Expert panel. Report of the National Cholesterol Education Program Expert Panel on detection, and treatment of high blood cholesterol in adults. Arch. Int. Med. 36:148–156; 1988.
27. Fantl, W. J.; Johnson, D. E.; Williams, L. T. Signaling by receptor tyrosine kinases. Annu. Rev. Biochem. 62: 453–481; 1993.
28. Genot, E. M.; Parker, P. J.; Cantrell, D. A. Analysis of the role of protein kinase C-alpha, -epsilon, and -zeta in T cell activation. J. Biol. Chem. 270:9833–9839; 1995.
29. Goldstein, J. L.; Brown, M. S. Regulation of the mevalonate pathway. Nature 343:425–430; 1990.
30. Hazalin, C. A.; Mahadevan, L. C. MAPK-regulated transcription: A continuously variable gene switch. Nat. Rev. 3:30–40; 2002.
31. Hill, C. S.; Treisman, R. Transcriptional regulation by extracellular signals: Mechanisms and specificity. Cell 80:199–211; 1995.
32. Heidenreich, K. A.; Toledo, S. P.; Brunton, L. L.; Watson, M. J.; Daniel-Issakani, S.; Strulovici, B. Insulin stimulates the activity of a novel protein kinase C, PKC-epsilon, in cultured fetal chick neurons. J. Biol. Chem. 265:15076–15082; 1990.
33. Inglese, J.; Koch, W. J.; Touhara, K.; Lefkowitz, R. J. Gvγ interaction with PH domain and Ras-MAPK signaling pathways. Trends Biochem. Sci. 20:151–156; 1995.
34. Kannel, A.; Castelli, W. P.; Gordon, T.; McNamara, P. M. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham Study. Ann. Int. Med. 74:1–12; 1971.
35. Kapoor, G. S.; Atkins, B. A.; Mehta, K. D. Activation of Raf-1/MEK-1/2/p42/44^{MAPK} cascade alone is sufficient to uncouple LDL receptor expression from cell growth. Mol. Cell. Biochem. (in press).
36. Kumar, A.; Chambers, T. C.; Cloud-Heflin, B. A.; Mehta, K. D. Phorbol-ester-induced low density lipoprotein receptor gene expression in HepG2 cells involves protein kinase C-mediated p42/44MAP kinase activation. J. Lipid Res. 38:2240–2248; 1997.
37. Kumar, A.; Middleton, A.; Chambers, T. C.; Mehta, K. D. Differential roles of extracellular signal-regulated kinase-1/2 and p38^{MAPK} in interleukin-1β- and tumor necrosis factor-α-induced low density lipoprotein receptor expression in HepG2 cells. J. Biol. Chem. 273:15742–15748; 1998.
38. Kyriakis, J. M.; Avruch, J. Sounding the alarm: Protein kinase cascades activated by stress and inflammation. J. Biol. Chem. 271:24313–24316; 1996.
39. Kyriakis, J. M.; Avruch, J. Protein kinase cascades activated by stress and inflammatory cytokines. Bioessays 18:567–577; 1996.
40. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 372:739–746; 1994.
41. Li, Y.; Davis, K. L.; Sytkowski, A. J. Protein kinase C-epsilon is necessary for erythropoietin's up-regulation of c-myc and for factor-dependent DNA synthesis. Evidence for discrete signals for growth and differentiation. J. Biol. Chem. 271:27025–27030; 1996.
42. Liu, J.; Ahlborn, T. E.; Briggs, M. R.; Kraemer, F. B. Identification of a novel sterol-independent regulatory element in the human low density lipoprotein receptor promoter. J. Biol. Chem. 275:5214–5221; 2000.
43. Mackova, M.; Man, J. R.; Chik, C. L.; Ho, A. K. p38^{MAPK} inhibition enhances basal and norepinephrine-stimulated p42/44^{MAPK} phosphorylation in rat pinealocytes. Endocrinology 141:4202–4208; 2000.
44. Margolis, B.; Skolnik, E. Y. Activation of Ras by receptor tyrosine kinases. Am. J. Soc. Nephrol. 5:1288–1299; 1994.
45. Marshall, J. M. MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. Curr. Opin. Genet. Dev. 4:82–89; 1994.
46. Mazzone, T.; Basheeruddin, K.; Ping, L.; Frazer, S.; Getz, G. S. Mechanism of the growth-related activation of the low density lipoprotein receptor pathway. J. Biol. Chem. 264:1787–1792, 1989.
47. Mehta, K. D.; Chang, C.; Underwood, J.; Wise, J.; Kumar, A. Identification of a novel cis-acting element participating in maximal induction of the human low density lipoprotein receptor gene transcription in response to low cellular cholesterol levels. J. Biol. Chem. 271:33616–33622; 1996.
48. Mehta, K. D.; Miller, L. Inhibition of stress-activated p38 mitogen-activated protein kinase induces low density lipoprotein receptor expression. Trends Cardiovasc. Med. 9:201–205; 1999.
49. Mehta, K. D.; Radominska, A.; Kapoor, G. S.; Dave, B.; Atkins, B. A. Critical role of diacylglycerol- and phospholipid-regulated protein kinase Ce in induction of LDL receptor transcription in response to depletion of cholesterol. Mol. Cell. Biol. (in press).
50. Mellor, H.; Parker, P. J. The extended protein kinase C superfamily. Biochem. J. 332:281–292; 1998.
51. Mendez, A. J.; Oram, J. F.; Bierman, E. L. Protein kinase C as a mediator of high density lipoprotein receptor-dependent efflux of intracellular cholesterol. J. Biol. Chem. 266:10104–10111; 1991.
52. Mischak, H.; Goodnight, J. A.; Kolch, W.; Martiny, B. G.; Schaechtle, C.; Kazanietz, M. G.; Blumberg, P. M.; Pierce, J. H.; Mushinski, J. F. Overexpression of protein kinase C-delta and -epsilon in NIH3T3 cells induces opposite effects on growth, morphology, an-

chorage-dependence, and tumorigenicity. J. Biol. Chem. 268:6090–6096; 1993.
53. Mollers, C.; Drobnik, T.; Resnik, T.; Schmitz, G. High-density lipoprotein and low-density lipoprotein-mediated signal transduction in cultured human skin fibroblasts. Cell Signal 7:695–707; 1995.
54. Murakami-Mori, K; Mori, S.; Nakamura, S. p38MAP kinase is a negative regulator for ERK-1/2-mediated growth of AIDS-associated Kaposi's sarcoma cells. Biochem. Biophys. Res. Commun. 264:676–682; 2000.
55. Nakajima, T.; Kinoshita, S.; Sasagawa, T.; Sasaki, K.; Naruto, M.; Kishimoto, T.; Akira, S. Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. Proc. Natl. Acad. Sci. USA 90:2207–2211; 1993.
56. Newton, A. C. Regulation of protein kinase C. Curr. Opin. Cell Biol. 9:161–167; 1997.
57. Nishizuka, Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614; 1992.
58. Ohno, S.; Mizuno, K.; Adachi, Y.; Hata, A.; Akita, Y.; Akimoto, K.; Osada, S.; Hirai, S.; Suzuki, K. Activation of novel protein kinases delta and epsilon upon mitogenic stimulation of quiescent rat 3Y1 fibroblasts. J. Biol. Chem. 269:17495–17501; 1994.
59. Olubadewo, J. O.; Wingard, M.; Washington, B.; Tsai, C. S.; Robinson, T. J.; Ochillo, R. F. The effects of stress on lipoproteins and catecholamines in rats. Cell. Mol. Biol. 40:1201–1206; 1994.
60. Pak, Y. K.; Kanuck, M. P.; Berrio, D.; Briggs, M. R.; Cooper, A. D.; Ellsworth, J. L. Activation of LDL receptor gene expression in HepG2 cells by hepatocyte growth factor. J. Lipid Res. 37:985–998; 1996.
61. Pawson, T.; Gish, G. D. SH2 and SH3 domains: From structure to function. Cell 71:359–362; 1992.
62. Perletti, G. P.; Concari, P.; Brusaferri, S.; Marras, E.; Piccinini, F.; Tashian, A. H. Protein kinase C epsilon is oncogenic in colon epithelial cells by interaction with the ras signal transduction pathway. Oncogene 16: 3345–3348; 1998.
63. Pulverer, B. J.; Kyriakis, J. M.; Avruch, J.; Nikolakaki, E.; Woodgett, J. R. Phosphorylation of c-jun mediated by MAP kinases. Nature 353:670–674; 1991.
63. Pumiglia, K. M.; LeVine, H.; Haske, T.; Habib, T.; Jove, R.; Decker, S. J. A direct interaction between G-protein βγ subunit and the Raf-1 protein kinase. J. Biol. Chem. 270:14251–14254; 1995.
64. Radominska-Pandya, A.; Chen, G.; Czernik, P. J.; Little, J. M.; Samokyszyn, V. M.; Carter, C. A.; Nowak, G. Direct interaction of all-trans-retinoic acid with protein kinase C (PKC). Implications for PKC signaling and cancer therapy. J. Biol. Chem. 275:22324–22330; 2000.
65. Razin, E.; Szallasi, Z.; Kazanietz, M. G.; Blumberg, P. M.; Rivera, J. Protein kinase C-beta and C-epsilon link the mast cell high-affinity receptor for IgE to the expression of c-fos and c-jun. Proc. Natl. Acad. Sci. USA 91:7722–7726; 1994.
66. Rodriguez-Lafrasse, C. R.; Rousson, S.; Valla, S.; Antignac, P.; Louisot, P.; Vanier, M. T. Modulation of protein kinase C by endogenous sphingosine: Inhibition of phorbol dibutyrate binding in Niemann-Pick C fibroblasts. Biochem. J. 325:787–791; 1997.
67. Rosemann, R. H. Relationship of neurogenic and psychological factors to the regulation and variability of serum lipids. Stress Med. 9:133–140; 1993.
68. Roth, G.; Kotzka, J.; Kremer, L.; Lehr, S.; Lohaus, C.; Meyer, H. E.; Krone, W.; Muller-Wieland, D. MAP kinases Erk1/2 phosphorylate sterol regulatory element-binding protein (SREBP)-1a at serine 117 in vitro. J. Biol. Chem. 275:33302–33307; 2000.
69. Schaeffer, H. J.; Weber, M. Mitogen-activated protein kinases: Specific messages from ubiquitous messengers. Mol. Cell. Biol. 19:2435–2444; 1999.
70. Schonwasser, D. C.; Marais, R. M.; Marshall, C. J.; Parker, P. J. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol. Cell. Biol. 18:790–798; 1998.
71. Scott-Burden, T.; Reisnik, T. J.; Hahn, A. W.; Baur, U.; Box, R. J.; Buhler, F. R. Induction of growth-related metabolism in human vascular smooth muscle cells by low density lipoprotein. J. Biol. Chem. 264: 12582–12589; 1989.
72. Seger, R.; Krebs, E. G. The MAPK signaling cascade. FASEB J. 9:726–735; 1995.
73. Seth, A.; Gonzalez, F. A.; Gupta, S.; Raden, D. L.; Davis, R. J. Signal transduction within the nucleus by mitogen-activated protein kinase. J. Biol. Chem. 267: 24796–24804; 1992.
74. Sewing, A.; Wiseman, B.; Llyod, A. C.; Land, H. High-intensity Raf signal causes cell cycle arrest mediated by p21Cip. Mol. Cell. Biol. 17:5588–5597; 1997.
75. Shiota, G.; Rhoags, D. B.; Wang, T. C.; Nakamura, T.; Schmidt, E. V. Hepatocyte growth factor inhibits growth of hepatocellular carcinoma cells. Proc. Natl. Acad. Sci. USA 89:373–377; 1992.
76. Singh, R. P.; Dhawan, P.; Golden, C.; Kapoor, G. S.; Mehta, K. D. One-way cross-talk between $p38^{MAPK}$ and $p42/44^{MAPK}$. Inhibition of $p38^{MAPK}$ induces low density lipoprotein receptor expression through activation of $p42/44^{MAPK}$ cascade. J. Biol. Chem. 274:19563–19600; 1999.
77. Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, P.; Haser, W. G.; King, F., Roberts, T.; Ratnofsky, S.; Lechleider, R. J.; Neel, B. G.; Birge, R. B.; Fajardo, E. J.; Chou, M. M.; Hanafusa, H.; Schaffhausen, B.; Cantley, L. C. SH2 domains recognize specific phosphopeptide sequences. Cell 72:767–7778; 1993.
78. Standaert, M. L.; Bandyopadhyay, G.; Zhou, X.; Galloway, L.; Farese, R. V. Insulin stimulates phospholipase D-dependent phosphatidylcholine hydrolysis, Rho translocation, de novo phospholipid synthesis, and diacylglycerol/protein kinase C signaling in L6 myotubes. Endocrinology 137:3014–3020; 1996.
79. Stokoe, D.; Campbell, D. G.; Nakielny, S.; Hidaka, H.; Leevers, S. J.; Marschall, C.; Cohen, P. MAPKAP ki-

nase-2: A novel protein kinase activated by mitogen-activated protein kinase. EMBO J. 11:3985–3994; 1992.
80. Stokoe, D.; Macdonald, S. G.; Cadwallder, K.; Symons, M.; Hancock, J. F. Activation of Raf as a result of recruitment to the plasma membrane. Science 264: 1463–1467; 1994.
81. Sturgill, T. W.; Ray, L. B.; Erikson, E.; Maller, J. L. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature 334: 715–718; 1988.
82. Sutherland, C.; Campbell, D. G.; Cohen, P. Identification of insulin-stimulated protein kinase-1 as the rabbit equivalent of rskmo-2; Identification of two threonines phosphorylated during activation by mitogen-activated protein kinase. Eur. J. Biochem. 212:581–588; 1993.
83. Thomas, F.; Pombo, C.; Avruch, J. A.; Bonventre, J. V.; Kyriakis, J. M. Stress-activated protein kinases in cardiovascular disease. Circ. Res. 78:947–953; 1996.
84. Toker, A.; Meyer, M.; Reddy, K. K.; Falck, J. R.; Aneja, R.; Aneja, S.; Parra, A.; Burns, D. J.; Treisman, R. Regulation of transcription by MAP kinase cascades. Curr. Opin. Cell Biol. 8:205–215; 1996.
85. Treisman, R. The SRE: A growth factor responsive transcriptional regulator. Semin. Cancer Biol. 1:47–58; 1990.
86. Wegner, M.; Cao, Z.; Rosenfeld, M. G. Calcium-regulated phosphorylation within the leucine zipper of C/EBPβ. Science 256:370–373; 1992.
87. Winitz, S.; Russel, M.; Qian, N. X.; Gardner, A.; Dwyer, L.; Johnson, G. L. Involvement of Ras and Raf in Gi-coupled acetylcholine muscarinic m2 receptor activation of mitogen-activated protein (MAP) kinase kinase and MAP kinase. J. Biol. Chem. 268:19196–19199; 1993.
88. Widmann, C.; Gibson, S.; Jarpe, M. B.; Johnson, G. L. Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human. Physiol. Rev. 79:143–180; 1999.
89. Woods, D.; Parry, D.; Cherwinski, H.; Bosch, E.; Lees, E.; McMahon, M. Raf-induced proliferation or cell cycle arrest is determined by the level of Raf activity with arrest mediated by p21Cip. Mol. Cell. Biol. 17: 5598–5611; 1997.
90. Yan, M.; Templeton, D. J. Identification of 2 serine residues of MEK-1 that are differentially phosphorylated during activation by raf and MEK kinase. J. Biol. Chem. 269:19067–19073; 1994.
91. Zhao, L.; Standaert, M. L.; Cooper, D. R.; Avignon, A.; Farese, R. V. Effects of insulin on protein-kinase C (PKC) in HIRC-B cells: Specific activation of PKC epsilon and its resistance to phorbol ester-induced down regulation. Endocrinology 135:2504–2510; 1994.
92. Zhang, F.; Strand, A.; Robbins, D.; Cobb, M. H.; Goldsmith, E. J. Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. Nature 367:704–710; 1994.
93. Zhang, H.; Shi, X.; Hampong, M.; Blanis, L.; Pelech, S. Stress-induced inhibition of ERK1 and ERK2 by direct interaction with p38 MAP kinase. J. Biol. Chem. 276:6905–6908; 2001.
94. Zheng, C. F.; Guan, K. L. Activation of MEK family kinases requires phosphorylation of two conserved Ser/Thr residues. EMBO J. 13:1123–1131; 1994.