

MOL Manuscript #12660

**Activation of mitogen-activated protein kinases by peroxisome proliferator-activated
receptor ligands: an example of non-genomic signaling^s**

Olivia S. Gardner, Brian J. Dewar, and Lee M. Graves

Curriculum in Toxicology and Department of Pharmacology

University of North Carolina at Chapel Hill

MOL Manuscript #12660

Running title: Non-genomic mechanism of PPAR ligand-induced MAPK activation

Corresponding author:

Lee M. Graves

CB# 7365, Department of Pharmacology
University of North Carolina at Chapel Hill

Chapel Hill, NC 27599

Tel: (919) 966-0915

Fax: (919) 966-5640

Email: lmg@med.unc.edu

Number of *text pages*: 13

Number of *figures*: 5

Number of *references*: 95

Number of *words in Abstract*: 114

Number of *words in Introduction*: 354

Abbreviations: PPAR, peroxisome proliferator-activated receptor; MAPK, mitogen-activated protein kinase; RXR, retinoic acid receptor; PPRE, peroxisome proliferator response element; MEK or MKK, mitogen-activated protein kinase kinase; Erk, extracellular signal-regulated kinase; 15dPGJ(2), 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂; JNK, c-jun N-terminal kinase; EGFR, epidermal growth factor receptor; Pyk2, proline-rich tyrosine kinase; CaMKII, calcium/calmodulin-dependent protein kinase II; ER, endoplasmic reticulum; PKR, double stranded RNA-activated protein kinase; eIF2 α , eukaryotic initiation factor 2 α

Abstract

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of nuclear hormone receptors that function as ligand-activated transcription factors to regulate lipid metabolism and homeostasis. In addition to their ability to promote gene transcription in a PPAR-dependent manner, ligands for this receptor family have recently been shown to induce mitogen-activated protein kinase (MAPK) phosphorylation. Interestingly, the transcriptional changes induced by PPAR ligands can be separated into distinct PPAR- and MAPK-dependent signaling pathways, suggesting that MAPKs alone mediate some of the effects of PPAR agonists in a non-genomic manner. This review will highlight recent studies that elucidate the non-genomic mechanisms of PPAR ligand-induced MAPK phosphorylation. The potential relevance of MAPK signaling in PPAR biology is also discussed.

Introduction

Peroxisome proliferator-activated receptors (PPARs) comprise a three-member subgroup (α , γ , and β/δ) within the nuclear hormone receptor family of ligand-activated transcription factors (Dreyer et al., 1992). As physiological lipid sensors and regulators of lipid metabolism, PPARs have recently emerged as attractive targets in the development of pharmaceutical agents to treat metabolic disorders, hypercholesterolemia, diabetes, inflammation, and cancer (Chinetti et al., 2000; Michalik et al., 2004; Vanden Heuvel, 1999). Indeed, ligands for PPARs have not only become a leading treatment for patients with type II diabetes and dyslipidemia, but also show promise as anti-inflammatory and anti-tumor drugs. While receptor activation was thought to be the primary mechanism of action of PPAR ligands, the direct role of PPARs in mediating these therapeutic effects has recently come under scrutiny. Specifically, PPAR α and γ agonists affect growth in cell types that lack their respective receptor (Palakurthi et al., 2001; Pauley et al., 2002). Similarly, while PPAR γ ligands improve insulin sensitivity by simultaneous, coordinated actions on adipose, muscle, and liver tissues (Evans et al., 2004; Picard and Auwerx, 2002), only adipose tissue expresses significant levels of PPAR γ (Vidal-Puig et al., 1996).

While it is well documented that PPAR ligands induce transcription of target genes in a PPAR-dependent manner, recent research has revealed that these drugs also elicit 'non-genomic', PPAR-independent effects. For example, PPAR ligands were shown to rapidly induce phosphorylation of mitogen-activated protein kinase (MAPK) family members (Lennon et al., 2002; Mounho and Thrall, 1999; Rokos and Ledwith, 1997; Teruel et al., 2003). This well characterized kinase family plays a pivotal role in signal transduction by relaying a variety of signals from the cell surface to the nucleus (Johnson and Lapadat, 2002). MAPKs are specifically involved in promoting cell growth and differentiation as well as coordinating

responses to cell stress. Importantly, the ability of PPAR ligands to activate MAPKs could contribute to their pharmacological mechanism of action and thus help to explain their apparent receptor-independent effects. This review will give a brief history of the relationship between PPARs and MAPKs, highlight the mechanisms of MAPK activation by PPAR ligands, and explain the potential relevance of kinase signaling to PPAR biology.

Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors were discovered as a class of receptors activated by a diverse group of rodent hepatocarcinogens (Issemann and Green, 1990). The name peroxisome proliferator-activated receptor was derived from the observation that these carcinogens induced proliferation of peroxisomes, the subcellular organelles primarily responsible for the oxidation of long chain fatty acids and subsequent detoxification of hydrogen peroxide. Since the cloning of PPAR α , two additional PPARs have been characterized: PPAR γ and PPAR β/δ . PPAR γ has been further subdivided into PPAR γ_1 , PPAR γ_2 , and PPAR γ_3 resulting from differential RNA splicing and alternative promoter use, giving rise to a total of five receptor isoforms (Fajas et al., 1997; Fajas et al., 1998).

PPARs possess the two characteristic functional domains present in all nuclear hormone receptors (Figure 1A), a superfamily that also includes receptors for estrogen, progesterone, vitamin D₃, and thyroid hormone among others (Evans, 1988; Issemann and Green, 1990). The N-terminal DNA-binding domain of PPARs contains two zinc fingers responsible for the recognition of specific hormone response elements within the promoter region of target genes, whereas the C-terminal domain controls ligand-dependent receptor activation and additional protein-protein interactions (Berg, 1989; Klug and Schwabe, 1995). The presence of these signature motifs indicates that PPARs function physiologically as nuclear hormone receptors or,

more specifically, intracellular ligand-activated transcription factors. Agonist binding to PPARs induces the formation of PPAR/retinoic acid receptor (RXR) heterodimers (Kliwer et al., 1992; Miyata et al., 1994), leading to the recruitment of cofactor proteins that facilitate the initiation of transcription (Xu et al., 1999). The active PPAR/RXR transcription complex binds to peroxisome proliferator response elements (PPREs), a direct repeat of two copies of a hexameric nucleotide sequence within the promoter region (Figure 1B), leading to transcription of these target genes (Tugwood et al., 1992).

PPAR α . PPAR α was identified following a screen of a mouse liver cDNA library for nuclear hormone receptors that were activated by a class of chemicals known as peroxisome proliferators (Issemann and Green, 1990). In addition to liver, this receptor is expressed in other highly metabolic tissues such as kidney, heart, skeletal muscle, and vascular smooth muscle cells where it functions specifically in the regulation of genes responsible for cellular uptake and β -oxidation of fatty acids (Braissant et al., 1996; Lee et al., 1995). Over 70 compounds, including synthetic hypolipidemic fibrate drugs (Wy-14,643, nafenopin, clofibrate), phthalate plasticizers (monoethylhexylphthalate), chlorinated hydrocarbons, and herbicides along with endogenous hormones and fatty acids (arachadonic acid), have been identified as PPAR α agonists (Table 1, Figure 2) (Citron, 1995; Kliwer et al., 1997; Krey et al., 1997; Zhou and Waxman, 1998).

PPAR γ . While no significant functional differences have been reported for the PPAR γ isoforms, expression of these variants is tissue specific. Similar to PPAR α , PPAR γ_1 is expressed to some extent in highly metabolic tissues whereas PPAR γ_2 is found nearly exclusively and at much higher levels in adipose tissue (Fajas et al., 1997). PPAR γ_3 is also expressed in adipose tissue as well as the colon and macrophages (Fajas et al., 1998). In addition to its ability to affect cellular energy homeostasis, PPAR γ is unique among this subfamily of nuclear hormone

receptors in that its main physiological function is to regulate adipocyte differentiation as well as insulin sensitivity (Masugi et al., 1999; Tontonoz et al., 1994).

In addition to its ability to influence fat cell proliferation, growth inhibitory actions of PPAR γ have also been observed in breast cancer cells (Mueller et al., 1998), advanced liposarcoma (Demetri et al., 1999), and most notably colon cancer (Sarraf et al., 1998). The role of PPAR γ in maintaining glycemic control, however, was not fully appreciated until it was shown that the antidiabetic and insulin-sensitizing agents known as thiazolidinediones (*e.g.*, ciglitazone and troglitazone) were high affinity PPAR γ ligands (Figure 3A) (Lehmann et al., 1995). The ability of thiazolidinediones to activate PPAR γ is thought to be a primary mechanism by which these agents exert their anti-diabetic effects. In addition to the synthetic thiazolidinediones, PPAR γ is also activated by endogenous ligands such as the eicosanoid 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (Kliewer et al., 1997; Krey et al., 1997).

PPAR β/δ . Expression of this PPAR isoform is ubiquitous and often found at higher levels than PPAR α and γ {Braissant, 1996 #46}. The biological functions of PPAR β/δ are the least understood of the three PPARs and no PPAR β/δ target genes have been identified. Moreover, a PPAR β/δ -selective ligand has yet to be discovered. Evidence collected thus far suggests that PPAR β/δ , like PPAR γ , appears to play a critical role in colon cancer and potentiates PPAR γ -stimulated adipocyte differentiation (Harman et al., 2004; Matsusue et al., 2004). Recently, targeted activation of PPAR β/δ in mice resulted in complete resistance to both high-fat, diet-induced and genetically predisposed obesity (Wang et al., 2003), suggesting that this receptor isoform is also important in the regulation of lipid metabolism.

PPAR ligands induce receptor independent signaling

Clearly the presence of PPARs in numerous tissues as well as the vast array of ligands

MOL Manuscript #12660

capable of activating these receptors suggests that PPARs play a critical role in maintaining normal cellular function on multiple levels. Interestingly, studies have shown that the effects of PPAR agonists are even more complex as these compounds not only induce receptor-mediated transcription, but also appear to exert PPAR-independent or ‘non-genomic’ effects. For example, the carcinogenicity of PPAR α agonists does not always correlate with the degree of peroxisome proliferation (Marsman et al., 1988), a PPAR α -dependent event (Lee et al., 1995). In addition, PPAR α ligands were found to induce expression of immediate early genes in cell lines that did not express this receptor (Pauley et al., 2002).

Similarly, while treatment with the thiazolidinedione class of PPAR γ ligands has been directly associated with up-regulation of insulin sensitizing genes and coordinate inhibition of genes promoting insulin resistance (Hofmann et al., 1994; Ribon et al., 1998; Smith et al., 2001), much still remains unclear as to how activation of PPAR γ improves insulin sensitivity. For example, the specific target tissue(s) of thiazolidinediones is unknown; candidates include adipose, skeletal muscle, and liver tissues as well as pancreatic beta cells (Evans et al., 2004). While each of these targets is significantly affected by thiazolidinediones, PPAR γ is expressed at disproportionately higher levels in adipocytes. Furthermore, the role of PPAR γ in mediating the growth inhibitory effects of thiazolidinediones has also been questioned for similar reasons. The PPAR γ agonists ciglitazone and troglitazone significantly inhibited cell growth to the same extent in PPAR $\gamma^{+/+}$ as well as PPAR $\gamma^{-/-}$ cell lines (Palakurthi et al., 2001).

While these apparently receptor-independent effects of PPAR ligands are contradictory to the classical mechanism of nuclear hormone receptor action, recent studies have implicated that a variety of other nuclear hormone receptor agonists exert similar non-genomic effects. For example, progesterone, estrogen, aldosterone, thyroid hormone, and vitamin D₃ have all been

shown to evoke rapid changes in signal transduction pathways that contribute to their biological mechanism of action (Losel and Wehling, 2003). In addition to the aforementioned effects, PPAR α and γ agonists, like other nuclear hormone receptor ligands, were recently shown to activate members of the MAPK family at times too rapid to account for new protein synthesis (Lennon et al., 2002; Mounho and Thrall, 1999; Rokos and Ledwith, 1997; Teruel et al., 2003). Since MAPKs themselves are known transcriptional regulators (Johnson and Lapadat, 2002), this finding provides a possible mechanism explaining how PPAR agonists are able to induce cellular effects in a PPAR-independent manner.

Activation of MAPKs by PPAR ligands

MAPKs alter the activity of a diverse array of transcription factors and the expression of their conjugate genes and represent one of the major cellular mechanisms effecting gene expression. There are currently four main groups of MAPKs in mammalian cells: extracellular signal regulated kinase (ERK), c-jun N-terminal kinase (JNK), p38, and extracellular signal regulated kinase-5 [ERK5, or Big MAP kinase-1 (BMK1)] (reviewed in (Lewis et al., 1998)). Alternate splicing variants of pre-mRNAs results in a number of gene products and isoforms within each group. Activation of ERK occurs in response to mitogenic stimuli such as growth factors and hormones, while stress stimuli predominantly activate JNK and p38. ERK5 activation occurs in response to both stress stimuli and growth factors (Kyriakis and Avruch, 2001). In addition to the direct regulation of transcription factors through phosphorylation, MAPKs additionally affect other kinases, including ribosomal S6 kinase (RSK), MAPK-interacting kinase (MNK), MAPK-activated protein kinase (MAPKAP) and mitogen and stress-activated protein kinase (MSK), which in turn regulate gene expression through phosphorylation of histones and transcriptional regulatory proteins (Kyriakis and Avruch, 2001; Lewis et al.,

1998). For a detailed list of transcriptional targets regulated by MAPK pathways see (Yang et al., 2003). The ability of PPAR ligands to activate MAPKs independent of their ability to modulate PPAR-dependent genes consequentially implies that numerous other target genes may be subject to transcriptional control by PPAR ligands.

Both PPAR α and γ ligands have been shown to activate MAPK family members in a variety of different cell types (Table 2). MAPK activation is thought to facilitate some of the pharmacological as well as toxicological effects associated with these agents. Specifically, the PPAR α agonists Wy-14,643, monoethylhexylphthalate, and clofibrate induced mitogen-activated protein kinase kinase (MEK or MKK) and extracellular-signal regulated kinase (Erk) phosphorylation in mouse liver cells (Rokos and Ledwith, 1997). In addition, nafenopin was demonstrated to activate Erk as well as p38 in primary rat hepatocytes (Cosulich et al., 2000). Inhibition of MAPK signaling in these models prevented both increases in immediate early gene expression and DNA synthesis suggesting that MAPKs play an important role in promoting the hepatoproliferative effects of PPAR α ligands. Similarly, the PPAR γ agonist rosiglitazone was shown to increase uncoupling protein-1 expression in fetal rat brown adipocytes via a p38 MAPK-dependent pathway (Teruel et al., 2003). Uncoupling proteins are involved in the control of energy expenditure in response to nutritional status and are known PPAR target genes (Sears et al., 1996). Furthermore, the anti-proliferative effect of troglitazone on colon cancer cell growth was demonstrated to require Erk (Baek et al., 2003; Kim et al., 2002). Specifically, troglitazone-dependent induction of early growth response-1, a transcription factor linked to apoptosis, was prevented by Erk inhibition. Similarly, the MEK inhibitor PD98059 prevented troglitazone-induced p21^{Cip/WAF1} translocation to the nucleus, an event correlated with reduced cell viability in this model. Troglitazone-induced hepatotoxicity in a human liver cell line was

also shown to involve c-jun N-terminal kinase (JNK) (Bae and Song, 2003). Clearly, the ability of PPAR ligands to activate MAPKs plays an important role in mediating the biological effects of these compounds.

Mechanisms of MAPK Activation by PPAR ligands

Interestingly, studies with MAPK inhibitors have revealed that some of the transcriptional changes induced by PPAR agonists can be dissociated into distinct MAPK- and PPAR-dependent pathways (Baek et al., 2003). This suggests that PPAR ligand-dependent PPAR activation alone cannot account for simultaneous MAPK phosphorylation. Additional evidence also suggests that MAPK and PPAR activation by PPAR agonists are separate events. For example, ciglitazone activated Erk, p38, and JNK in astrocytes whereas rosiglitazone, a structurally similar PPAR γ ligand, failed to induce phosphorylation of any of these kinases in this model (Lennon et al., 2002). While kinase activation by PPAR agonists thus appears to play an important role in the mechanism of action of these compounds, few studies have investigated the potential non-genomic mechanism responsible for MAPK phosphorylation by PPAR ligands. An understanding of this mechanism is necessary due to the widespread and clinical use of these agents. MAPK expression is also ubiquitous; thus, these ligands have the potential to induce effects in multiple cell types regardless of PPAR expression.

The epidermal growth factor receptor. A classical mechanism for Erk activation is dependent upon the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (Prenzel et al., 2001). Typically, an extracellular ligand (*i.e.*, epidermal growth factor or EGF) binds to the EGFR leading to receptor autophosphorylation on multiple tyrosine residues, which is followed by activation of downstream kinase signaling cascades (Ullrich and Schlessinger, 1990). In addition to its role in relaying EGF-dependent signals to the cytosol, the EGFR has

recently emerged as a critical transducer of intracellular signals in the absence of physiological ligands via a mechanism termed EGFR ‘transactivation’ (Carpenter, 1999; Zwick et al., 1999). Interestingly, Pauley *et al.* recently noted that a nonspecific EGFR kinase inhibitor blocked Erk activation by the PPAR α agonist Wy-14,643, suggesting possible crosstalk between the EGFR and PPAR ligand-induced signaling (Pauley et al., 2002). Furthermore, it has been proposed that EGF and PPAR α ligands act synergistically to promote the clonal expansion of hepatocytes (James and Roberts, 1994). Collectively, these studies suggest a potential role for the EGFR in mediating MAPK activation by PPAR ligands.

Increases in intracellular calcium. In addition to the EGFR, increases in intracellular calcium are also associated with MAPK phosphorylation as a number of protein kinases are directly affected by changes in calcium homeostasis. The calcium-regulated protein kinase C family of serine/threonine kinases can activate Erk through both Raf-dependent as well as Raf-independent mechanisms (Cobb and Goldsmith, 1995). Similarly, the calcium-activated proline-rich tyrosine kinase or Pyk2 as well as calcium/calmodulin-dependent kinase II (CaMKII) are known to be an upstream activators of p38 in glomerular mesangial cells and neurons, respectively (Pandey et al., 1999; Sorokin et al., 2001; Takeda et al., 2004). Moreover, expression of a calcium-dependent tyrosine kinase such as Pyk2 was shown to link calcium signals to JNK activation in rat liver epithelial cells (Zohn et al., 1995). Interestingly, exposure of different cell types to PPAR ligands was observed to cause increases in intracellular calcium. Specifically, treatment of macrophages with the PPAR α agonist Wy-14,643 led to an influx of extracellular calcium (R.G. Thurman, unpublished observation), an ability that was correlated with Wy-14,643-dependent protein kinase C activation (Rose et al., 1999). The PPAR γ ligands ciglitazone and troglitazone were recently shown to increase intracellular calcium by directly

promoting its release from the endoplasmic reticulum (ER), initiating a stress response (Palakurthi et al., 2001). For a detailed summary of ER stress, the reader is referred to the following review articles (Rutkowski and Kaufman, 2004; Zhang and Kaufman, 2004). Interestingly, this ER-associated stress response has been coupled to activation of JNK (Nishitoh et al., 2002) as well as p38 (Yamamoto et al., 2003). Thus, while the ability of these agents to mobilize calcium is clear, it remains unknown whether such effects are important for PPAR ligand-dependent MAPK activation.

PPAR-independent activation of MAPK signaling pathways by PPAR ligands in liver epithelial cells

Using GN4 rat liver epithelial cells, our laboratory has recently shown that PPAR α and γ ligands activate two, distinct kinase signaling cascades that culminate in either Erk or p38 MAPK phosphorylation (Gardner et al., 2003). Consistent with earlier studies suggesting a connection between the EGFR and MAPK signaling by PPAR α ligands (James and Roberts, 1994; Orellana et al., 1993; Pauley et al., 2002), inhibition of EGFR kinase activity prevented Erk activation by both PPAR α and γ agonists. In addition to PPAR α ligands, these studies also provided the first evidence that PPAR γ agonists influence Erk activity through specific phosphorylation of the EGFR. Further investigation into this mechanism of EGFR-dependent Erk activation revealed that PPAR ligands transactivate the EGFR, a process that requires Src and reactive oxygen species (Gardner et al., 2003). While other kinases such as protein kinase C (Shah and Catt, 2002) as well as proteolytic cleavage of diffusible EGF-like ligands by metalloproteases (Prenzel et al., 1999) have been associated with EGFR transactivation, there was no evidence to suggest that these mechanisms were important for EGFR phosphorylation in response to PPAR ligands (Gardner, unpublished data). Other mechanisms, such as the possible

role of Pyk2 and changes in intracellular calcium concentration remain undetermined. Furthermore, expression of a dominant negative Ras as well as inhibition of MEK1/2 with U0126 blunted PPAR agonist-induced Erk phosphorylation without an observable effect on the EGFR. Collectively, this evidence suggests that PPAR α and γ ligands promote Src-dependent EGFR transactivation in GN4 cells leading to downstream Ras, MEK, and ultimately Erk phosphorylation (Figure 4).

In addition to Erk, PPAR α and γ ligands also activated p38 GN4 cells (Gardner et al., 2003). Interestingly, inhibition of EGFR signaling had no effect on the ability of these agents to induce p38 phosphorylation suggesting that two signaling pathways facilitate PPAR agonist-dependent MAPK activation in this model (Figure 5). This hypothesis is supported by the observation that PPAR ligand-dependent p38 activation requires increases in intracellular calcium, CaMKII, and MKK3/6, signaling components whose inhibition conversely did not prevent EGFR and Erk phosphorylation by these compounds (Gardner et al., 2005). Further examination into the mechanism responsible for calcium-dependent p38 activation revealed that PPAR γ ligands induce ER stress in GN4 cells. Collectively, these data not only suggest that the ER is the likely source of calcium necessary for PPAR agonist-dependent CaMKII and downstream p38 activation, but also provide novel, mechanistic evidence that induction of ER stress and p38 phosphorylation are potentially tightly-coupled signaling events. Specifically, both CaMKII and the ER stress-sensitive kinase PKR or double stranded RNA-activated protein kinase were required for PPAR γ ligand-induced p38 as well as eukaryotic initiation factor 2 α (eIF2 α) phosphorylation. Furthermore, the data suggest that CaMKII may regulate PKR, which then leads to downstream phosphorylation of both p38 and eIF2 α . Future studies are needed to characterize the specific role of CaMKII in PKR activation.

MAPK activation by PPAR agonists in liver epithelial cells was both rapid and transient as the onset of kinase phosphorylation was first observed as early as 5 minutes after exposure to these drugs and supports previous ideas that nuclear hormone receptor ligands have non-genomic, PPAR-independent signaling effects (Losel and Wehling, 2003). Studies utilizing the protein synthesis inhibitor cycloheximide, pharmacological antagonists of PPAR α and γ , as well as thiazolidinedione structural derivatives that lack receptor ligand-binding activity (Figure 3B) (Δ 2-troglitazone, Δ 2-ciglitazone) were unable to demonstrate a necessary role for PPAR transcriptional activity in MAPK phosphorylation (Gardner et al., 2003; Gardner et al., 2005). Moreover, the ability of certain thiazolidinediones (ciglitazone and troglitazone) but not other higher affinity PPAR γ ligands (rosiglitazone and pioglitazone) to selectively activate MAPKs in this model also speaks to the PPAR-independent nature of these signaling events. Together, these data provide further evidence to support the current hypothesis that nuclear hormone receptor ligands possess unanticipated and diverse signaling capacity that must be recognized and appreciated in order to fully understand their mechanism of action.

Potential biological significance of MAPK activation by PPAR ligands

As MAPKs are well-known growth regulators (Johnson and Lapadat, 2002), the ability of PPAR α and γ ligands to activate members of this kinase family may be important for their effects on cell proliferation. Indeed, PPAR α agonist-dependent increases in immediate early gene expression were previously shown to require Erk (Rokos and Ledwith, 1997). Our work elaborates on this mechanism and identifies a key role for the EGFR in triggering downstream Erk phosphorylation. In contrast to PPAR α ligands, the thiazolidinedione class of PPAR γ agonists promotes growth inhibition by a mechanism that is not well understood. Interestingly, the ability of thiazolidinediones to promote ER stress as well as down-regulate G1 cell cycle

MOL Manuscript #12660

regulators was previously correlated with decreased cell viability (Palakurthi et al., 2001). Our work not only supports these earlier findings, but also suggests that ER stress and p38 signaling are part of the same pathway in liver epithelial cells. It is thus possible to speculate that MAPKs play a role in growth inhibition by PPAR γ ligands. Future studies are necessary to determine a causal role for p38 in this mechanism.

In addition to their potential role in influencing PPAR ligand-dependent changes in cell proliferation, all three of the well-known MAPK family members (Erk, p38, and JNK) are known to phosphorylate PPARs leading to changes in transcriptional activity. Specifically, Erk- and p38-dependent PPAR α phosphorylation resulted in increased transcriptional activity (Barger et al., 2001; Juge-Aubry et al., 1999), whereas PPAR γ phosphorylation by MAPKs decreased transcriptional activity (Camp et al., 1999; Hu et al., 1996). These data are intriguing as they suggest that PPAR agonists not only directly activate PPARs via ligand binding, but also stimulate kinases that indirectly modulate receptor activity. This may be a universal feature for a number of nuclear receptors as extensive work has shown that in addition to the ability of estrogen to bind and activate the estrogen receptor, activation of MAPKs or coactivators occurs, thereby amplifying the transcriptional activity of the estrogen receptor (Coleman and Smith, 2001). The identification of genes up- or down-regulated in response to PPAR phosphorylation by MAPKs as well as how MAPK-dependent changes in receptor activity influence the biological effects of PPAR ligands requires further study. This presents an exciting new area of research that has only been initially explored in the literature. In light of the current studies, it would be of interest to determine whether the mechanisms of MAPK activation by PPAR agonists described herein also contribute to MAPK-dependent PPAR phosphorylation.

Conclusions

The ability of PPAR ligands to activate MAPK signaling pathways is an attractive hypothesis to explain, at least in part, how these agents induce non-genomic effects. Yet, additional studies provide evidence that the non-genomic actions of PPAR ligands are not limited to influencing MAPK activity. Thiazolidinediones inhibit the production of inflammatory mediators in both wild-type and PPAR γ -deficient macrophages (Chawla et al., 2001) and inhibit pancreatic cell invasiveness via mechanisms that involve matrix metalloproteases and plasminogen activator inhibitor-1 as opposed to PPAR γ (Galli et al., 2004). Both troglitazone and ciglitazone and the $\Delta 2$ -derivatives were recently shown to specifically ablate cyclin D1 protein in breast cancer cells (Huang et al., 2005). Similarly $\Delta 2$ -ciglitazone and $\Delta 2$ -troglitazone induced apoptosis of PPAR γ expressing (PC-3) and PPAR γ deficient (LNCaP) prostate cancer cell lines through effects on Bcl-x1 and Bcl-2 (Shiau et al., 2005). Thus, it is becoming clear that PPAR ligands have the potential to induce a variety of different effects in multiple cell types irrespective of their ability to act as nuclear hormone receptor agonists. An understanding of these additional PPAR-independent signaling pathways is necessary as these mechanisms would support the use of PPAR ligands for not only treating metabolic disorders but also as therapies for inflammation and cancer.

Acknowledgments

[§]This work was supported by the Susan G. Komen Breast Cancer Foundation (O.S.G.) and the Environmental Protection Agency Science to Achieve Results Award (L.M.G.).

References

- Bae MA and Song BJ (2003) Critical role of c-Jun N-terminal protein kinase activation in troglitazone-induced apoptosis of human HepG2 hepatoma cells. *Mol Pharmacol* **63**:401-408.
- Baek SJ, Wilson LC, Hsi LC and Eling TE (2003) Troglitazone, a peroxisome proliferator-activated receptor γ (PPAR γ) ligand, selectively induces the early growth response-1 gene independently of PPAR γ . *J Biol Chem* **278**:5845-5853.
- Barger P, Browning A, Garner A and Kelly D (2001) p38 Mitogen-activated protein kinase activates peroxisome proliferator-activated receptor α . *J Biol Chem* **276**:44495-44501.
- Berg JM (1989) DNA binding specificity of steroid hormone receptors. *Cell* **57**:1065-1068.
- Braissant O, Fougelle F, Scotto C, Dauca M and Wahli W (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**:354-366.
- Camp HS, Tafuri SR and Leff T (1999) c-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor- γ 1 and negatively regulates its transcriptional activity. *Endocrinology* **140**:392-397.
- Carpenter G (1999) Employment of the epidermal growth factor receptor in growth factor-independent signaling pathways. *J Cell Biol* **146**:697-702.
- Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P and Evans RM (2001) PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med* **7**:48-52.

MOL Manuscript #12660

Chinetti G, Fruchart J-C and Staels B (2000) Peroxisome proliferator-activated receptors

(PPARs): Nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* **49**:497-505.

Citron M (1995) Perplexing peroxisome proliferators. *Environ Health Perspect* **103**:232-235.

Cobb MH and Goldsmith EJ (1995) How MAP kinases are regulated. *J Biol Chem* **270**:14843-14846.

Coleman KM and Smith CL (2001) Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. *Front Biosci* **6**:D1379-91.

Cosulich S, James N and Roberts RA (2000) Role of MAP kinase signaling pathways in the mode of action of peroxisome proliferators. *Carcinogenesis* **21**:579-584.

Demetri GD, Fletcher C, Mueller E, Sarraf P, Naujoks R, Campbell N, Spiegelman BM and Singer S (1999) Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma. *PNAS* **96**:3951-3956.

Devchand PR, Keller H, Peters JM, Varquez M, Gonzalez FJ and Wahli W (1996) The PPAR α -leukotriene B₄ pathway to inflammation control. *Nature* **384**:39-43.

Dreyer C, Krey G, Keller H, Givel F, Helftenbein G and Wahli W (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **68**:879-887.

Evans RM (1988) The steroid hormone and thyroid hormone receptor superfamily: transcriptional regulators of development and physiology. *Science* **240**:889-895.

MOL Manuscript #12660

Evans RM, Barish GD and Wang YX (2004) PPARs and the complex journey to obesity. *Nat Med* **10**:1-7.

Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H and Auwerx J (1997) The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* **272**:18779-18789.

Fajas L, Fruchart JC and Auwerx J (1998) PPARgamma3: A distinct PPARgamma mRNA subtype transcribed from an independent promoter. *FEBS Lett* **438**:55-60.

Fujita T, Sugiyama Y, Taketomi S, Sohda T, Kawamatsu Y, Iwatsuka H and Suzjoka Z (1983) Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexyl-methoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone), a new antidiabetic agent. *Diabetes* **32**:804-810.

Galli A, Ceni E, Crabb DW, Mello T, Salzano R, Grappone C, Milani S, Surrenti E, Surrenti C and Casini A (2004) Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARgamma independent mechanisms. *Gut* **53**:1688-1697.

Gardner OS, Dewar BJ, Earp HS, Samet JM and Graves LM (2003) Dependence of peroxisome proliferator-activated receptor ligand-induced mitogen-activated protein kinase signaling on epidermal growth factor transactivation. *J Biol Chem* **278**:46261-46269.

Gardner OS, Shiau CW, Chen CS and Graves LM (2005) Peroxisome proliferator-activated receptor gamma-independent activation of p38 MAPK by thiazolidinediones involves calcium/calmodulin-dependent protein kinase II and protein kinase R: correlation with endoplasmic reticulum stress. *J Biol Chem* **280**:10109-18.

MOL Manuscript #12660

Harman FS, Nicol CJ, Marin HE, Ward JM, Gonzalez FJ and Peters JM (2004) Peroxisome proliferator-activated receptor-delta attenuates colon carcinogenesis. *Nat Med* **10**:481-483.

Hofmann C, Lorenz K, Braithwaite S, Colca J, Palazuk B, Hotamisligil G and Spiegelman BM (1994) Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* **134**:264-270.

Hu E, Kim JB, Sarraf P and Spiegelman BM (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPAR γ . *Science* **274**:2100-2103.

Huang JW, Shiau CW, Yang YT, Kulp SK, Chen KF, Brueggemeier RW, Shapiro CL and Chen CS (2005) Peroxisome proliferator-activated receptor gamma-independent ablation of cyclin D1 by thiazolidinediones and their derivatives in breast cancer cells. *Mol Pharmacol* **67**:1342-8.

Huang W, Chio C, Chi K, Wu H and Lin W (2002) Superoxide anion-dependent Raf/MEK/ERK activation by peroxisome proliferator activated receptor γ agonists 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, ciglitazone, and GW1929. *Exp Cell Res* **277**.

Issemann I and Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**:645-650.

James NH and Roberts RA (1994) The peroxisome proliferator class of non-genotoxic hepatocarcinogens synergize with epidermal growth factor to promote clonal expansion of initiated rat hepatocytes. *Carcinogenesis* **15**:2687-2694.

Johnson GL and Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* **298**:1911-1912.

MOL Manuscript #12660

Juge-Aubry CE, Hammar E, Siegrist-Kaiser CA, Pernin A, Takeshita A, Chin WW, Burger AG and Meier CA (1999) Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor α by phosphorylation of a ligand-independent *trans*-activating domain. *J Biol Chem* **274**:10505-10510.

Jung KM, Park KS, Oh JH, Jung SY, Yang KH, Song YS, Son DJ, Park YH, Yun YP, Lee MK, Oh KW and Hong JT (2003) Activation of p38 mitogen-activated protein kinase and activator protein-1 during the promotion of neurite extension of PC-12 cells by 15-deoxy-delta12,14-prostaglandin J2. *Mol Pharmacol* **63**:607-16.

Kim JA, Park KS, Kim HI, Oh SY, Ahn Y, Oh JW and Choi KY (2002) Troglitazone activates p21^{Cip/WAF1} through the ERK pathway in HCT15 human colorectal cancer cells. *Cancer Lett* **179**:185-195.

Kliwer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand PR, Wahli W, Willson TM, Lenhard JM and Lehmann JM (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *PNAS* **94**:4318-4323.

Kliwer SA, Umesono K, Noonan DJ, Heyman RA and Evans RM (1992) Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. *Nature* **358**:771-774.

Klug A and Schwabe JWR (1995) Protein motifs 5. Zinc fingers. *FASEB J* **9**:597-604.

Kohlroser J, Mathai J, Reichheld J, Banner BF and Bonkovsky HL (2000) Hepatotoxicity due to troglitazone: report of two cases and review of adverse events reported to the United States Food and Drug Administration. *Am J Gastroenterol* **95**:272-276.

MOL Manuscript #12660

Krey G, Braissant O, L'Horsset F, Kalkhoven E, Perroud M, Parker MG and Wahli W (1997)

Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* **11**:779-791.

Kyriakis JM and Avruch J (2001) Mammalian mitogen-activated protein kinase signal

transduction pathways activated by stress and inflammation. *Physiol Rev* **81**:807-869.

Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H

and Gonzalez FJ (1995) Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* **15**:3012-3022.

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM and Kliewer SA (1995)

An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* **270**:12953-12956.

Lennon AM, Ramage M, Dessouroux A and Pierre M (2002) MAP kinase cascades are

activated in astrocytes and preadipocytes by 15-deoxy- Δ^{12-14} -prostaglandin J₂ and the thiazolidinedione ciglitazone through peroxisome proliferator-activated receptor γ -independent mechanisms involving reactive oxygenated species. *J Biol Chem* **277**:29681-29685.

Lewis TS, Shapiro PS and Ahn NG (1998) Signal transduction through MAP kinase cascades.

Adv Cancer Res **74**:49-139.

Losel R and Wehling M (2003) Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol*

4:46-56.

MOL Manuscript #12660

- Marsman DS, Cattley RC, Conway JG and Popp JA (1988) Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (Wy-14,643) in rats. *Cancer Res* **48**:6739-6744.
- Masugi J, Tamori Y and Kasuga M (1999) Inhibition of adipogenesis by a COOH-terminally truncated mutant of PPAR γ 2 in 3T3-L1 cells. *Biochem Biophys Res Commun* **264**:93-99.
- Matsusue K, Peters JM and Gonzalez FJ (2004) PPARbeta/delta potentiates PPARgamma-stimulated adipocyte differentiation. *FASEB J* **18**:1477-1479.
- Michalik L, Desvergne B and Wahli W (2004) Peroxisome proliferator-activated receptors and cancer: complex stories. *Nat Rev Cancer* **4**:61-70.
- Miyata KS, McCaw SE, Marcus SL, Rachubinski RA and Capone JP (1994) The peroxisome proliferator-activated receptor interacts with the retinoid X receptor in vivo. *Gene* **148**:327-330.
- Mounho BJ and Thrall BD (1999) The extracellular signal-regulated kinase pathway contributes to mitogenic and antiapoptotic effects of peroxisome proliferators in vitro. *Toxicol Appl Pharmacol* **159**:125-133.
- Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M, Fletcher C, Singer S and Spiegelman BM (1998) Terminal differentiation of human breast cancer through PPAR gamma. *Mol Cell* **1**:465-470.
- Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, Hori S, Kakizuka A and Ichijo H (2002) ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* **16**:1345-1355.

MOL Manuscript #12660

- Orellana A, Holuigue L, Hidalgo PC, Faundez V, Gonzalez A and Bronfman M (1993) Ciprofibrate, a carcinogenic peroxisome proliferator, increases the phosphorylation of epidermal growth factor receptor in isolated rat hepatocytes. *Eur J Biochem* **215**:903-906.
- Palakurthi SS, Aktas H, Grubissich LM, Mortensen RM and Halperin JA (2001) Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor γ and mediated by inhibition of translation initiation. *Cancer Res* **61**:6213-6218.
- Pandey P, Avraham S, Kumar S, Nakazawa A, Place A, Ghanem L, Rana A, Kumar V, Majumder PK, Avraham H, Davis RJ and Kharbanda S (1999) Activation of p38 mitogen-activated protein kinase by Pyk2/related focal adhesion tyrosine-kinase dependent mechanism. *J Biol Chem* **274**:10140-10144.
- Pauley CJ, Ledwith BJ and Kaplanski C (2002) Peroxisome proliferators activate growth regulatory pathways largely via peroxisome proliferator-activated receptor alpha-independent mechanisms. *Cell Signal* **14**:351-358.
- Picard F and Auwerx J (2002) PPAR γ and glucose homeostasis. *Annu Rev Nutr* **22**:167-97.
- Prenzel N, Fischer OM, Streit S, Hart S and Ullrich A (2001) The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. *Endocr Relat Cancer* **8**:11-31.
- Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C and Ullrich A (1999) EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**:884-888.
- Rao GN, Alexander RW and Runge MS (1995) Linoleic acid and its metabolites, hydroperoxyoctadecadienoic acids, stimulate c-Fos, c-Jun, and c-Myc mRNA expression,

- mitogen-activated protein kinase activation, and growth in rat aortic smooth muscle cells. *J Clin Invest* **96**:842-7.
- Ribon V, Johnson J, Camp HS and Saltiel A (1998) Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor gamma activation stimulates expression of the CAP gene. *PNAS* **95**:14751-14756.
- Rokos CL and Ledwith BJ (1997) Peroxisome proliferators activate extracellular signal-regulated kinases in immortalized mouse liver cells. *J Biol Chem* **272**:13452-13457.
- Rose M, Rivera C, Bradford B, Graves LM, Cattley RC, Schoonhoven R, Swenberg J and Thurman RG (1999) Kupffer cell oxidant production is central to the mechanism of action of peroxisome proliferators. *Carcinogenesis* **20**:27-33.
- Rutkowski DT and Kaufman RJ (2004) A trip to the ER: coping with stress. *Trends Cell Biol* **14**:20-28.
- Sarraf P, Mueller E, Jones D, King F, DeAngelo D, Partridge J, Holden S, Chen L, Singer S, Fletcher C and Spiegelman BM (1998) Differentiation and reversal of malignant changes in colon cancer through PPARgamma. *Nat Med* **4**:1046-1052.
- Scheen AJ (2001) Thiazolidinediones and liver toxicity. *Diabetes Metab* **27**:305-313.
- Sears IB, MacGinnitie MA, Kovacs LG and Graves RA (1996) Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. *Mol Cell Biol* **16**:3410-3419.
- Shah BH and Catt KJ (2002) Calcium-independent activation of extracellularly regulated kinases 1 and 2 by angiotensin II in hepatic C9 cells: roles of protein kinase C δ , Src/proline-rich tyrosine kinase 2, and epidermal growth factor receptor *trans*-activation. *Mol Pharmacol* **61**:343-351.

MOL Manuscript #12660

- Shiau CW, Yang CC, Kulp SK, Chen KF, Chen CS and Huang JW (2005) Thiazolidinediones mediate apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 functions independently of PPARgamma. *Cancer Res* **65**:1561-9.
- Smith U, Gogg S, Johansson A, Olausson T, Rotter V and Svalstedt B (2001) Thiazolidinediones (PPARgamma agonists) but not PPARalpha agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. *FASEB J* **15**:215-220.
- Sohda T, Mizuno K, Imamiya E, Sugiyama Y, Fujita T and Kawamatsu Y (1982) Studies on antidiabetic agents. II. Synthesis of 5-[4-(1-methylcyclohexylmethoxy-benzyl)]thiazolidine-2,4-dione (ADD-3878) and its derivatives. *Chem Pharm Bull* **30**:3580-3600.
- Sorokin A, Kozlowski P, Graves LM and Philip A (2001) Protein-tyrosine kinase Pyk2 mediates endothelin-induced p38 MAPK activation in glomerular mesangial cells. *J Biol Chem* **276**:21521-21528.
- Takeda K, Ichiki T, Tokunou T, Iino N and Takeshita A (2001) 15-Deoxy-delta 12,14-prostaglandin J2 and thiazolidinediones activate the MEK/ERK pathway through phosphatidylinositol 3-kinase in vascular smooth muscle cells. *J Biol Chem* **276**:48950-5.
- Takeda K, Matsuzawa A, Nishitoh H, Tobiume K, Kishida S, Ninomiya-Tsuji J, Matsumoto K and Ichijo H (2004) Involvement of ASK1 in Ca²⁺-induced p38 MAP kinase activation. *EMBO Rep* **5**:161-166.
- Teruel T, Hernandez R, Benito M and Lorenzo M (2003) Rosiglitazone and retinoic acid induce uncoupling protein-1 (UCP-1) in a p38 mitogen-activated protein kinase-dependent manner in fetal primary brown adipocytes. *J Biol Chem* **278**:263-269.

MOL Manuscript #12660

- Tontonoz P, Hu E and Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid activated transcription factor. *Cell* **79**:1147-1156.
- Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL and Green S (1992) The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *EMBO J* **11**:433-439.
- Ullrich A and Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**:203-212.
- Vanden Heuvel JP (1999) Peroxisome proliferator-activated receptors: a critical link between among fatty acids, gene expression, and carcinogenesis. *J Nutr* **129**:575S-580S.
- Vidal-Puig A, Jimenez-Linan M, Lowell BB, Hamann A, Hu E, Spiegelman BM, Flier JS and Moller DE (1996) Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J Clin Invest* **97**:2553-2561.
- Walgren JE, Kurtz DT and McMillan JM (2000) Expression of PPAR(alpha) in human hepatocytes and activation by trichloroacetate and dichloroacetate. *Res Commun Mol Pathol Pharmacol* **108**:116-132.
- Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H and Evans R (2003) Peroxisome proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* **113**:159-170.
- Watkins PB and Whitcomb RW (1998) Hepatic dysfunction associated with troglitazone. *N Engl J Med* **338**:916-917.
- Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, Beck KD, Moore LB, Kliewer SA and Lehmann JM (1996) The structure-activity relationship between

- peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. *J Med Chem* **39**:665-668.
- Wilmer WA, Dixon C, Lu L, Hilbelink T and Rovin BH (2001) A cyclopentenone prostaglandin activates mesangial MAP kinase independently of PPARgamma. *Biochem Biophys Res Commun* **281**:57-62.
- Xu L, Glass CK and Rosenfeld MG (1999) Coactivator and corepressor complexes in nuclear receptor function. *Curr Opin Genet Dev* **9**:140-147.
- Yamamoto K, Hamada H, Shinkai H, Kohno Y, Koseki H and Aoe T (2003) The KDEL receptor modulates the endoplasmic reticulum stress response through mitogen-activated protein kinase signaling cascades. *J Biol Chem* **278**:34525-34532.
- Yang SH, Sharrocks AD and Whitmarsh AJ (2003) Transcriptional regulation by the MAP kinase signaling cascades. *Gene* **320**:3-21.
- Yin F, Bruemmer D, Blaschke F, Hsueh WA, Law RE and Herle AJ (2004) Signaling pathways involved in induction of GADD45 gene expression and apoptosis by troglitazone in human MCF-7 breast carcinoma cells. *Oncogene* **23**:4614-23.
- Zhang K and Kaufman RJ (2004) Signaling the unfolded protein response from the endoplasmic reticulum. *J Biol Chem* **279**:25935-25938.
- Zhou YC and Waxman DJ (1998) Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids. *Environ Health Perspect* **106 (Suppl 4)**:983-988.
- Zohn IE, Yu H, Li X, Cox AD and Earp HS (1995) Angiotensin II stimulates calcium-dependent activation of c-jun N-terminal kinase. *Mol Cell Biol* **15**:6160-6168.

Zwick E, Hackel PO, Prenzel N and Ullrich A (1999) The EGF receptor as a central transducer of heterologous signaling systems. *Trends Pharmacol Sci* **20**:408-412.

Legends for Figures

Figure 1. PPARs as nuclear hormone receptors. *A*, Functional domains of PPARs. *B*, Mechanism of ligand-dependent PPAR/RXR heterodimerization and DNA binding. In the absence of ligand (*L*), nuclear hormone receptors are maintained in the inactive state via interactions with co-repressors (**1**). Ligand binding to PPARs results in a conformational change leading to dissociation of co-repressors (*R*), heterodimerization of RXR, and recruitment of transcriptional co-activators (*A*)(**2**). The active transcription complex binds PPREs, two copies of a direct repeat hexanucleotide sequence, in the promoter region of target genes leading to increases in gene transcription (**3**).

Figure 2. PPAR α ligands. Chemical structures for synthetic PPAR α agonists such as Wy-14,643, nafenopin, and clofibrate, as well as arachidonic acid, a natural ligand, are shown. Despite noticeable structural diversity, most PPAR α agonists have a carboxylic acid group coupled to a large, hydrophobic side chain.

Figure 3. The thiazolidinedione class of PPAR γ ligands and structurally inactive derivatives. *A*, Thiazolidinediones are anti-diabetic agents that increase insulin sensitivity in adipose, liver, and muscle tissue in animals and human patients with non-insulin-dependent diabetes. Ciglitazone, a clofibrate (Figure 2) analog, was the first of these compounds to be synthesized (Sohda et al., 1982). While both clofibrate and ciglitazone had potent lipid-lowering

activity, ciglitazone was unique in that it also exhibited unexplained glucose-lowering properties (Fujita et al., 1983). This insulin sensitizing effect was later attributed to the ability of thiazolidinediones to bind and activate PPAR γ (Lehmann et al., 1995). Indeed, the ability of thiazolidinediones to bind PPAR γ in vitro correlates well with their anti-diabetic activity in vivo (Willson et al., 1996). Although the discovery of ciglitazone presented a promising treatment for type II diabetes, ciglitazone was abandoned as a potential therapy due to liver toxicity. Troglitazone (Rezulin®; Parke-Davis), a derivative of ciglitazone, was the first thiazolidinedione to demonstrate clinical efficacy, yet was promptly withdrawn from the market due to idiosyncratic hepatotoxicity and other mechanisms that remain unclear (Kohlroser et al., 2000; Watkins and Whitcomb, 1998). Rosiglitazone (Avandia®; GlaxoSmithKline) and pioglitazone (Actos®; Lilly), which are at least 100-fold more potent ligands for PPAR γ than troglitazone (Willson et al., 1996), are currently available in the U.S. While the ability of all these agents to activate PPAR γ and promote effective glycaemic control is clear, thiazolidinedione-specific effects have also been documented that may be a consequence of differing chemical structures and/or potency for PPAR γ . Interestingly, rosiglitazone and pioglitazone do not induce liver failure (Scheen, 2001). Also, rosiglitazone is metabolized differently than pioglitazone and troglitazone, greatly decreasing the potential for unwanted drug interactions. **B**, Structure of Δ 2-ciglitazone and Δ 2-troglitazone that completely lack PPAR γ binding capability (Shiau et al., 2005).

Figure 4. Mechanisms of epidermal growth factor receptor transactivation leading to Erk activation. A diverse array of signals has been shown to induce EGFR transactivation. Work from our laboratory provides evidence for a PPAR α and γ ligand activation of ROS/ Src-

MOL Manuscript #12660

dependent transactivation of EGFR leading to Erk activation through Ras. There is currently no evidence to suggest that additional mechanisms such as protein kinase C activation or proteolytic generation of diffusible EGF-like ligands by metalloproteases is important for EGFR transactivation in response to PPAR ligands.

Figure 5. Working hypothesis of thiazolidinedione-induced ER stress p38 activation.

PPAR ligands stimulate the release of intracellular calcium and activation of CaMKII, followed by subsequent activation of PKR, MKK3/6 and p38. Increases in intracellular calcium correlate with PPAR ligand-induced ER stress as seen by the activation of known ER stress signals PERK and eIF2 α . PPAR ligand-induced p38 activation is not dependent upon EGFR transactivation events. Activation of MAPK by PPAR γ ligands may alter gene transcription due to phosphorylation of PPARs or by activation of downstream p38 effectors.

Tables

Table 1. *PPAR α* agonists are a diverse class of natural and synthetic compounds.

Class of compound	Name	Reference
<i>Hypolipidemic fibrates</i>	Clofibrate	(Krey et al., 1997)
	Gemfibrozil	(Krey et al., 1997)
	Nafenopin	(Krey et al., 1997)
	Wy-14,643	(Issemann and Green, 1990)
<i>Industrial plasticizers</i>	Monoethylhexylphthalate	(Issemann and Green, 1990)
	Diethylhexylphthalate	(Issemann and Green, 1990)
<i>Industrial Solvents</i>	Trichloroacetate	(Walgren et al., 2000)
	Dichloroacetate	(Walgren et al., 2000)
<i>Eicosanoids</i>	8(S)-hydroxyeicosatraenoic acid	(Krey et al., 1997)
	Docosaheptaenoic acid	(Krey et al., 1997)
	Leukotiene B ₄	(Devchand et al., 1996)
<i>Fatty Acids</i>	Linoleic acid	(Krey et al., 1997)
	Linolenic acid	(Krey et al., 1997)
	Arachidonic acid	(Krey et al., 1997)

TABLE 2. Activation of MAPKs, or non-genomic effects, by various PPAR ligands in different cell model systems.

LIGAND	CELL SYSTEM	MAPK ACTIVATED	REFERENCE
PPARα			
docosahexaenoic acid	rat VSMC	p38	(Diep et al., 2000)
retinoic acid	rat adipocytes	ERK, p38	(Teruel et al., 2003)
linoleic acid	rat aortic SMC	ERK	(Rao et al., 1995)
WY-14,643	ML457	ERK	(Rokos and Ledwith, 1997)
	primary mouse hepatocytes	ERK	(Mounho and Thrall, 1999)
	primary rat hepatocytes	ERK, p38	(Pauley et al., 2002)
clofibrate	ML457	ERK	(Rokos and Ledwith, 1997)
nafenopin	primary rat hepatocytes	ERK, p38	(Cosulich et al., 2000)
PPARγ			
15d-PGJ(2)	human mesangial cells	ERK, no effect on p38 or JNK	(Wilmer et al., 2001)
	primary rat astrocytes	ERK, p38, JNK	(Lennon et al., 2002)
	PC-12	P38	(Jung et al., 2003)
	Rat VSMC	ERK	(Takeda et al., 2001)
	C ₂ C ₁₂	ERK	(Huang et al., 2002)
Ciglitazone	human mesangial cells	No effect	(Wilmer et al., 2001)
	primary rat astrocytes	ERK, p38, JNK	(Lennon et al., 2002)
	GN4	ERK, p38, JNK	(Gardner et al., 2003)
	C ₂ C ₁₂	ERK	(Huang et al., 2002)
Troglitazone	HCT-15	ERK	(Baek et al., 2003; Kim et al., 2002)
	Rat VSMC	ERK	(Takeda et al., 2001)
	HepG2	p38, JNK, no effect on ERK	(Bae and Song, 2003)
	GN4	p38, ERK, no effect on JNK	(Gardner et al., 2005)
	MCF-7	ERK, p38, JNK	(Yin et al., 2004)
Rosiglitazone	fetal rat adipocytes	ERK, p38	(Teruel et al., 2003)

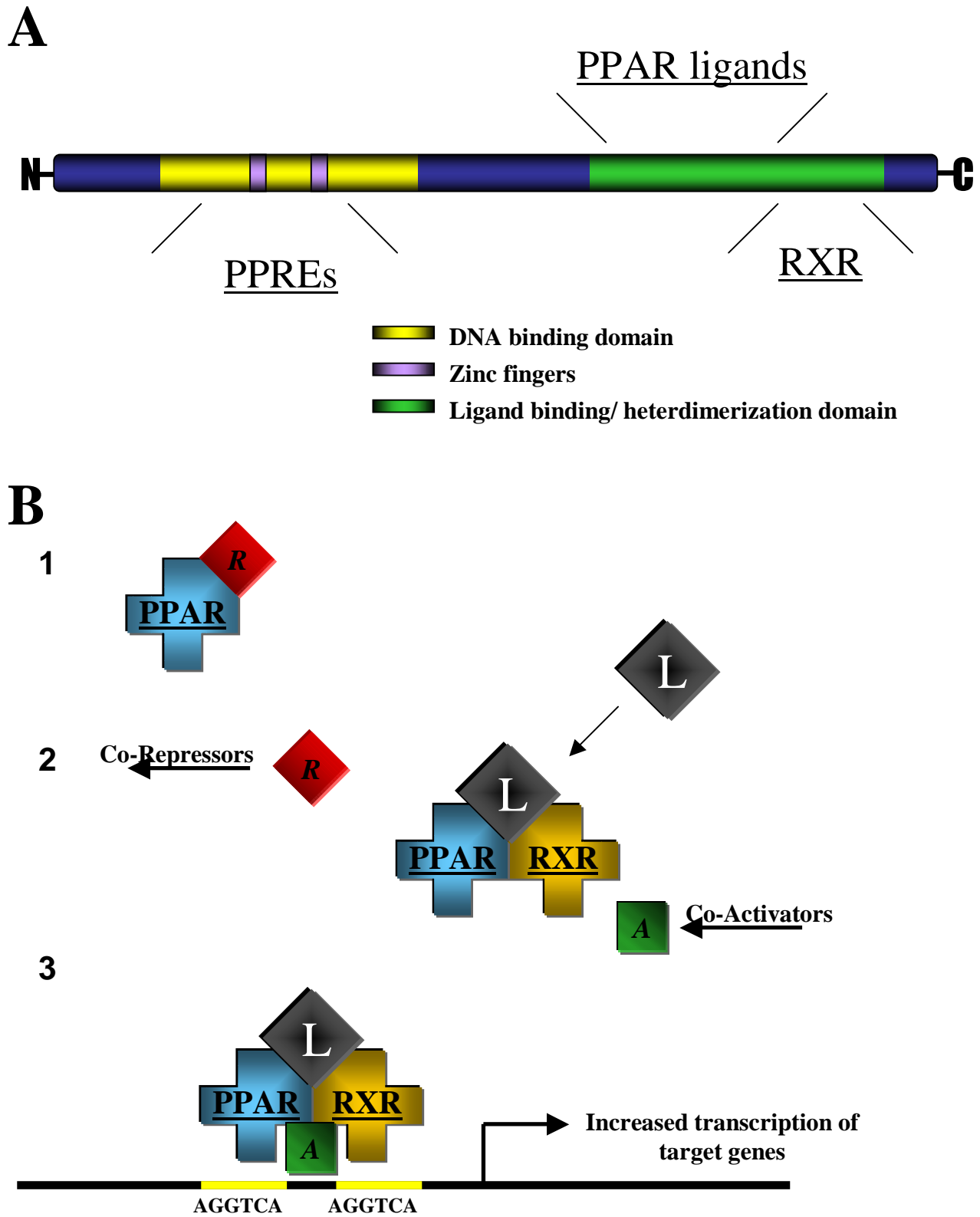
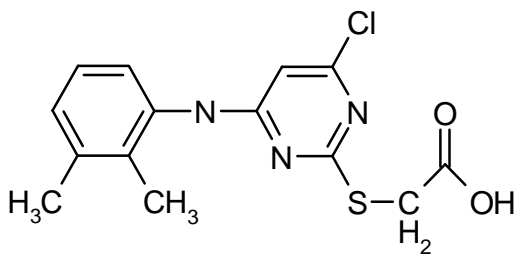
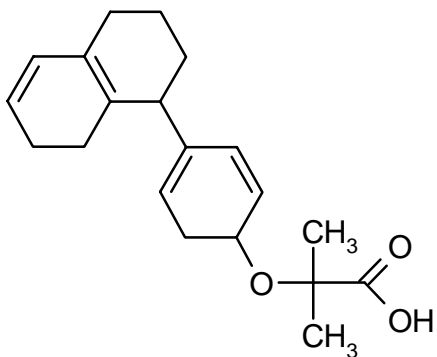


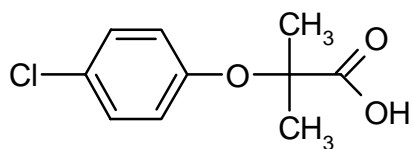
Figure 1



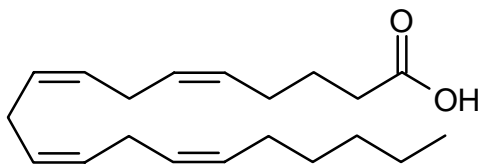
Wy-14,643



Nafenopin



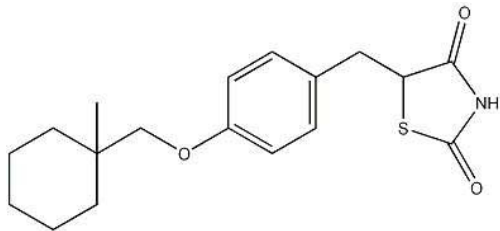
Clofibrate



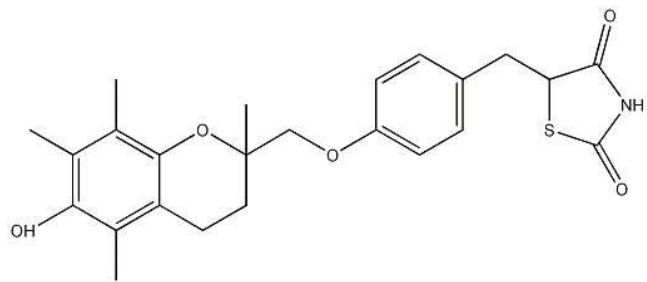
Arachidonic Acid

Figure 2

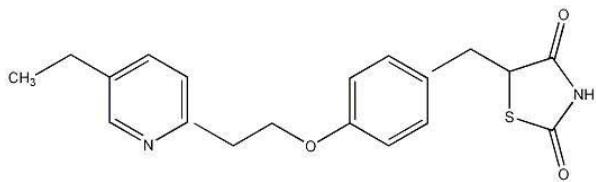
A



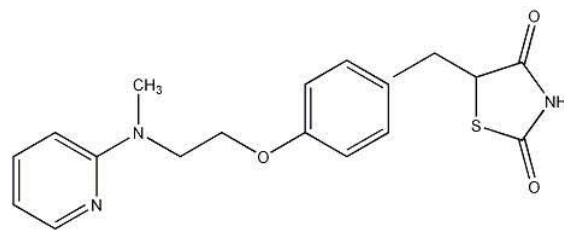
Ciglitazone



Troglitazone

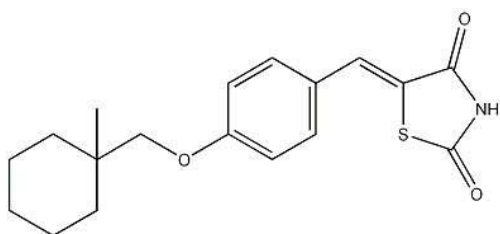


Pioglitazone

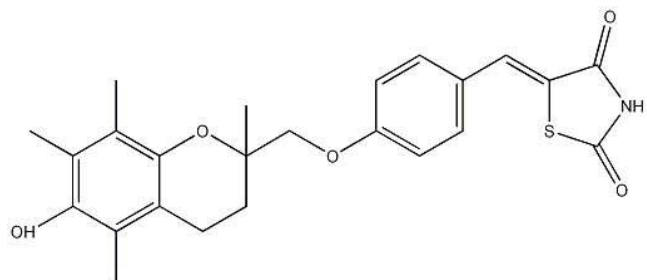


Rosiglitazone

B



Δ^2 -Ciglitazone



Δ^2 -Troglitazone

Figure 3

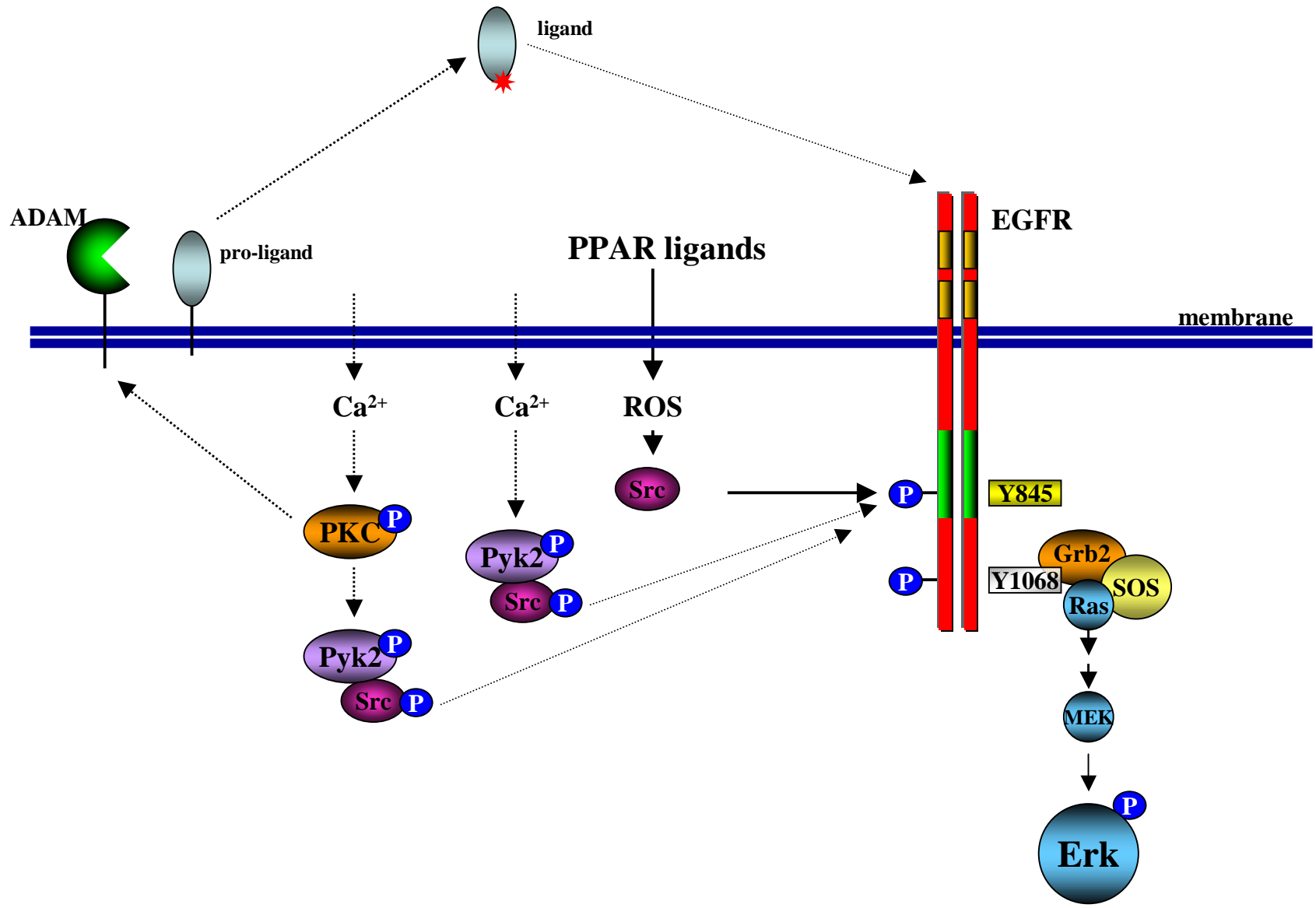


Figure 4

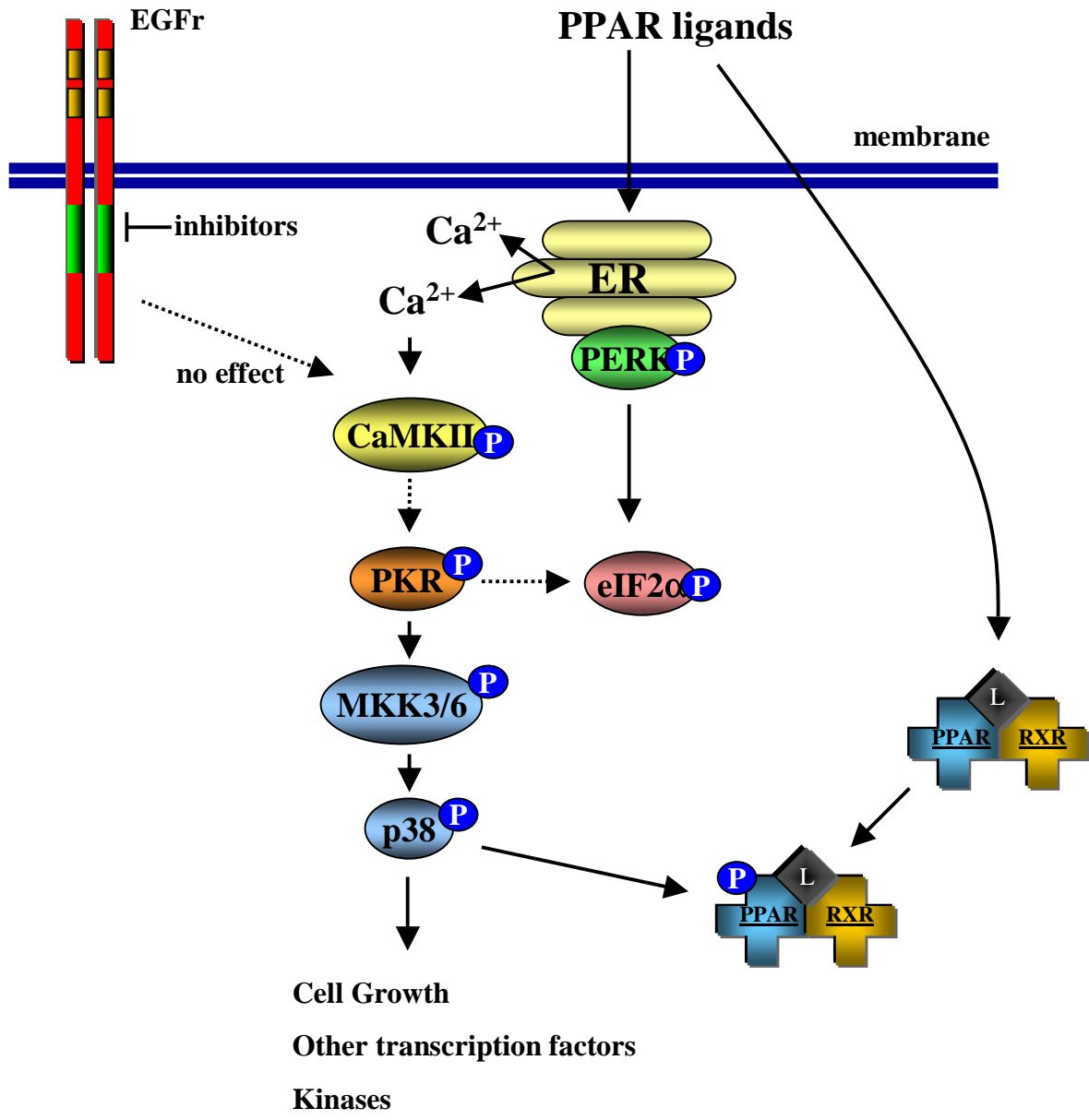


Figure 5